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DEVELOPMENT OF NASAL, FECAL AND SERUM ISOTYPE-SPECIFIC ANTIBODIES IN CALVES CHALLENGED WITH BOVINE CORONAVIRUS OR ROTAVIRUS

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ABSTRACT

Saif, L.J., 1987. Development of nasal, fecal and serum isotype-specific antibodies in calves challenged with bovine coronavirus or rotavirus. *Vet. Immunol. Immunopathol.*, 17: 425-439.

Unsuckled specific pathogen free calves were inoculated at 3-4 weeks of age, either intranasally (IN) or orally (O) with bovine coronavirus or O plus IN (O/IN) or O with bovine rotavirus. Shedding of virus in nasal or fecal samples, and virus-infected nasal epithelial cells were detected using immunofluorescent staining (IF), ELISA or immune electron microscopy (IEM). Isotype-specific antibody titers in sera, nasal and fecal samples were determined by ELISA. Calves inoculated with coronavirus shed virus in feces and virus was detected in nasal epithelial cells. Nasal shedding persisted longer in IN-inoculated calves than in O-inoculated calves and longer than fecal shedding in both IN and O-inoculated calves. Diarrhea occurred in all calves, but there were no signs of respiratory disease. Calves inoculated with rotavirus had similar patterns of diarrhea and fecal shedding, but generally of shorter duration than in coronavirus-inoculated calves. No nasal shedding of rotavirus was detected.

Peak IgM antibody response, in most calves, were detected in fecal and nasal specimens at 7-10 days post-exposure (DPE), preceding peak IgA responses which occurred at 10-14 DPE. The nasal antibody responses occurred in all virus-inoculated calves even in the absence of nasal shedding of virus in rotavirus-inoculated calves. Calves inoculated with coronavirus had higher titers of IgM and IgA antibodies in fecal and nasal samples than rotavirus-inoculated calves. In most inoculated calves, maximal titers of IgM or IgA antibodies correlated with the cessation of fecal or nasal virus shedding. A similar sequence of appearance of IgM and IgA antibodies occurred in serum, but IgA antibodies persisted for a shorter period than in fecal or nasal samples. Serum IgG<sub>1</sub> antibody responses generally preceded IgG<sub>2</sub> responses and were predominant in most calves after 14-21 DPE.

INTRODUCTION

The tropism of bovine coronavirus and rotavirus for intestinal epithelial cells is well-established, as is the viruses' ability to cause villous atrophy and malabsorptive diarrhea in young calves (Mebus et al., 1973; Doughri and Storz, 1977; Mebus and Newman, 1977; Bridger et al., 1978). Recent reports suggest bovine coronavirus also possesses a tropism for respiratory epithelial cells (Thomas et al., 1982; McNulty et al., 1984; Reynolds et al., 1983; Saif

et al., 1986), a characteristic shared by certain coronaviruses infecting other species (McIntosh et al., 1974; Kemeny et al., 1975). Bovine coronaviruses have been isolated from respiratory tissues of calves with respiratory disease in the United Kingdom (Thomas et al., 1982). However experimental intranasal (IN) challenge of calves with bovine coronaviruses generally produced only mild or no signs of respiratory disease, even though all calves developed upper respiratory tract infections and diarrhea (McNulty et al., 1984; Reynolds et al., 1985; Saif et al., 1986). Although several authors have reported respiratory symptoms preceding or concurrent with rotavirus infections in children (Tallett et al., 1977; Hieber et al., 1978; Lewis et al., 1979) there is no evidence that rotaviruses multiply in human or porcine respiratory tissues either in vivo (Theil et al., 1978; Lewis et al., 1979; Stals et al., 1984) or in vitro (Tallett et al., 1977). Studies of such localized infections of either respiratory and intestinal tissues (bovine coronavirus) or intestinal tissues alone (rotavirus) should provide comparative information on development of active immune responses at both the local mucosal site of viral replication and a distant mucosal site. Both rotaviruses and coronaviruses cause localized mucosal infections and there is no evidence for hematogenous spread of these viruses to a distant mucosal site (Mebus et al., 1973; Doughri and Storz, 1977; Mebus and Newmann, 1977).

