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Enteric Viral Infections of Calves and Passive Immunity¹

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ABSTRACT

At least eight viruses have been identified, four within the last 5 yr, that produce diarrhea and pathological intestinal lesions in experimentally inoculated calves. Coronavirus and rotavirus frequently are associated with the neonatal calf diarrhea syndrome, but the etiologic role of the newly identified viruses is undefined. All diarrheal viruses replicate within small intestinal epithelial cells, resulting in variable degrees of villous atrophy.

Immunity against these viral infections, therefore, must be directed toward protection of the susceptible intestinal epithelial cells. Because most of these viral infections occur in calves <3 wk of age, passive lactogenic immunity within the gut lumen plays an important role in protection. This report reviews methods of boosting rotavirus antibody responses in bovine mammary secretions and analyses of passive and active immunity in calves supplemented with colostrum and challenged by rotavirus. Results indicate rotavirus immunoglobulin G₁ antibodies in colostrum and milk were elevated after intramuscular and intramammary vaccination of pregnant cows with an Ohio Agricultural Research and Development Center rotavirus vaccine but not after intramuscular immunization with a commercial rota-coronavirus vaccine. Feeding colostrum from intra-

muscular plus intramammary immunized cows to newborn calves challenged by rotavirus prevented diarrhea and shedding of rotavirus.

INTRODUCTION

Electron microscopy for detection of enteric viruses from diarrheic calves has led to the discovery of a number of new viruses within the past decade (61, 63, 81, 83). Whereas both rotaviruses and coronaviruses are primary enteric pathogens frequently associated with the neonatal calf diarrhea (NCD) syndrome (3, 28, 32, 43), the etiologic role of these recently identified viruses in the NCD syndrome is undefined. Inability to cultivate these often fastidious new viruses in tissue culture precludes development of many commonly used serologic tests for detection of these agents or their antibodies.

Knowledge of the pathogenesis, epidemiology, and mechanisms of immunity to enteric viruses is important in developing rational approaches to immunization and control of these viral diarrheas. Because most of these infections commonly occur as enzootics in calves <3 wk of age, enhancement of passive immunity may be an effective approach for their control. Passive immunity to enteric viruses such as rotaviruses and coronaviruses is associated with ingestion of colostrum or milk containing high concentrations of specific antibodies (lactogenic immunity). Antibodies in blood serum generally appear to be of less value in protection against these viruses (67, 68).

This review describes the currently known diarrheagenic viruses of calves including their epidemiology, pathogenicity, and pathogenesis. It also focuses on passive lactogenic immunity, including ways to enhance this immunity by immunization of pregnant cows. An analysis of passive protection in calves fed various colostrum supplements is reported.

Received September 26, 1983.

¹Salaries and research support provided by State and Federal Funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University. Journal Article No. 104-83.

Bovine Enteric Diarrheal Viruses: Description and Antigenic Characterization

Summarized in Table 1 is a description of each of the currently known bovine enteric diarrheagenic viruses. Half of these viruses have been identified only within the last 5 yr, so only limited information is available about their antigenicity, pathogenicity, or epidemiology.

As further illustrated in Table 1, only one serotype of most of these viruses has been reported with the exception of rotavirus and possibly Bredavirus and calicivirus. At least two bovine rotavirus serotypes were distinguished by crossprotection studies and by virus neutralization tests based on presence of type specific antigens thought to be primarily associated with the viral outer capsid layer (44). However, rotaviruses from all species also share common group-specific antigens detectable by complement fixation, immunofluorescence, enzyme-linked immunosorbent assay (ELISA), and other serological tests (24). Because rotaviruses from one species experimentally can crossinfect and cause clinical disease in another species (38), a potential may exist for cattle to be infected with rotaviruses that originated in other species.

A bovine rotavirus-like agent (RVLA) identified in our laboratory was morphologically identical to rotavirus but differed antigenically and in the double-stranded ribonucleic acid electropherotype (63). However, this RVLA did not react antigenically or have a similar RNA electropherotype to a different RVLA which we previously identified in pigs (8, 60), suggesting the existence of several antigenically distinct categories of RVLA. Whether the bovine and porcine RVLA that we identified share common antigens with recently described RVLA from other species (10) is unknown.

Bovine coronavirus does not crossreact antigenically with other enteric coronaviruses such as transmissible gastroenteritis (TGE) virus of swine, canine coronavirus, or feline infectious peritonitis virus. However, it is antigenically related to hemagglutinating encephalomyelitis of swine and human respiratory coronavirus OC43 (51). Two reports suggested possible transmission of coronaviruses between calves and man (50, 71), but the epidemic significance of these possible cross-species infections is uncertain.

Bovine parvovirus does not crossreact with enteric parvoviruses from other species. Antigenic relationships of the newly described viruses to similar viruses from different species generally is undefined (Table 1). As serologic tests are developed to characterize these newly described viruses, additional serotypes may be detected and antigenic relationships revealed.

Epidemiology and Pathogenicity. A number of viruses originally isolated from feces or intestinal contents of calves cause diarrhea in experimentally inoculated gnotobiotic or colostrum-deprived calves (Table 2). Several of these viruses have been associated consistently with diarrhea and pathological intestinal lesions in studies of conventional calves (28, 43). Enteroviruses and reoviruses (Table 2) frequently have been detected in calf feces but have not been reported to cause diarrhea. By analogy to similar viruses in other species these two viruses presumably replicate in intestinal lymphoid tissue (80), and this may explain their failure to cause diarrhea. All viruses capable of causing diarrhea replicate in either villous or crypt intestinal epithelial cells (Table 2); the cell tropism of these viruses will be discussed further in a later section.

The age incidence of infection of calves reported for the various viruses or other enteric disease agents is in Table 3. All viruses in Table 3 are endemic. Most adult cattle are seropositive for antibodies to rotavirus (2, 17, 82) coronavirus (56), and parvovirus (70), and about 60% of cattle have neutralizing antibodies to bovine viral diarrhea virus (BVD) (39). None of the recently described viruses (Bredavirus, RVLA, calicivirus, or astrovirus) has been passaged serially in cell culture. This precludes use of routine serologic tests to assay for presence of antibodies to these agents. Thus, their prevalence is unknown.

The ubiquitous and stable nature of the enteric viruses, which results in infections of calves under the protective umbrella of passive antibodies, may explain the generally high morbidity but low mortality associated with these infections (3, 43). This is in marked contrast to epidemics of the swine coronavirus, TGE, which causes high mortality of seronegative piglets less than 1 wk of age (21). Widespread occurrence of antibodies to these enteric viruses in bovine colostrum may explain partially the age incidence of rotavirus, coronavirus, and

TABLE 1. Bovine enteric diarrheagenic viruses: description and antigenic characterization.

Bovine virus	Virus shape and size	Nucleic acid ^a	First description	No. known serotypes	Antigenic relationships
Togavirus (bovine viral diarrhea virus)	Pleomorphic 30–100 nm	SS RNA	Olafson et al., 1946 (49)	1	Hog cholera virus
Parvovirus	Icosahedral 18–25 nm	DNA	Abinanti and Warfield, 1961 (1)	2	No crossreaction with parvoviruses from other species
Rotavirus	Spherical 55–70 nm	DS RNA	Mebus et al., 1960 (36) Murakami et al., 1983 (44)	2	All rotaviruses from other species
NCDV ^b Coronavirus	Pleomorphic 70–120 nm	SS RNA	Stair et al., 1972 (69)	1	Hemagglutinating encephalomyelitis of swine; Human respiratory coronavirus OC 43
Newly described viruses					
Calicivirus (Newbury agent)	Spherical –33 nm	?	Woode and Bridger, 1978 (81)	2 (?)	Unknown – does not crossreact with porcine enteric calicivirus
Astrovirus	Spherical 28–30 nm	SS RNA	Woode and Bridger, 1978 (81)	1	Unknown
Bredavirus	Pleomorphic 70–100 nm	?	Woode et al., 1982 (83)	2 (?)	Unknown – does not crossreact with bovine coronavirus
Rotavirus-like agent	Spherical 55–70 nm	DS RNA	Saif et al., 1982 (63)	1	Unknown – does not crossreact with porcine pararotavirus

^aSS = Single stranded; RNA = ribonucleic acid; DNA = deoxyribonucleic acid; DS = double stranded.

^bNCDV = Nebraska Calf Diarrhea Virus.

TABLE 2. Pathogenicity and classification of enteric viruses of calves according to intestinal cell tropism.

	Infection of enterocytes		Diarrhea in:		
	Villous	Crypt	Conventional calves	Colostrum-deprived	Gnotobiotic calves
Type 1: Infect villous enterocytes					
Rotavirus	+	-	+	+	+
Rotavirus-like agent	+	-	? ^a	+	+
Coronavirus	+	±	+	+	+
Bredavirus	+	±	?	+	+
Calicivirus	+	-	?	NT ^b	+
Astrovirus	+	-	?	NT	-
Type 2: Infect crypt enterocytes					
Bovine viral diarrhea virus	-	+	+	+	+
Parvovirus	-	+	+(?)	+	?
Type 3: Infect intestinal lymphoid cells					
Enterovirus	-	-	-	-	-
Reovirus	-	-	-	-	-

^a? = Not enough data available.