The purpose of this study was first to examine the nasal and fecal shedding of coronavirus and rotavirus in calves inoculated with virus either orally (O), IN or by the two routes combined (O/IN). Secondly, the appearance of isotype-specific antibodies to rotavirus and coronavirus in serum, nasal and fecal samples from the inoculated calves was compared.

## MATERIAL AND METHODS

### Calves and virus challenge

Eight unsuckled specific pathogen-free (SPF) male Holstein calves were assigned in equal numbers to 1 of 4 virus challenge groups. Calves in Groups A & B were challenged with bovine coronavirus either O or IN. Those in Groups C & D were given bovine rotavirus either O or O/IN. Calves were 3-4 weeks old at the time of challenge with the DB2 strain of coronavirus or NCDV strain of rotavirus. Procurement and housing of the SPF calves; viral strains, doses and methods of inoculation; clinical observation and sample collections were as described previously (Saif et al., 1983; Saif et al., 1986). Calves were fed 1% normal bovine colostrum in Similac (commercial infant formula) 2X/day from birth through 5 days of age, and Similac alone thereafter.

### Assays for virus shedding

Nasal and rectal swabs or fecal samples were collected daily through 14 days

post-exposure (DPE) and placed in cell culture medium (2 swabs in 8 ml) (Saif, 1986). Nasal epithelial cells were collected from the nasal swab samples, processed and stained for IF using fluorescein-conjugated bovine anti-coronavirus or anti-rotavirus sera as described previously (Saif et al., 1986). The processed nasal swab fluid was tested for rotavirus or coronavirus using a cell culture immunofluorescence (CCIF) test (Saif et al., 1986; Bohl et al., 1982). Feces or rectal swab samples were processed as described previously and tested for presence of rotavirus using CCIF and ELISA assays (Bohl et al., 1982; Saif et al., 1986), or for coronavirus using immune electron microscopy on 10% fecal suspensions (IEM, Saif et al., 1977).

#### Antibody assays

A plaque reduction virus neutralization (VN) test was used to assay rotavirus and coronavirus antibody titers (80% neutralization end points) in serum, and in processed fecal and nasal samples as described previously for bovine rotavirus (Saif et al., 1984). The VN antibody titers to bovine coronavirus were assayed in Madin Darby bovine kidney (MDBK) cells using plaque-purified NCDV bovine coronavirus (L.J. Saif and T. Mohamed, unpublished). IgG<sub>1</sub>, IgG<sub>2</sub>, IgA and IgM antibodies to each virus were determined in sera, and in processed fecal and nasal samples using isotype-specific ELISA. Monoclonal antibodies to each bovine isotype (except IgM) were used in ELISA to validate the specificity and sensitivity of the absorbed polyclonal anti-bovine Ig heavy chain-specific sera used (Saif et al., 1983; Saif et al., 1984).

## RESULTS

### Nasal and fecal shedding of virus and clinical signs of disease

Information on the onset and duration of nasal and fecal shedding of coronavirus and rotavirus, and diarrhea is summarized in Table 1. Responses of individual calves (1 from each group) are shown (Figures 1-4). Calves inoculated either IN or O with coronavirus shed virus in feces and as infected nasal epithelial cells. These infected nasal cells were detected longer than fecal shedding of virus and both fecal and nasal shedding persisted longer in IN-inoculated than in O-inoculated calves. All calves developed diarrhea which persisted for 5-6 days. Clinical signs of respiratory disease were not observed in any calf.

Calves inoculated with rotavirus showed no evidence of nasal shedding of virus or virus-infected nasal cells. Calves inoculated O/IN or O with rotavirus showed similar patterns of fecal shedding and diarrhea, the latter persisting for a shorter period than in coronavirus-inoculated calves.

TABLE 1

Development of diarrhea and detection of virus in nasal and fecal specimens from calves inoculated with bovine coronavirus or rotavirus.

Inoculum Group-Rt <sup>b</sup>	Diarrhea		Fecal shedding <sup>a</sup>		Nasal shedding <sup>a</sup>	
	Onset (DPE) <sup>c</sup>	Duration (Tot. da)	Onset (DPE)	Duration (Tot. da)	Onset (DPE)	Duration (Tot. da)
<b>Coronavirus</b>						
A-IN	3	6	3	7	2	13.5
B-O	3	5	3	4.5	2.5	9.5
<b>Rotavirus</b>						
C-O/IN	2.5	4.5	3	5.5	None	None
D-O	2.5	4	2.5	5.5	None	None

<sup>a</sup>Infected nasal epithelial cells were detected using immunofluorescence; fecal shedding was detected using immune electron microscopy or ELISA. (Times shown are mean responses of 2 calves).

<sup>b</sup>Rt = route of inoculation: IN = intranasal; O = oral; O/IN = Oral and intranasal.

<sup>c</sup>DPE = days post-exposure

#### Antibody isotypes and titers in fecal and nasal secretions

The fecal and nasal isotype-specific IgM, IgA, IgG<sub>1</sub> and IgG<sub>2</sub> and VN antibody responses in calves sampled at 0 through 21 or 35 DPE are shown in separate figures for individual calves (1 calf/group, Figures 1-4). In fecal and nasal samples of most calves, IgM antibodies were detected at 7 DPE, either prior to, or of higher initial titer, than IgA antibodies. However the IgM response was transient and no longer detected by 21 DPE, except for 2 calves (Groups A & C, data not shown) in which the response persisted through at least 21 DPE. At 10 DPE, IgA antibodies predominated or were equal in titer to IgM antibodies in fecal and nasal samples from all but 1 calf (Feces from #186, Figure 3). IgM and IgA rotavirus antibodies were detected in nasal samples from all rotavirus-inoculated calves, even in the absence of nasal shedding of rotavirus by these calves (Figures 3 and 4). The IgA antibody levels in nasal and fecal samples were similar in most calves, but IgM titers were consistently lower in nasal samples than in fecal samples from rotavirus-inoculated calves. IgA antibodies declined to low levels by the end of the sampling period in nasal and fecal samples from all calves except nasal samples from #181 (Figure 4). The VN antibody titers in fecal and nasal samples closely paralleled the IgA antibody titers in these samples. No IgG<sub>1</sub>, or IgG<sub>2</sub> fecal or nasal antibodies were