^bNT = Not tested.

parvovirus infections, which occur at a time (7 to 21 days of age) when titers of passive milk antibodies have decreased to unprotective concentrations (2, 17, 28, 56, 70). Infection of younger calves (<7 days) with these viruses may relate to low colostrum antibody titers, higher doses of virus, or failure of transfer of passive immunity.

Existence of animals that are seronegative to BVD virus (39) or occasionally coronavirus (72) explains the ability of these viruses to infect animals of various ages including adults, depending on their serologic status. Bovine viral diarrhea virus may occur in young calves (2 to 3 days old) as a result of a late gestation in utero infection of seronegative dams (27).

Viral Replication Sites and Pathogenesis of Disease

Enteric diarrheagenic viruses may be categorized according to their sites of replication, both vertical and longitudinal, within the intestinal tract. The first type of virus (Type 1, Table 2) infects villous epithelial cells and usually does not cause systemic infections. This includes coronavirus, rotavirus, and the newly described viruses. In contrast, viruses of the second type infect primarily crypt enterocytes and intestinal lymphoid cells and usually cause systemic infections. Type 2 viruses presently include parvovirus and BVD virus (Table 2).

TABLE 3. Age associated incidence of diarrhea infections in calves.

Pathogen	Age incidence			
	<7 days	7-14 days	15-21 days	>3 mo
<i>Escherichia coli</i>	+	-	-	-
Rotavirus	±	+	±	±
Coronavirus	±	+	+	+
Bovine viral diarrhea virus	+	+	+	+
Parvovirus	+	+	+	+
Cryptosporidia	-	+	+	+

Type 1 viruses are thought to be transmitted by the fecal-oral route resulting in direct infection of villous epithelial cells via the luminal surface of these cells. Whether certain of these viruses also can be transmitted by aerosol exposure, with virus initially replicating in the oropharynx leading to massive doses of viruses being swallowed, is unclear and needs to be investigated. In preliminary studies in our laboratory, experimental oral/intranasal (IN) or IN exposure of colostrum-deprived or gnotobiotic calves to bovine coronavirus led to coronavirus antigen not only in the small intestine and colon but also in tracheal and nasal smears prepared at 2 to 3 days post-exposure (DPE) (L. J. Saif, D. R. Redman, and K. W. Theil, unpublished observations). Replication of the coronavirus TGE also occurs in tissues of the upper respiratory tract (25). These findings suggest the possibility that more labile enveloped enteric viruses such as coronavirus may undergo initial replication in the oropharynx contributing massive doses of virus to the small intestine. Viremia generally has not been reported for Type 1 viruses.

There are no definite explanations to account for the villous epithelial cell tropism of Type 1 viruses. Possible explanations include the role of cell receptors and various enzymes within the brush borders of the enzymatically mature villous epithelial cells. These enzymes may be required for viral uncoating or exposure of cell receptors in a manner similar to the *in vitro* requirement for intestinal enzymes such as trypsin, chymotrysin, or pancreatin for replication of most rotaviruses (73, 84) and human astrovirus (30) in cell culture. Additionally, in the neonate, some viruses might be taken up in absorptive villous epithelial cells nonspecifically via pinocytosis during the period of colostrum absorption.

Type 1 viruses infect and destroy villous epithelial cells, and result in villous atrophy. Loss of these mature absorptive cells leads to a malabsorption diarrheal syndrome typical of these viral infections. In a review of diarrheal pathogenesis, Moon (40) drew a corollary between the severity of various viral infections and the extent of viral replication in epithelial cells lining both the sides and tips of the villi. Whereas rotavirus mainly infects cells on the apical half of the villi (the more enzymatically mature cells) producing a milder, transient

diarrhea, coronaviruses infect epithelial cells throughout the length of the villi causing more pronounced villous atrophy and also diarrhea. Epithelial cells on the villi tips constantly are being replaced by cells that proliferate in the crypts and migrate up the sides of the villi. The turnover rate of these cells is slower in younger or gnotobiotic animals, leading to less rapid repair of villous atrophy, which may contribute to the enhanced susceptibility of neonates to these viral diarrheas (40). Increased frequency of villous epithelial cell replacement and loss of the capacity to absorb macromolecules (Ig) from colostrum are age-related factors that may increase the resistance of older animals to viral diarrheas. Loss of the absorptive capacity may reduce the animal's susceptibility to enteric viral infections by eliminating the possible "nonspecific" uptake of viral or bacterial pathogens via the same pinocytotic pathway as the Ig are absorbed.

The constant replacement of damaged villous epithelial cells by cells originating in the crypts, which are refractory to further rotavirus infection (34), suggests that in most animals these infections will be self-limiting. However, a cause of some concern might be multiple infections that occur naturally with several Type 1, or Type 2 viruses that destroy both villous and crypt enterocytes (32). Such infections presumably could lead to much greater morbidity and mortality than one agent alone, and in one study mortality rates increased with number of enteric viruses detected (76). There are few experimental studies of the pathogenesis or possible additive or synergistic interactions of multiple viral infections in calves, but detection of several viruses from a single diarrheic calf is common (3, 32, 43, 76, 81).

Besides shared intestinal cell tropisms, Type 1 viruses also possess common physicochemical and biological characteristics as described in Table 4. Properties such as stability to low pH and proteolytic enzymes enable these viruses to function well in the intestinal environment. Because of inability to propagate many of these fastidious enteric viruses in cell culture, electron microscopy has been instrumental in their detection and identification.

Viruses of the second type that infect mainly crypt epithelial and intestinal lymphoid cells (Table 2) also are thought to be transmitted via the fecal-oral and respiratory routes.

TABLE 4. Characteristics of type 1 bovine enteric viruses.

Physicochemical
1. Heat labile – partially explain their peak occurrence in winter months.
2. Stable at low pH (3–4).
3. Stable to proteolytic enzymes – often key to their propagation in cell culture.
Biological
4. Difficult to propagate in conventional cell culture systems (electron microscopy usually led to their initial detection).
5. Generally cause more severe infections in younger animals than in adults.
6. Generally occur as endemic infections in partially immune animals.

Studies of BVD and feline and canine parvoviruses suggested the initial site of viral replication is in the upper respiratory tract [(48), oropharyngeal tissue]. From there, virus may be disseminated to intestinal lymphoid tissues, crypt epithelial cells, or other secondary sites either via a viremia, infected leukocytes, or both (48). The predilection of BVD and parvovirus for crypt epithelial and intestinal lymphoid cells probably is related to the rapid proliferative rate of these cells as *in vivo* replication of these viruses requires a population of cells undergoing frequent mitosis (23).

As discussed by Moon (40), infection of crypt epithelial cells by Type 2 viruses also leads to severe villous atrophy with loss of crypt and consequently replacement villous epithelial cells. This results in a state of total mucosal collapse.

Besides this vertical stratification of virus replication sites within intestinal epithelial cells, there also appears to be longitudinal differentiation of viral replication sites in the intestine (Table 5). In general, viruses that replicate only in limited portions of the intestine or that infect only scattered epithelial cells cause only mild or no villous atrophy and subsequently mild or no diarrhea. In comparison, viruses that replicate throughout the entire intestine or that infect a high percentage of epithelial cells might be expected to cause more severe villous atrophy and diarrhea. For example, a virus such as astrovirus produces only a patchy fluorescence (indicative of only scattered infected cells), and

in the calf this limited replication results in little villous atrophy or diarrhea (Table 5). Coronavirus, however, produces an almost continuous infection of entire villous enterocytes throughout the distal portion of the small intestine and colon. It usually is associated with a severe diarrheal syndrome in experimentally inoculated or naturally infected calves (28, 35, 50). Bovine Bredavirus has a similar predilection for cells in the distal portion of the small intestine and colon, but it generally produces a milder diarrheal syndrome (61, 83).

Rotavirus and RVLA generally replicate and cause villous atrophy in the distal half of the small intestine, but not the colon, whereas the bovine calicivirus replicates and produces villous atrophy in the proximal portion of the small intestine (Table 5).

As for cell tropisms, reasons for the predilections of different viruses for certain regions of the intestine are unclear. Physiologic factors such as different enzyme concentrations, pH, and presence of bile salts all may play a role. Studies have shown longitudinal differentiation in the small intestine in regard to absorption of nutrients and cell turnover with both activities increased in the duodenum (5). All these variables may influence virus stability, receptors, and replication. Immunologic factors such as regional variation in concentrations of local antibodies, cell-mediated immunity, or interferon also may influence intestinal viral replication sites.

Immunity Against Enteric Viral Infections: Bovine Immunoglobulins in Mammary Secretions

There are numerous detailed reviews of the ruminant humoral immune system (29, 42). To summarize briefly, IgG₁ and IgG₂ are predominant in bovine serum where they occur in similar concentrations. The IgG₁, the major isotype in bovine mammary secretions, is transported actively from serum into colostrum (52) and also into milk (31, 47), although less. Prevalence of IgG₁ in milk is unique to ruminants, and IgA predominates in mammary secretions of monogastrics including humans (74). In monogastric species, secretory IgA (SIgA) antibodies occur in milk after antigenic stimulation of the gastrointestinal tract of the dam, such as following an enteric infection (4, 7, 58, 59). Parenteral immunization generally

TABLE 5. Viral replication sites of bovine enteric viruses and their distribution in the intestine.