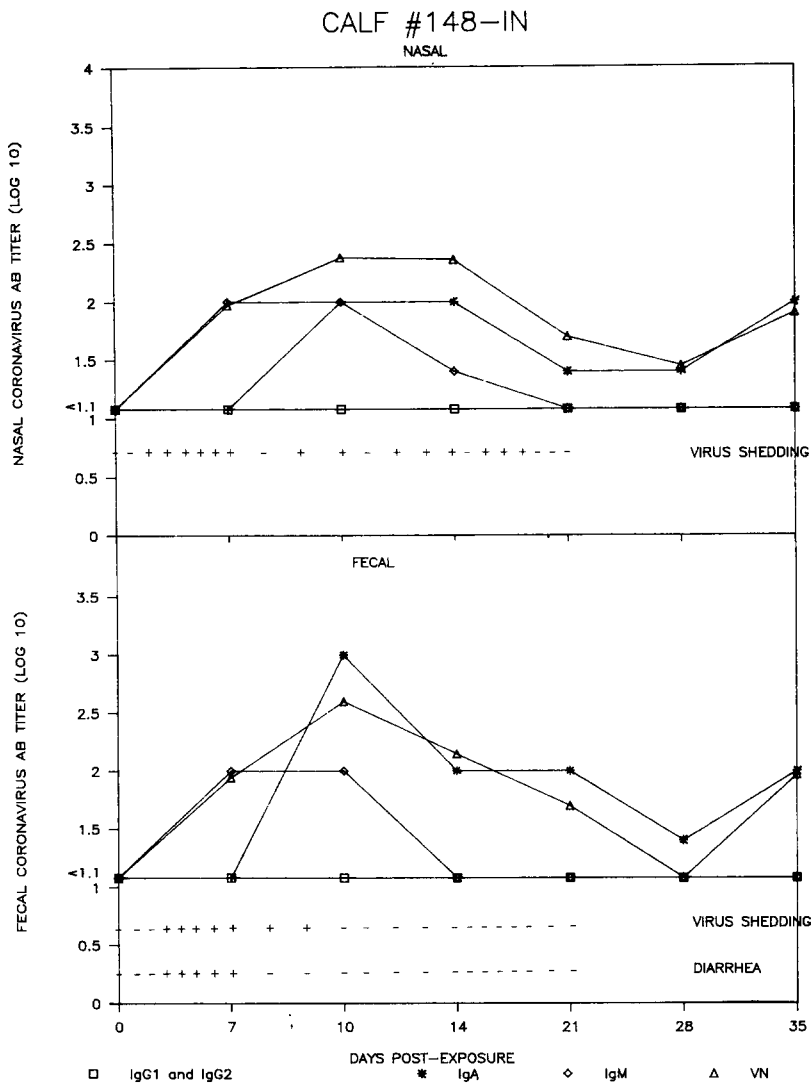


Figure 1. Coronavirus antibody isotype-specific responses detected using ELISA, and VN tests on nasal (upper panel) and fecal (lower panel) samples from calf #148 inoculated intranasally (IN) with coronavirus. Presence (+) or absence (-) of virus shedding and diarrhea is shown.

detected, except for low transient IgG<sub>1</sub> rotavirus antibody titers in feces from #186 (Figure 3).

The various routes of virus inoculation did not evoke major differences in the immune responses: calves inoculated 0 compared with 0/IN or IN-inoculated