Virus	Cell type infected	Extent of infection	Primary regions of intestine infected ^a	Primary site of villous atrophy ^a
Rotavirus (34)	Villous enterocytes	patchy-continuous	J, I	J, I
Rotavirus-like agent (63)	Villous enterocytes	patchy-continuous	J, I	J
Bovine coronavirus (35)	Entire Villous enterocyte Crypt enterocytes (colon)	continuous	J, I, Colon	J, I, Colon
Bovine Bredavirus (62, 83)	Lower villous and crypt enterocytes	patchy-continuous	J, I, Colon	J, I
Calicivirus (81)	Lower villous enterocytes	patchy	D, J	D, J
Astrovirus (81)	Dome enterocytes	patchy	I	J(±)
Bovine viral diarrhea virus (48)	Crypt enterocytes	patchy-continuous	I, Colon	? ^b
Parvovirus (70)	Crypt enterocytes	patchy	J, I, Colon	?

^aD = Duodenum; J = jejunum; I = ileum.

^b? = Not reported.

elicits only IgG milk antibodies (7, 58, 59). Following local intestinal antigenic stimulation, IgA immunocytes migrate from the gut to the mammary gland where they secrete SIgA antibodies into the milk (57), thus establishing an "enteromammary SIgA link". Thus, the antibody specificities of milk IgA reflect antigenic challenges in the intestinal tract of the dam.

Few studies have addressed the question of a similar enteromammary SIgA link in the bovine such that antigenic stimulation by the oral route would elicit IgA milk antibodies. In one study, oral vaccination of pregnant cows with live or dead *Escherichia coli* of the K99 pilus type resulted in little or no K99 antibody response in serum or colostrum and no antibody response in milk (41). However, a subsequent parenteral booster immunization of these cows during lactation resulted in blood serum and low milk antibody responses that were higher in the live *E. coli* vaccine cows. The Ig isotypes of *E. coli* antibodies were not determined.

In another study, jejunal infusion of pregnant cows with a nonreplicating bacteriophage antigen elicited IgG₁ and IgG₂ but not IgA antibodies in serum and mammary secretions

(13). We found similar results in a preliminary study of oral administration of live virulent bovine rotavirus to five pregnant rotavirus seropositive cows; only IgG₁ rotavirus antibodies were elevated in mammary secretions of two of five cows (L. J. Saif, K. L. Smith, and D. R. Redman, unpublished observations). The source of elevated IgG₁ colostral and milk antibodies in these two studies is unclear. The former study demonstrated few IgG₁ producing cells in mammary secretions in spite of the predominately IgG₁ response (13). A possible explanation might be that IgG₁ plasma cells, which occur in relatively large numbers in the intestines of adult ruminants (46), contribute IgG₁ antibodies (with specificity to enteric antigens) to serum and these then are transported actively to colostrum and milk. This also would explain the occurrence of predominately IgG₁ rotavirus or coronavirus antibodies in mammary secretions of naturally infected (presumably via the oral route) cows (Figures 1 and 2, Table 6). A similar mechanism recently has been proposed for the selective transport of dimeric serum IgA (most of which was produced by intestinal IgA plasma cells) to the colostrum in lactating mice (18). These preliminary

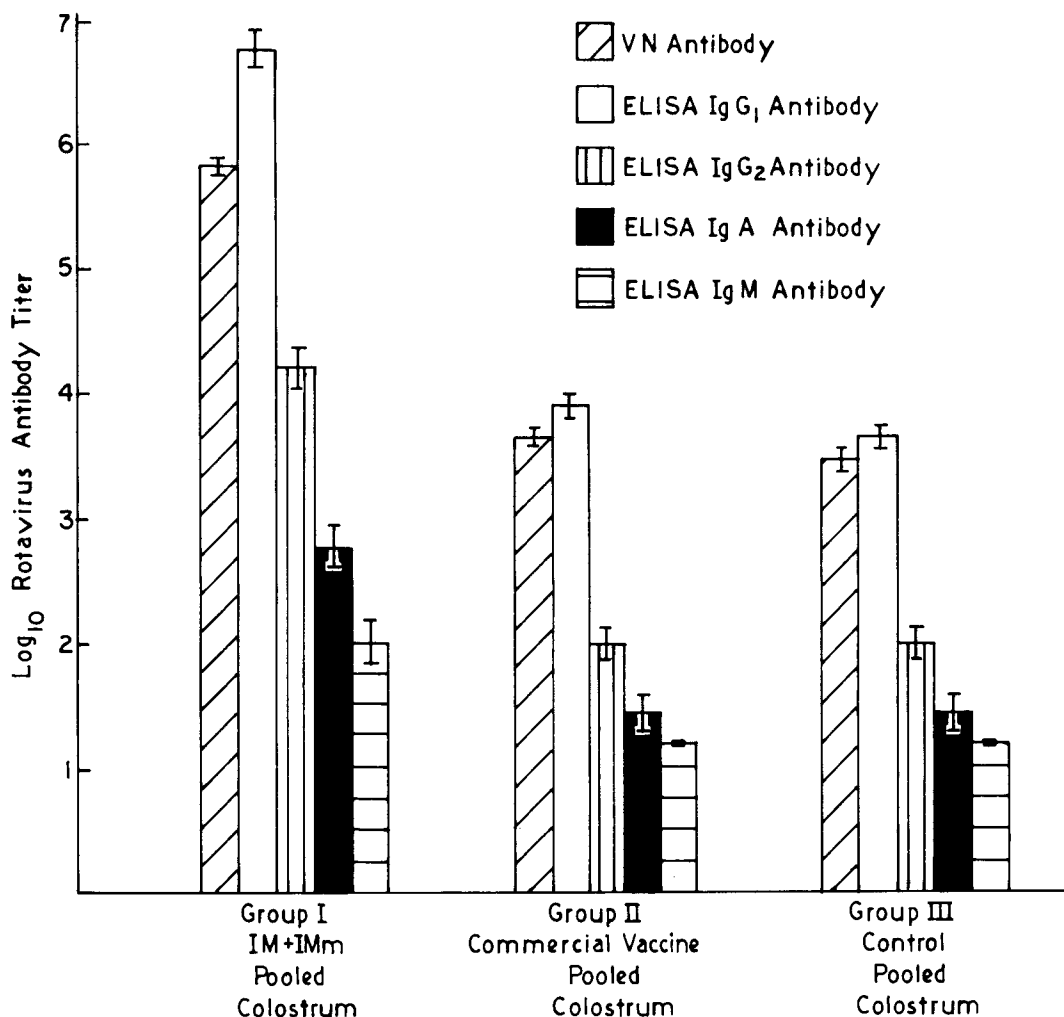


Figure 1. Enzyme-linked immunosorbent assay (ELISA) immunoglobulin (Ig)G₁, IgG₂, IgA, and IgM rotavirus geometric mean antibody titers (GMT = bars) and virus neutralization (VN) GMT \pm the standard error of the mean (lines) in pooled bovine colostrum from Group I, i.m.+IMm (immunized by intramuscular injection and intramammary infusion) Ohio Agricultural Research and Development Center rotavirus-immunized cows; Group II, commercial rota-coronavirus vaccine-immunized cows; and Group III, control cows.

findings suggest enteric priming or boosting may not be an effective means of eliciting SIgA antibodies in bovine mammary secretions. Indeed, the bovine mammary gland generally has been described as deficient of IgA, which may relate to rumen development (53). Although several studies have indicated bovine IgA milk antibodies are produced locally after intramammary (IMm) infusion of antigen (29), the origin of these cells producing IgA is undefined.

In the following discussion, attention will be directed primarily toward passive immunity against Type 1 viruses. In these viral infections, passive immunity is contingent upon continuing adequate amounts of antibodies in the gut lumen for protection of villous enterocytes (21). Serum-derived antibodies generally play a minor role, and there is usually little correlation between serum antibodies and infection (21, 37). This is the reverse of the situation for Type 2 viruses. In studies of BVD and canine parvo-

viral infections, there was a positive correlation between virus neutralizing antibody titers in blood serum (actively or passively acquired) and protection against infection (12, 55). Presumably this is because the virus infects intestinal cells via the hematogenous route. Therefore, neutralization of the virus before it reaches susceptible intestinal cells may be a key factor in immunity against Type 2 viral infections.

Rotavirus and Coronavirus Antibodies in Bovine Mammary Secretions: Antibody Isotypes and Titers in Naturally Infected Cows

Because bovine rotavirus and coronavirus are two well-characterized enteric viral infections,

they serve as useful models for studies of passive immunity to Type 1 viruses. Studies in our laboratory (62) and others (56, 64) using isotype specific ELISA and virus neutralization (VN) tests have confirmed the predominance of IgG₁ rotavirus and coronavirus antibodies in mammary secretions of naturally infected seropositive cows (Figures 1 and 2, Table 6). These were followed in prevalence by IgG₂ and IgA antibodies (Figure 1). Colostral rotavirus antibody titers decrease precipitously in the transition to milk (Figure 2). This is in contrast to monogastrics such as humans and swine in which IgA rotavirus antibodies predominate in milk (presumably due to the enteromammary SIgA link), and titers do not decrease dramatically during the transition to milk (7, 58, 59).

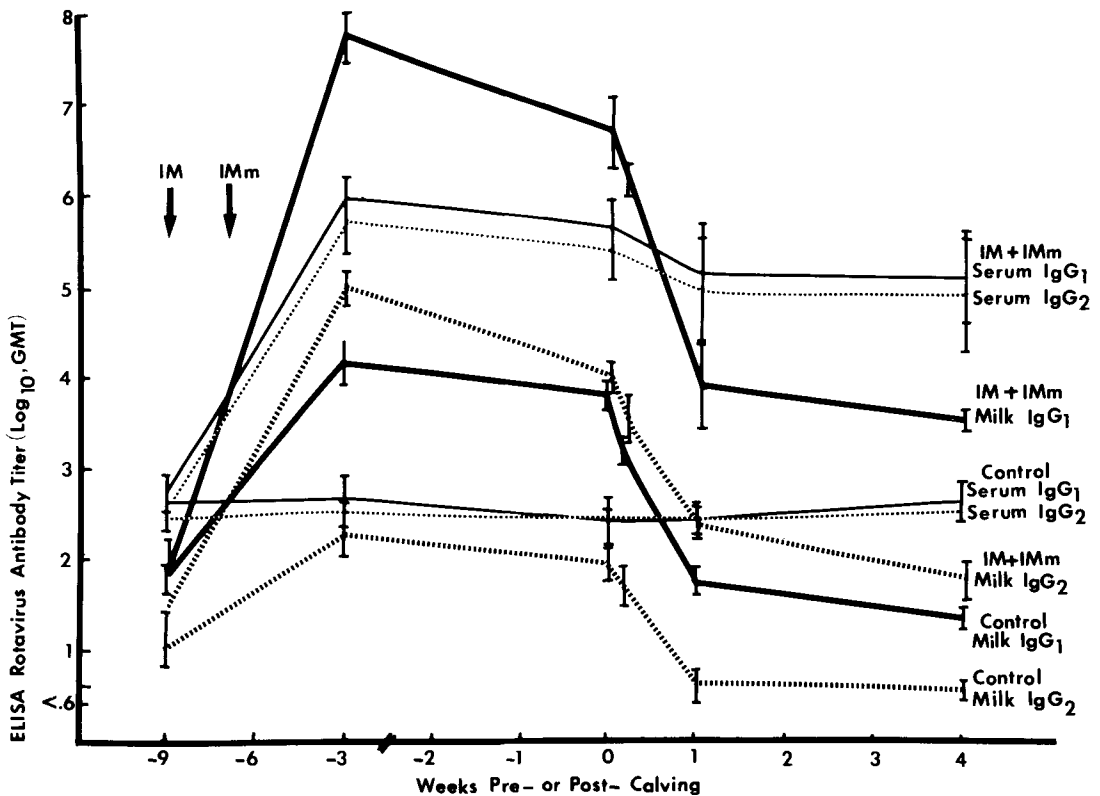


Figure 2. Rotavirus immunoglobulin (Ig)G₁, and IgG₂ enzyme-linked immunosorbent assay (ELISA) antibody titers in serum and mammary secretions from i.m.+IMm (immunized by intramuscular injection and intramammary infusion) Ohio Agricultural Research and Development Center rotavirus immunized cows or nonimmunized control cows (bars represent ± 1 SD about the geometric mean titer). Arrows indicate the time of immunization.

TABLE 6. Enzyme-linked immunosorbent assay (ELISA) immunoglobulin G₁ (IgG₁) rotavirus and coronavirus antibody titers in pooled bovine colostrum.^a

Immunization group	Rotavirus		Coronavirus	
	ELISA IgG ₁		ELISA IgG ₁	
	GMT ^b	95% CI ^c	GMT	95% CI
i.m. + IMm OARDC ^d rotavirus vaccine	2,413,682 ^A	[1,621,810; 3,548,133]	6,209 ^A	[3,845– 10,025]
Commercial Rota-coronavirus vaccine	8,192 ^B	[4,897; 13,803]	8,185 ^A	[4,853; 13,804]
Control	4,705 ^B	[2,691; 8,316]	7,132 ^A	[4,273; 11,901]

^{A,B}Means followed by the same letters are not significantly different ($P > .05$); those followed by different letters are significantly different ($P < .05$). Comparisons are made only within each given antibody test (vertical columns).

^aRepresents entire pooled first milking colostrum from five cows in each group.

^bGMT = Geometric mean titer computed on a minimum of five replicates for each sample.

^cBrackets = 95% confidence interval (CI).

^dOARDC = Ohio Agricultural Research and Development Center.

Enhancement of Rotavirus Antibodies in Mammary Secretions: Influence of Route and Time of Immunization

Because IgG₁ is transported selectively from serum to colostrum and milk, a rational approach for boosting rotavirus or coronavirus antibodies in bovine mammary secretions would be parenterally to immunize the pregnant dam with these viruses, thereby enhancing serum and, hence, colostrum rotavirus antibodies. Studies then would be required to evaluate the protective capacity of these IgG₁ antibodies in the calf against viral challenge.

A number of variables need to be considered in attempting to maximize antibody responses in mammary secretions. The first of these is the route and time of immunization. As in Figures 1 and 2, extremely high colostrum rotavirus IgG₁ antibody titers have been achieved by intramuscular (i.m.) immunization at ~9 wk prepartum and immunization by IMm infusion 2 wk post-i.m. of the pregnant dam with an Ohio Agricultural Research and Development Center

(OARDC) propagated modified-live NCDV bovine rotavirus administered in Freund's incomplete adjuvant. There were significant but lower increases of the other three colostrum antibody isotypes as well (Figure 1). Of further importance was the finding that these antibody titers remained significantly elevated in milk (compared to controls) for at least 30 days postpartum (Figure 2). In comparison, i.m. administration of the same modified-live rotavirus strain and coronavirus in a commercial vaccine² (administered without adjuvant at 6 and 3 wk prepartum) resulted in no significant increase of colostrum or milk rotavirus or coronavirus antibody titers compared to controls (Figure 1, Table 6). The rationale for the i.m. and IMm schedule was based on the hypothesis that a peripheral stimulus i.m. administered near involution could result in seeding of sensitized cells to the mammary gland. Subsequent IMm presentation of antigen then might result in further expansion of this clone of cells effectively increasing antibody in mammary secretions (78). Immunization studies in sheep and cattle demonstrated enhanced antibody responses of all isotypes in milk after this regimen (78).

²Calf Guard, Norden Laboratories, Lincoln, NE.

Although IMM immunization may be applicable in dairy cows, particularly during the dry period, it would not be practical in beef cows. To this end, other studies have reported significantly enhanced blood serum or mammary secretion rotavirus antibody titers after other parenteral routes, including i.m. or subcutaneous (S.C.) injection of adjuvanted inactivated virulent (37) or adjuvanted inactivated or live modified bovine rotavirus (19, 20, 64, 77). However, rotavirus antibody titers in mammary secretions were not as great as in our studies, and in two studies these antibodies failed to

protect nursing or colostrum-supplemented calves against rotavirus diarrhea (19, 64). However, two other studies (66, 75) reported decreased rotavirus incidence, duration, and severity after daily feeding of rotavirus immune colostrum supplements to dairy calves.

Use of Adjuvant

In addition to route of immunization, another important variable in our studies and others is the use of adjuvanted vaccines for enhancing rotavirus antibodies in mammary