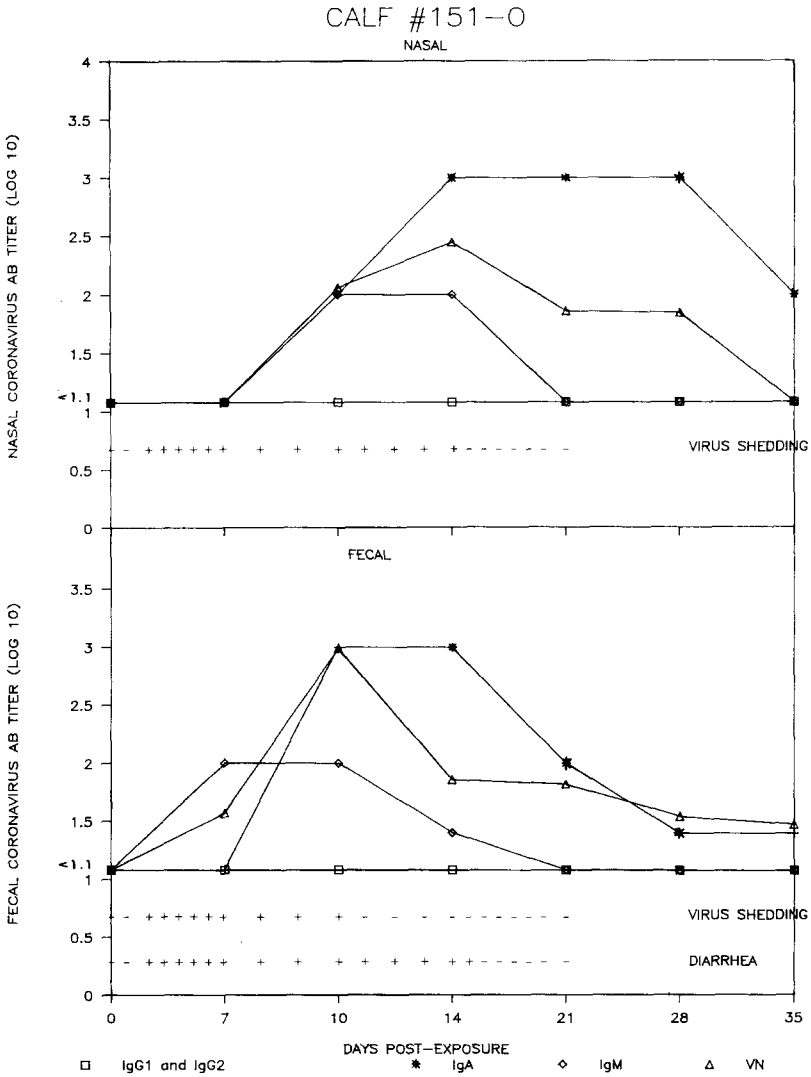


Figure 2. Coronavirus antibody isotype-specific responses detected using ELISA, and VN tests on nasal (upper panel) and fecal (lower panel) samples from calf #151 inoculated orally (0) with coronavirus. For further details, see Figure 1 legend.

calves, had similar nasal and fecal antibody responses. However, calves inoculated 0 alone with either virus had slightly delayed IgM and IgA nasal antibody responses, first detected at 10-14 DPE. In a comparison of the antibody responses of calves inoculated with either coronavirus (Groups A & B) or rotavirus (Groups C & D), the only differences noted were detection of higher IgM and IgA antibody titers in fecal and nasal secretions from the