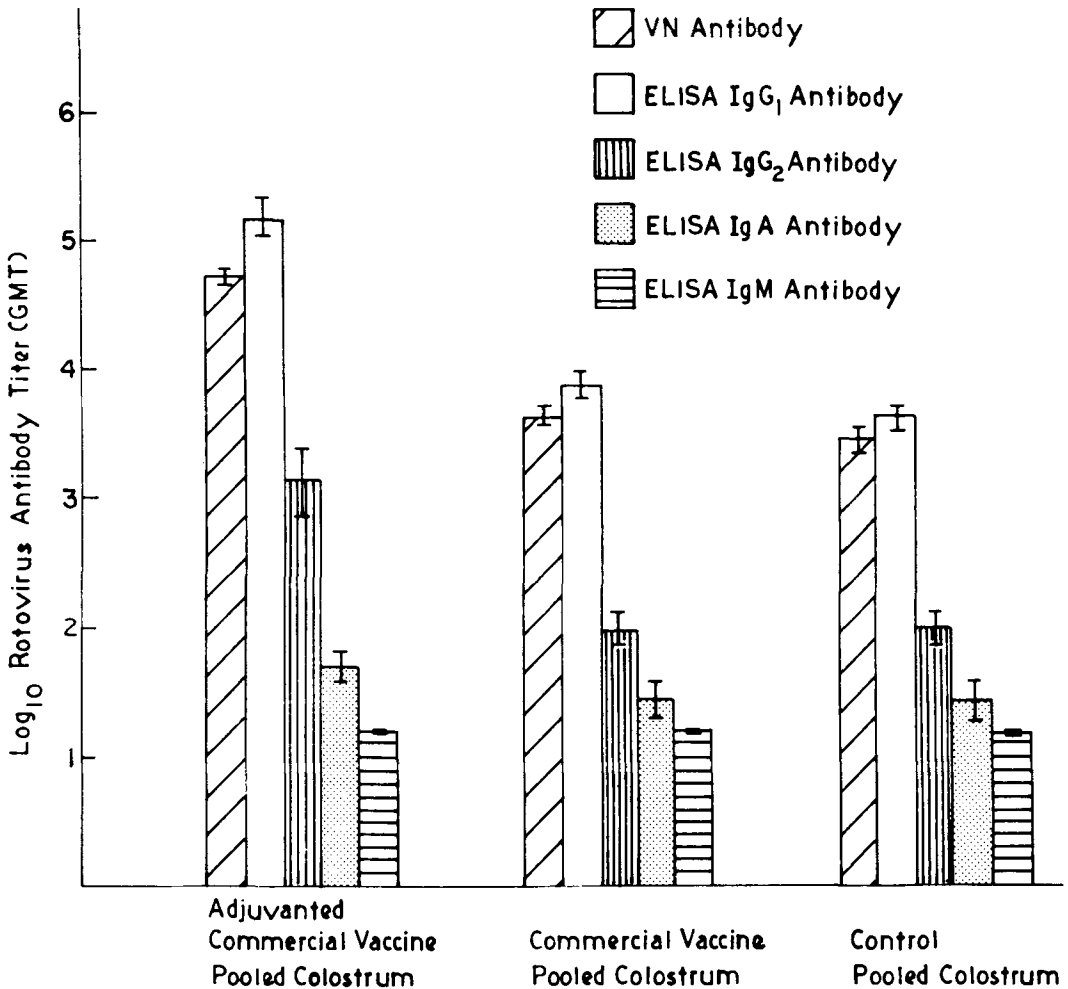


Figure 3. Enzyme-linked immunosorbent assay (ELISA) immunoglobulin (Ig)G₁, IgG₂, IgA, and IgM rotavirus geometric mean antibody titers (GMT = bar) and virus neutralization (VN) GMT ± the standard error of the mean (lines) in pooled bovine colostrum from five cows immunized with the commercial rota-coronavirus vaccine with Freund's incomplete adjuvant, five cows immunized with the commercial vaccine alone, and five control cows.

secretions. Investigations by Hess et al. (20) demonstrated little increase of colostrum or milk antibody titers in cows immunized with rotavirus alone. Their studies and another (65) showed various adjuvants differed in their ability to enhance rotavirus antibody titers in mammary secretions. Our findings concur with these results; incorporation of Freund's incomplete adjuvant with the commercial rotavirus vaccine led to enhanced colostrum antibody titers associated primarily with IgG₁, whereas use of unadjuvanted vaccine did not increase significantly colostrum antibody titers compared to controls (Figure 3). However, antibody titers were still lower with the adjuvanted commercial vaccine than with the OARDC adjuvanted vaccine i.m. plus IMm (Figures 1 and 3). Results of our studies did not demonstrate that use of adjuvant could boost nonspecifically antibody titers to an unrelated virus in seropositive animals, as concluded by others (77). As in Table 6, administration of the adjuvanted OARDC rotavirus vaccine did not enhance significantly coronavirus colostrum IgG₁ antibody titers in this group compared to controls.

Viral Dose and Form

A third variable of significance in rotavirus maternal vaccination procedures is the virus titer or dose and form (live or inactivated). In our experiments the titer of the OARDC vaccine (1×10^8 pfu/ml) was 10,000 times greater than the rotavirus titer of the commercial vaccine (1×10^4 pfu/ml), and this difference in titer also may have influenced the rotavirus antibody responses in mammary secretions. Also, in considering the optimal virus dose, use of inactivated vaccines may necessitate use of higher titered virus or larger amounts to achieve the same effect as the live attenuated virus because the antigenicity of some viral proteins may be destroyed in the inactivation process. The effect of different inactivating agents also needs to be analyzed. In preliminary studies, we found differences in the antibody response to binary ethylenimine (BEI) inactivated versus B-propiolactone (BPL) inactivated bovine rotavirus. Although both agents successfully inactivated rotavirus, antibody titers were about 10 times greater in mammary secretions of cows vaccinated with

BEI inactivated rotavirus compared to those vaccinated with BPL inactivated rotavirus.

Passive Immunity Against Type 1 Viruses: Lactogenic Immunity

The newborn calf is agammaglobulinemic and is dependent upon colostrum immunoglobulins for both local intestinal and humoral immunity. Uptake of colostrum Ig across the intestinal epithelium into the circulation occurs for a limited time (about 24 h) after birth. These passively acquired humoral antibodies, primarily of the IgG₁ isotype, persist for several weeks, protecting the newborn, especially against systemic infections. Circulating antibodies, although not essential for local protection against enteric infections, may moderate the severity or duration of these infections if in high titer (68).

Numerous studies have documented the importance of the almost continuous presence of colostrum or milk antibodies (lactogenic immunity) within the gut lumen for effective protection against enteric viruses (21). In swine, SIgA milk antibodies provided optimal passive protection against TGE virus, but high IgG milk antibodies were also protective if their titers could be maintained throughout lactation (58, 59). Few studies of the bovine animal have attempted to correlate antibody isotypes in colostrum or milk with passive immunity against enteric viral infection.

We investigated the ability of colostrum supplements from i.m. plus IMm OARDC rotavirus vaccine immunized cows or unimmunized control cows to protect newborn colostrum-deprived (CD) dairy calves against experimental rotavirus challenge. Colostrum supplements were fed at a 1% (vol/vol) concentration either 2 or 3 times/day beginning within 2 to 6 h after birth and continuing for 5 to 7 days. The rationale for this feeding regimen was: 1) feeding of normal control colostrum (to rotavirus challenged calves) at this concentration 2 or 3 times/day did not protect against rotavirus diarrhea or shedding, whereas feeding higher concentrations of control colostrum did provide partial immunity; and 2) rotavirus antibody titers in the 7- and 30-day milk samples from all cows declined to about 1% of the initial colostrum antibody titers (Figure 2). This decrease of milk antibody

titers is similar to the decrease of total IgG₁ in the transition from colostrum to 7- to 28-day milk (79). One group of experimental calves received no supplemental colostrum feeding. All calves were challenged orally with virulent NCDV bovine rotavirus at 12 to 30 h age and then monitored daily for occurrence of diarrhea and fecal rotavirus shedding by ELISA. Stools were characterized as abnormal based on color (yellow or bright green) and consistency (semiliquid or liquid).

Results of calf challenge studies are summarized in Table 7 and Figures 4 to 7. Colostrum-deprived calves fed no supplemental colostrum had no rotavirus serum IgG₁ antibody titers (<4) at challenge. These calves developed rotavirus-associated diarrhea by 1 DPE, and abnormal stools persisted for 5 to 9 DPE. Rotavirus shedding was detected for an average of 8.5 DPE. A typical serum and fecal antibody response of a CD calf (CD #26) is illustrated in Figure 4. By the isotype specific ELISA, rotavirus antibodies in serum following infection were associated primarily with IgM and IgA early in the response (7 DPE), but these antibodies were undetectable by 27 DPE. Serum antibodies to IgG₁, also detectable at 7 DPE, were prevalent at 14 to 27 DPE. Serum antibodies to IgG₂ were not detected until 14 DPE, and titers increased second only to IgG₁ at 21 to 27 DPE. Virus neutralizing rotavirus antibodies were detectable at 7 to 27 DPE, but titers were consistently lower than ELISA IgG₁ titers. A transient fecal IgM and IgA antibody response occurred at 5 to 14 DPE and was detectable even with continued shedding of viral antigen from 1 to 6 and 9 to 10 DPE. No fecal IgG₁ or IgG₂ antibodies were detectable throughout the 27 DPE sampling period.

These results correlate with studies of rotavirus infections in children (54) and specific-pathogen-free pigs (14) that demonstrated only a transient fecal IgA and IgM antibody response. Investigators studying rotavirus (14, 16, 54, 58, 59) and TGE in swine (26) reported primarily IgG and transient IgA responses in convalescent serum following intestinal infections. This serum IgA response (mostly dimer IgA in swine and cattle) may represent transfer of intestinally synthesized IgA antibodies into the serum. Rapid disappearance of these serum IgA antibodies may relate to the reported

selective transfer of dimeric serum IgA to other secretory sites (18) or back into the intestine via the bile (22).

Supplemental feeding of 1% colostrum from control cows did not protect newborn calves challenged by rotavirus against either rotavirus associated diarrhea or shedding (Table 7, Figure 5). Blood serum and fecal rotavirus antibody responses in calves fed 1% supplemental control colostrum were similar to those of calves fed no supplemental colostrum, as in Figure 5 for CD #43 calf. The principal difference was the low IgG₁ serum antibody at the time of challenge (16 h of age) after two 1% control colostrum feedings.

Calves fed 1% colostrum supplements from cows immunized with commercial vaccine had similar responses to those fed the control colostrum. (Data not shown.) Rotavirus IgG₁ serum geometric mean titers (GMT) were similar at challenge in calves fed control or commercial vaccine colostrum (ranging from 4 to 32), further reflecting similarities of initial antibody titers of these two colostrum pools (Figure 1). However, calves fed these two colostrum pools had slightly delayed onset and shorter duration of virus shedding and diarrhea compared to calves fed no colostrum (Figure 4 and 5, Table 7). This suggests even low colostrum rotavirus antibody may provide a slight passive immunity against rotavirus infection. Snodgrass and Wells (66) reported similar findings; lambs fed serum with low antibody titers had some protection against challenge compared to lambs fed serum devoid of rotavirus antibodies.