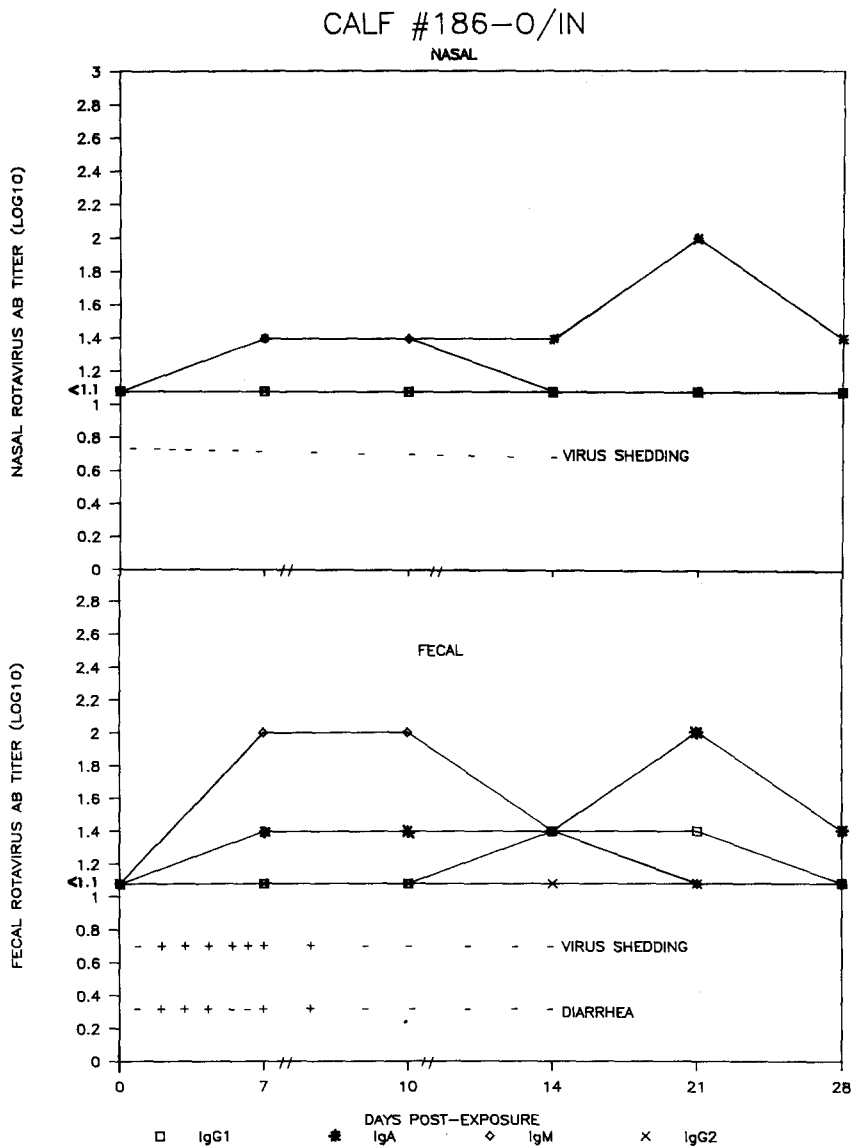


Figure 3. Rotavirus antibody isotype-specific responses detected using ELISA tests on nasal (upper panel) and fecal (lower panel) samples from calf #186 inoculated orally and intranasally (O/IN) with rotavirus. For further details see Figure 1 legend.

coronavirus-inoculated calves (Figures 1 & 2). In the latter calves, occurrence of peak levels of IgA antibodies generally correlated with failure to detect further fecal and nasal virus shedding. An exception was #148 which continued to shed coronavirus-infected nasal epithelial cells intermittently,



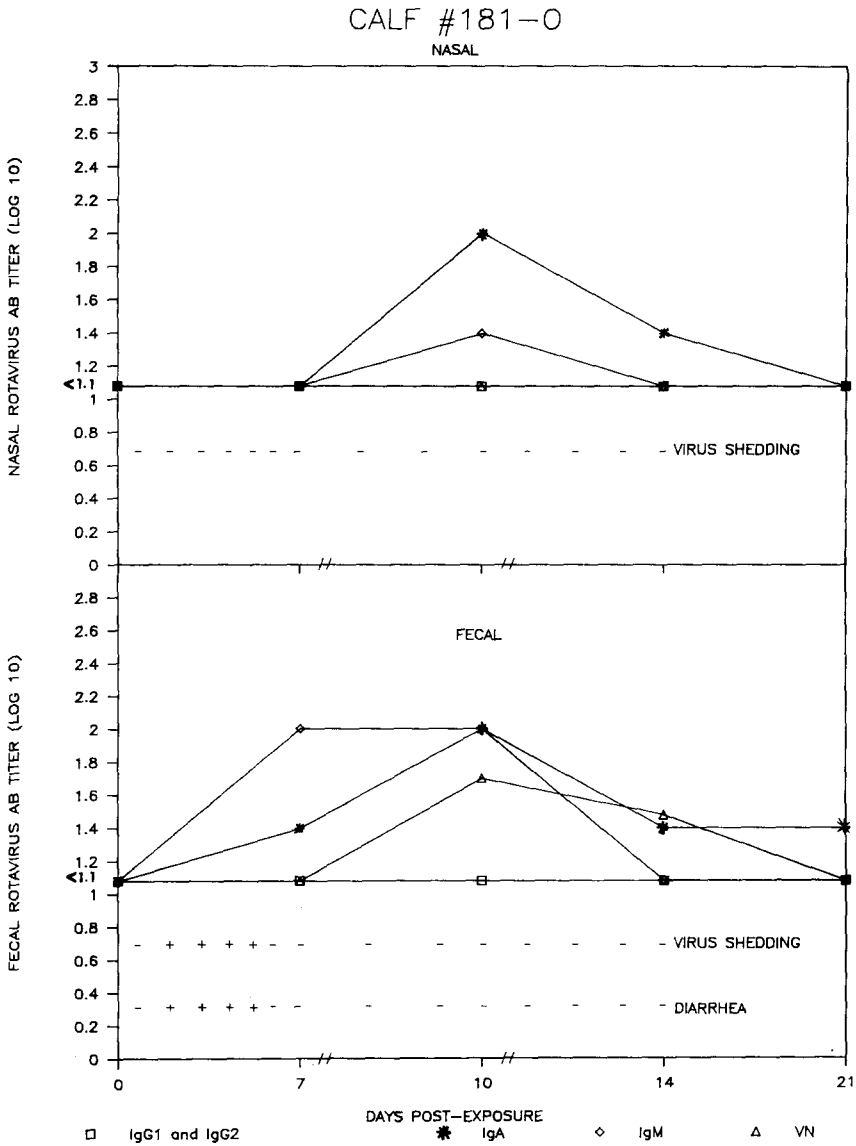


Figure 4. Rotavirus antibody isotype-specific responses detected using ELISA, and VN tests on nasal (upper panel) and fecal (lower panel) samples from calf #181 inoculated orally (0) with rotavirus. For further details, see Figure 1 legend.

even in the presence of peak levels of IgA antibodies in nasal samples (Figure 1). In rotavirus-inoculated calves, termination of fecal virus shedding and diarrhea correlated with peak titers of fecal IgM or IgA antibodies (Figures 3 & 4).

### Antibody titers and isotypes in serum

Serum isotype-specific antibody responses in calves in each of the 4 groups were similar (Figures 5 & 6). IgM antibodies were detected at peak levels in all calves by 7 DPE after which they declined to low levels. IgA antibodies, first appeared at 14 DPE in all but 2 calves (Groups B and C), reached undetectable levels by 21-35 DPE in all calves.

Low levels of passive IgG<sub>1</sub> antibodies in serum at the time of challenge, due to the initial colostrum feeding, were detected in 6/8 calves. Actively-produced IgG<sub>1</sub> antibodies were first detected in all calves at 14 DPE, whereas IgG<sub>2</sub> antibodies were first detected in 2/8 calves at 14 DPE, and the remaining calves (except #181, Figure 6) at 21 DPE. IgG<sub>1</sub> antibodies predominated in all but 1 calf (in which IgM predominated) by the end of the sampling period. The VN antibody responses closely paralleled IgG<sub>1</sub> responses, but were detected earlier (7 DPE), and were of lower titers than IgG<sub>1</sub> antibodies except at 7 DPE.

### DISCUSSION

The patterns of diarrhea and fecal shedding of rotavirus and coronavirus observed in this study were similar to those reported in other studies of SPF or gnotobiotic calves (Saif et al., 1983; McNulty et al., 1984; Reynolds et al., 1985; Saif and Smith, 1985; Saif et al., 1986; Van Zaane et al., 1986). As noted in previous studies nasal shedding of coronavirus was detected in both 0 and IN-inoculated calves. However, no respiratory shedding of rotavirus was evident in the present study in calves or in prior studies in children (Lewis et al., 1979; Stals et al., 1984).

The appearance of isotype-specific antibodies in feces and serum following intestinal infection of calves with rotavirus were similar to those reported in our previous studies (Saif and Smith, 1984; Saif et al., 1986) and one other report (Van Zaane et al., 1986) in which younger calves were used. Predominance of IgA or IgM antibodies in calf feces correlates with the predominance of IgA rotavirus antibody-producing cells and IgM and IgA containing cells in the intestinal mucosa of rotavirus-inoculated or normal calves, respectively (Allen and Porter, 1975; Vonderfecht and Osburn, 1982). Patterns of serum and fecal antibody responses in calves were similar to those noted following infection of children (Sonza, 1980; Riepenhoff-Talty et al., 1981; Stals et al., 1984), pigs (Corthier and Vannier, 1983) and mice (Sheridan et al., 1983) with rotavirus.