Feeding calves 1% pooled colostrum supplements from i.m. plus IMM OARDC vaccine immunized cows conferred both clinical protection and prevented rotavirus shedding (Table 7). Two of three calves remained clinically normal and failed to excrete rotavirus following a second homologous challenge at about 2 wk of age, indicating they probably initially had developed subclinical infection with subsequent active immunity (Figure 6). The active immunity was indicated by a transient increase of blood serum IgA and IgM antibody following virus challenge. Observations were similar in studies of passive immunity in rotavirus-challenged gnotobiotic lambs fed homologous colostrum (67) or gnotobiotic piglets fed bovine colostrum (9). As further illustrated in Figure 6 for CD #54 calf, calves had high serum IgG₁ and virus

TABLE 7. Summary of response of newborn colostrum-deprived (CD) calves fed various colostrum supplements to rotavirus (RV) challenge.

Source of pooled colostrum supplement fed ^a	No. of CD calves	\bar{X} Age at first challenge	RV IgG ₁ antibody titers ^b at challenge	Rotavirus shedding ^c	Peak rotavirus titer	Abnormal stool	Age of second challenge	Rotavirus shedding second challenge	Abnormal stools
		(h)	(GMT)	(\bar{X} day of onset DPE)	(\bar{X} Total no. days)	(\bar{X} days DPE)	(\bar{X} no. days)	(days)	(\bar{X} no. days)
None	8 ^d	22.5	<4	1.06	8.5	1.9	6.8	NC	...
Unimmunized Control cows	6 ^e	18.3	13.5	1.6	5.5	2.3	4.7	16.8	0
i.m. plus IMm ^f	5 ^g	20	4096	...	04	13.5 ^h	3.5
OARDC ⁱ RV	3 ^j	19.5	2048	...	0	...	0	12.0 ^h	5.0
Vaccine cows	6 ^k	23.8	203	3.8	4.0		2.8	14.8	1.0

^aEntire first milking colostrum was pooled from five cows in each group.

^bRotavirus IgG₁ antibody titers of serum collected immediately before challenge was determined by the antibody isotype specific ELISA test. GMT = Geometric mean titer.

^cAn ELISA test was used to quantitate RV in daily rectal swab samples. DPE = Days postexposure.

^dCalves were not fed any colostrum supplement.

^eCalves were fed 1% colostrum (20 ml + 2 liters Similac) 3X per day for 5 days and challenged with 2 ml of virulent RV between the second and third feedings.

^fi.m. plus IMm = Intramuscular plus intramammary infusion immunization.

^gCalves were fed 1% colostrum 3X day for 7 days and challenged with virulent rotavirus as above.

^hFour of eight calves in these two groups began shedding rotavirus spontaneously at 10 to 14 days of age.

ⁱOARDC = Ohio Agricultural Research and Development Center.

^jCalves were fed 1% colostrum 2X day for 5 days and challenged with virulent rotavirus as above.

^kCalves were fed .1% colostrum (2 ml + 2 liters Similac) 3X day for 5 days and challenged with virulent rotavirus as above.

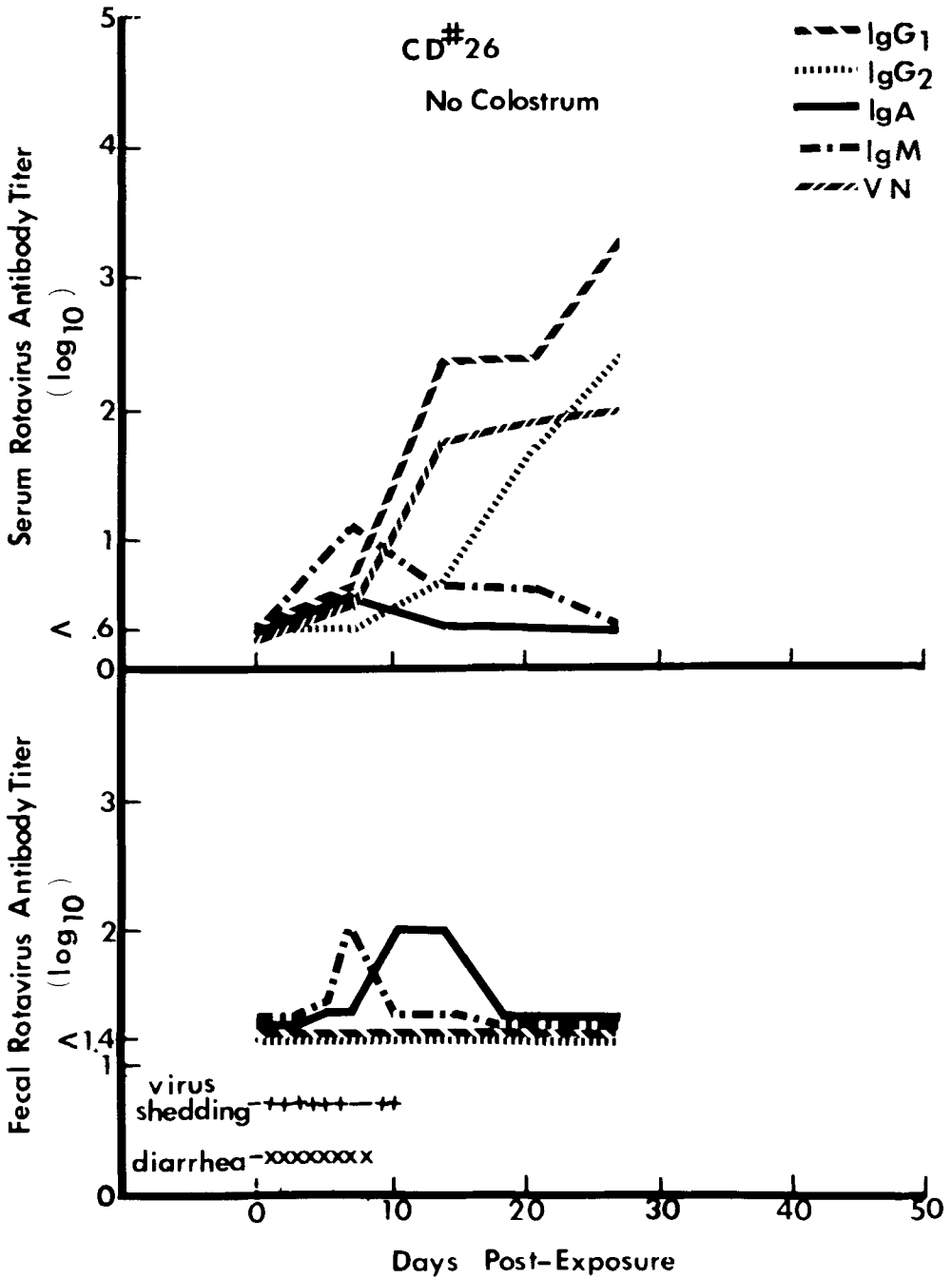


Figure 4. Serum and fecal rotavirus antibody isotypes (Enzyme-linked immunosorbent assay and virus neutralization titer log₁₀) after rotavirus challenge of a 17-h-old colostrum-deprived calf (CD #26).

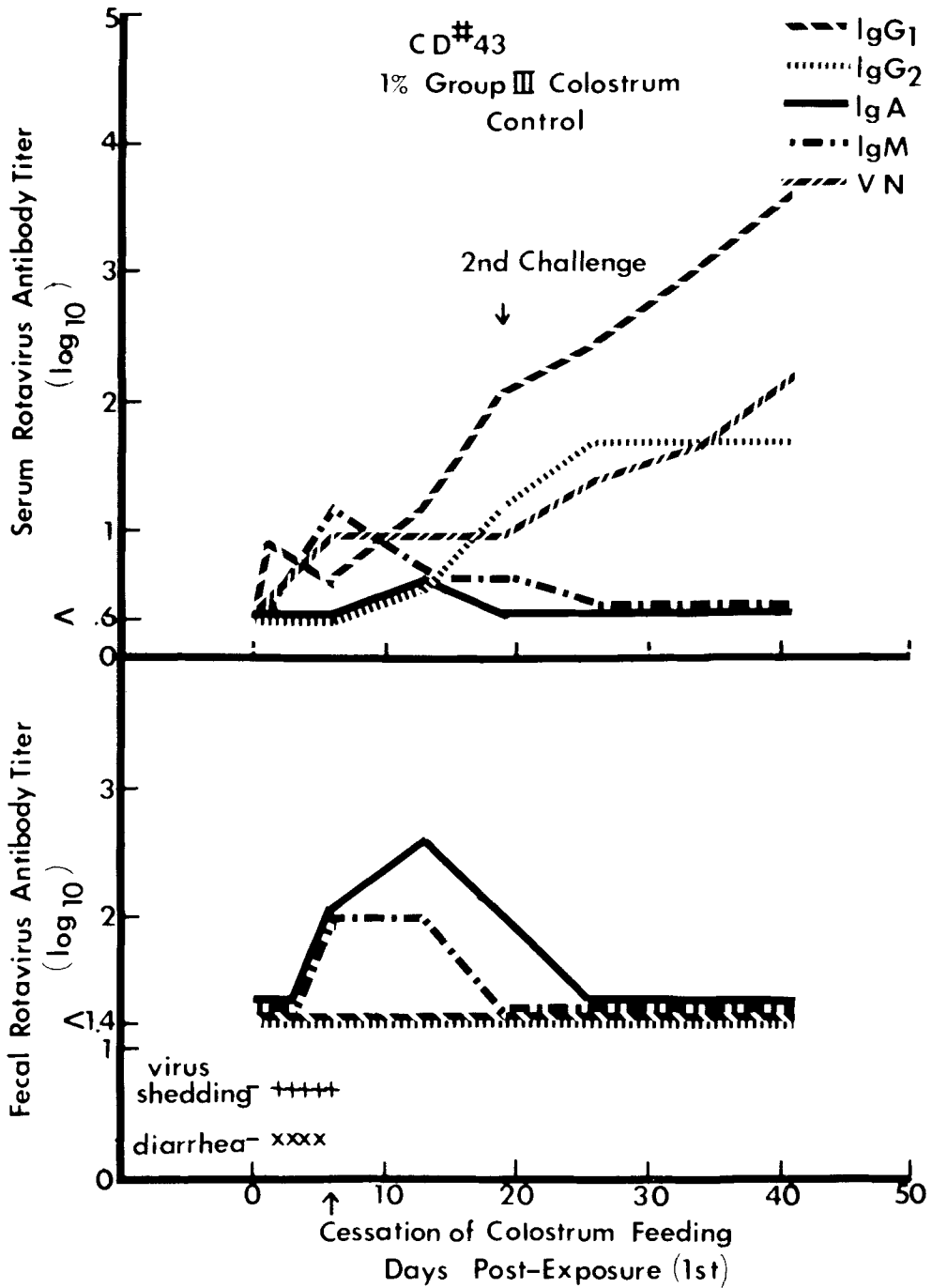


Figure 5. Serum and fecal rotavirus antibody isotypes (Enzyme-linked immunosorbent assay and virus neutralization titer log₁₀) after rotavirus challenge of a 16-h-old calf (CD #43) fed 1% control supplemental colostrum, 3 times per day for the first 7 days.

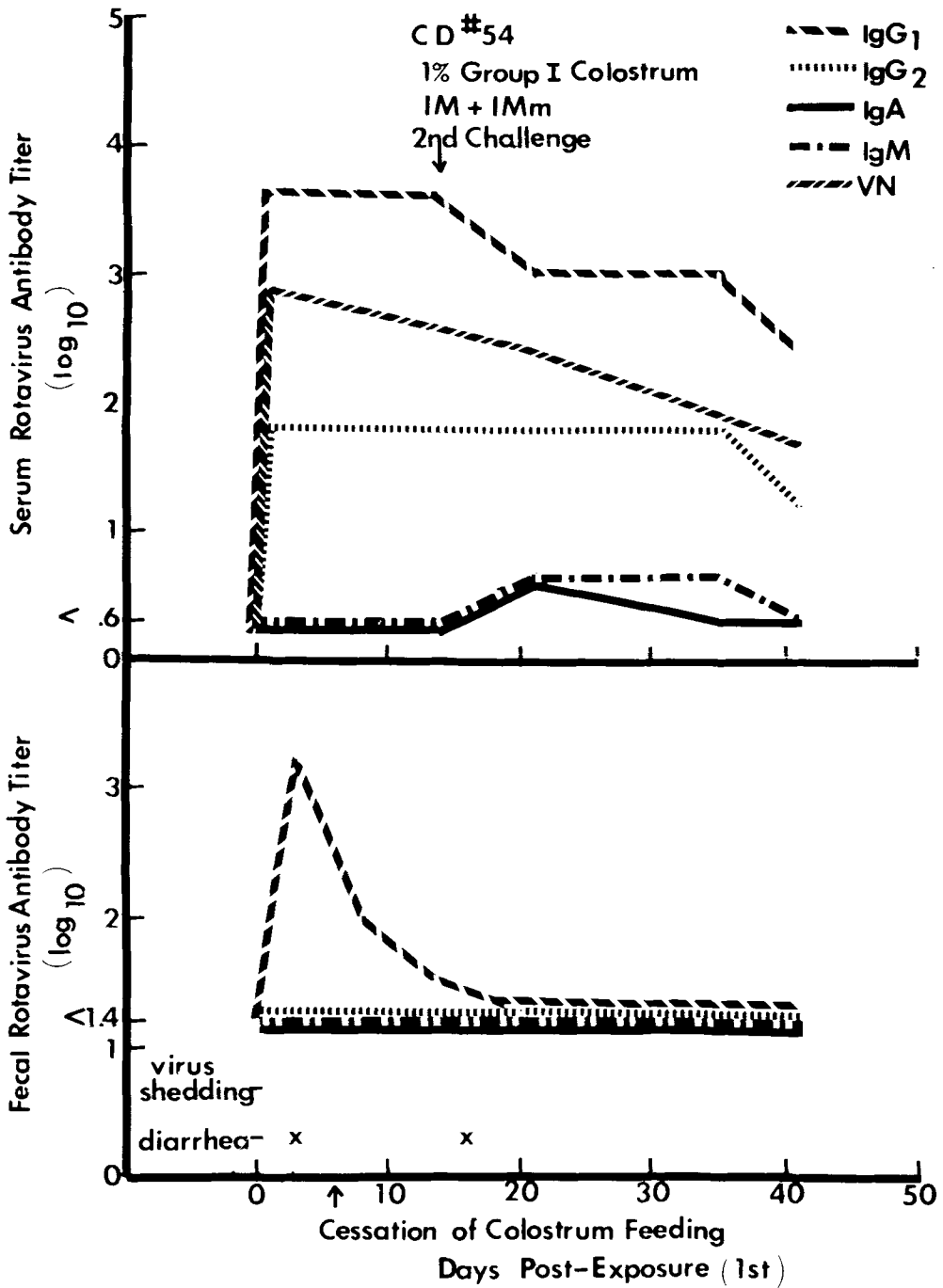


Figure 6. Serum and fecal rotavirus antibody isotypes (Enzyme-linked immunosorbent assay and virus neutralization titer log₁₀) after rotavirus challenge of a 12-h-old calf (CD #54) fed 1% supplemental colostrum from i.m.+IMm (immunized by intramuscular injection and intramammary infusion) Ohio Agricultural Research and Development Center vaccine-immunized cows, 3 times per day for the first 7 days.

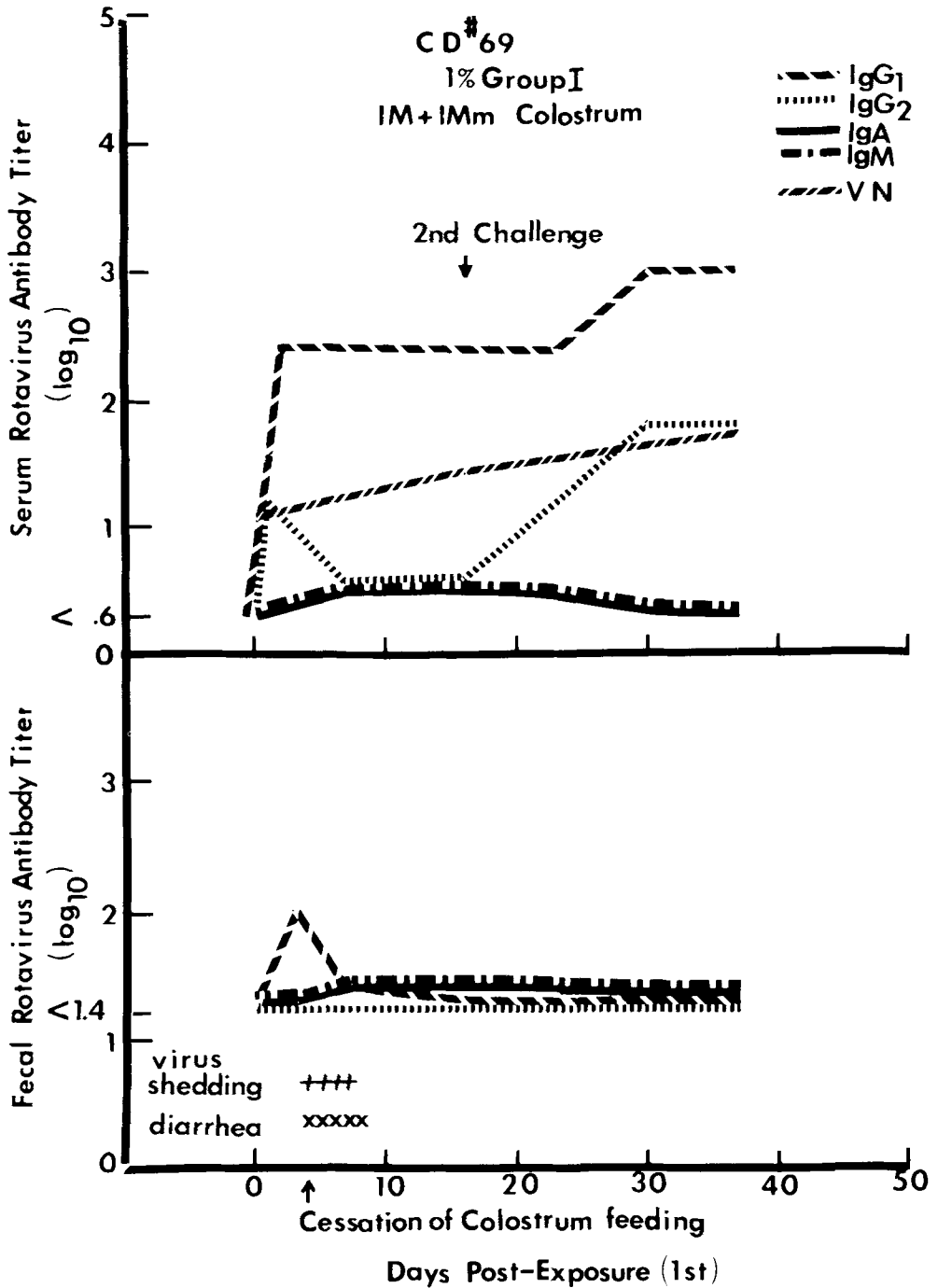


Figure 7. Serum and rotavirus antibody isotypes (enzyme-linked immunosorbent assay and virus neutralization titer log₁₀) after rotavirus challenge of a 22-h-old calf (CD #69) fed .1% supplemental colostrum from i.m.+IMm (immunized by intramuscular injection and intramammary infusion) Ohio Agricultural Research and Development Center vaccine-immunized cows, 3 times per day for the first 5 days.

neutralizing antibody titers after 2 i.m. plus IMm OARDC vaccine colostrum feedings; this was indicative of passively absorbed colostrum antibodies. Such antibodies declined throughout the 41-day sampling period but were still detectable at this time.

Calves fed 1% colostrum from the i.m. plus IMm cows had high passive IgG₁ rotavirus antibodies in feces compared with calves in the two previous feeding groups. Titers decreased after challenge but were still present for up to 7 days after termination of supplemental colostrum feeding (Figure 6). Furthermore, there were no detectable IgM or IgA rotavirus antibodies in feces (titer <25) after challenge. This failure may relate to suppression of active local intestinal IgA and IgM antibodies by passively acquired colostrum IgG₁ or development of very low undetectable (titer <25) IgA and IgM fecal antibodies in response to a subclinical infection.

Calves fed .1% supplemental i.m. plus IMm colostrum had a delayed onset and shorter duration of diarrhea and rotavirus shedding compared to controls (Table 7). Their serum and fecal rotavirus antibody responses were intermediate between those of 1% i.m. plus IMm or control colostrum-supplemented calves (Figure 7). Passively acquired IgG₁ rotavirus antibodies were lower than in the 1% i.m. plus IMm colostrum-fed calves but higher than in calves fed control colostrum. However, serum and fecal IgA and IgM antibody responses were lower than in the control group or in calves fed no colostrum. From these observations it appears that more prolonged diarrhea and virus shedding correlate with greater IgA and IgM fecal antibody responses. These findings concur with similar results in a study of rotavirus infections in children (54).

A number of calves (four of eight) began shedding rotavirus spontaneously within 3 to 9 days after termination of feeding 1% supplemental colostrum from i.m. plus IMm OARDC vaccine cows. Virus excretion persisted for several days but was accompanied by mild diarrhea for only 1 day. A similar sequence of virus shedding occurred in two calves fed .1% colostrum from i.m. plus IMm cows and a calf fed 50% control colostrum. However, two of these latter three animals shed virus the day after colostrum feeding ceased. These observations raise the question as to the source of

the rotavirus infection. The possibility that rotavirus was transmitted to these calves by the caretaker cannot be excluded totally. However, another plausible explanation may be that after initial rotavirus challenge, these animals developed a limited subclinical infection as indicated by development of IgA and IgM serum antibodies. This infection, kept in abeyance by daily colostrum feedings, sometimes resulted in a mild overt infection when colostrum feeding ceased. Other evidence supporting these conclusions is from protection studies in two rotavirus challenged gnotobiotic piglets fed bovine colostrum (9). Both animals remained clinically normal during colostrum feeding, but after cessation, a similar subclinical excretion of virus commenced in one piglet, and both developed serum antibodies.

Results of our studies concur with other reports that documented the importance of lactogenic immunity for effective protection against Type 1 enteric viruses (21, 37, 68, 82). Cessation of colostrum feeding led to susceptibility to disease or infection in several calves. Calves fed smaller amounts of i.m. plus IMm colostrum (.1%) or lower titered colostrum (50% control) usually commenced virus shedding earlier after colostrum feeding ceased than animals fed 1% high titered colostrum (i.m. plus IMm cows). These results may relate to higher titers of serum antibodies in this latter group of calves (1024 to 4096) compared to the previous calves (64 to 513). Such antibodies may be transferred from the serum back into the intestine, accounting for the persistence of IgG₁ fecal antibodies after termination of colostrum feeding and providing transient passive protection. Other studies have documented the transport of serum IgG₁ into the intestine in both sheep and cattle (15, 46). Studies also indicated passively acquired serum rotavirus antibodies, in high titer, could protect against rotavirus-associated diarrhea in lambs (67), and high serum Ig concentrations correlated with absence of diarrhea in young calves (33). An alternative or additional explanation for the delayed onset of virus shedding and persistence of IgG₁ fecal antibodies after cessation of 1% colostrum feeding might be that rotavirus IgG₁ colostrum antibodies selectively bind to enterocytes, similar to colostrum IgA in rats (45), these high titered antibodies effectively blocking rotavirus infection.

Results of studies of possible mechanisms of rotavirus pathogenesis under field conditions have suggested that the dam may shed rotavirus near parturition, thus serving as a source of virus for susceptible neonates (6). Infection of the neonate may occur at birth or shortly thereafter, the infection remaining subclinical as long as adequate colostrum or serum antibodies persist. In cows colostrum rotavirus antibodies decrease dramatically in the transition to milk, and this coincides with the peak occurrence of calf rotavirus infections at 5 to 14 days of age (3). This age-related susceptibility also may be influenced by a limited local protection provided by reverse transport of higher titered (in calves receiving adequate amounts of colostrum shortly after birth) serum IgG₁ antibodies back into the intestine. Depending on a number of poorly understood variables, calves then may begin shedding rotavirus with or without accompanying disease. The present investigation and others (9, 67) indicate that whereas most animals that are protected while receiving passive immunity may develop active immunity, other animals may remain susceptible to a subsequent infection, becoming a source of virus for additional animals. This might explain partially the occasional occurrence of sequential rotavirus infections in the same animal.

Our findings showing a correlation between colostrum antibody titers, amounts of colostrum fed, and passive protection have important implications for passive immunity against enteric infections in both dairy and beef calves. Because 30-day milk antibody titers from i.m. plus IMM OARDC rotavirus vaccine immunized cows represented approximately 1% of VN or ELISA colostrum antibody titers (which were protective at 1%), extrapolation of these results suggests this milk should be protective against natural rotavirus infections when fed undiluted to naturally or artificially suckled calves. In dairy herds, daily feeding of fresh or frozen colostrum supplements from immunized cows to calves should provide effective passive immunity for the duration of colostrum feeding (66). This supplemental feeding for only 3 to 4 wk may be sufficient, as older animals generally possess a high age-specific resistance to symptomatic rotavirus infections (64).

In our experiments, colostrum with high titers of rotavirus IgG₁ antibodies provided

effective passive immunity to rotavirus challenged calves. This suggests that in the bovine IgG₁ and not IgA may play a major role in passive mucosal immunity. Other observations that support this concept include the predominance of IgG₁ in bovine mammary secretions, specificity of bovine milk IgG₁ for enteric antigens in naturally infected cows (56, 64), and resistance of IgG₁ to certain proteolytic enzymes (11), all typical characteristics of SIgA in other species.

Although colostrum and milk rotavirus antibody were enhanced following maternal immunization in several studies, results were variable in passive protection studies under field conditions (19, 20, 64, 66, 75). Explanations for the equivocal results after challenge of nursing or immune colostrum supplemented calves are uncertain. The influence of a number of variables needs to be considered, including experimental determination of the minimal protective dose of colostrum or milk necessary to provide homologous protection against symptomatic infections. Our studies indicate high titers were necessary for complete protection [VN antibody titers of >678 (.1% colostrum supplements of initial VN titer = 678,828) and <6788 (1% colostrum supplements of initial VN titer = 678,828)]. Second, additional studies are needed to determine the amount of heterologous cross-protection provided against challenge with different newly recognized serotypes of bovine rotavirus. The timing of colostrum feeding versus challenge, the challenge dose and virulence of the virus strain, and other concurrent infections all may play a role in susceptibility to infection, even under controlled experimental conditions (64, 66, 67, 68).

ACKNOWLEDGMENTS

This investigation was supported in part by Cooperative Research Agreement No. 82-CRSR-2-2019 from the SEA, USDA, and by a Public Health Service Grant No. AI 10735-09 from the National Institute of Allergy and Infectious Disease.

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