Fecal and serum antibody responses of coronavirus-infected calves were similar to those of the rotavirus-infected group, but were generally of higher magnitude. The higher IgA and IgM fecal antibody responses correlated with a longer period of fecal virus shedding and diarrhea observed in most coronavirus-infected calves, and were similar to rotavirus infection data in

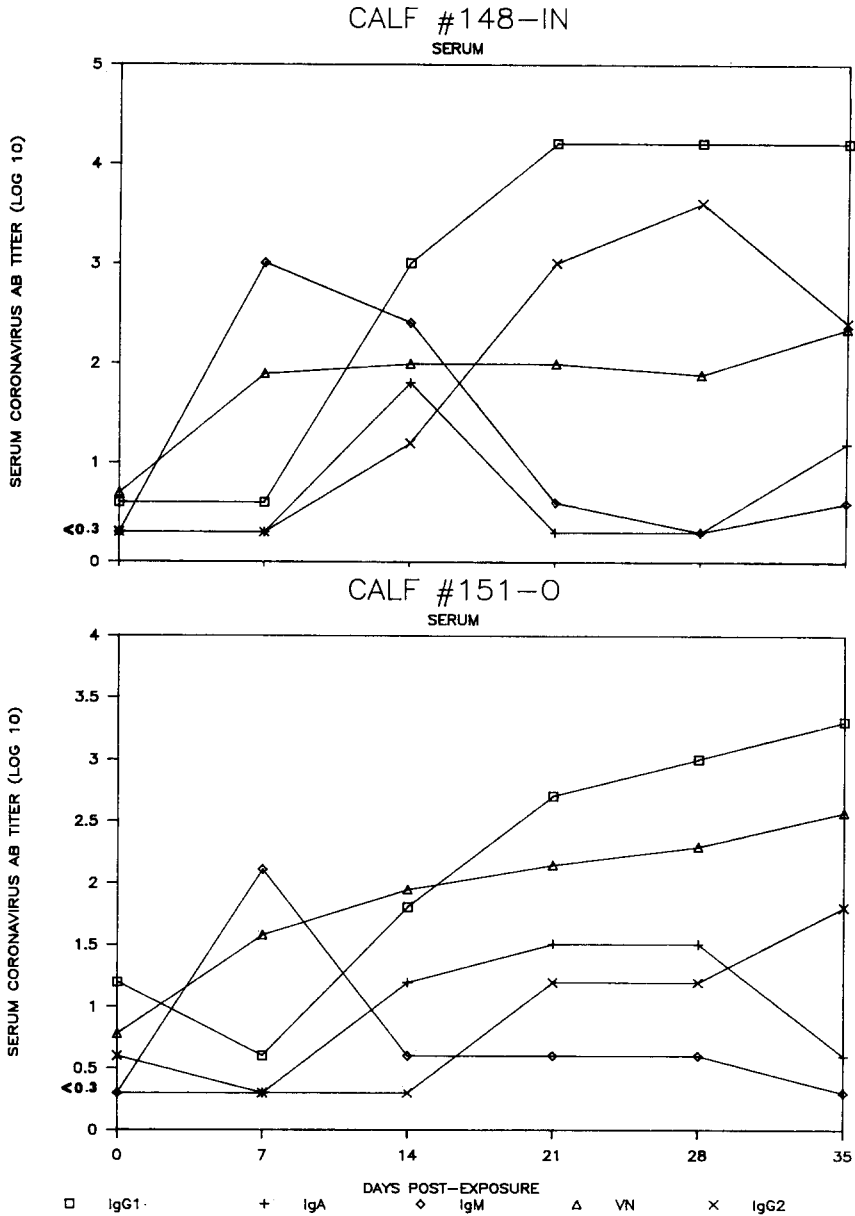


Figure 5. Rotavirus antibody isotype-specific responses detected using ELISA, and VN tests on sera from calves #148 (upper panel) and #151 (lower panel) inoculated with coronavirus.

children (Riepenhoff-Talty et al., 1981). Failure to detect fecal IgG<sub>1</sub> (except for 1 calf) and IgG<sub>2</sub> antibody responses, agrees with the results of several previous studies of rotavirus infections in calves (Saif and Smith, 1985; Saif et al., 1986; Van Zaane et al., 1986). Reasons for occurrence of fecal IgG<sub>1</sub>

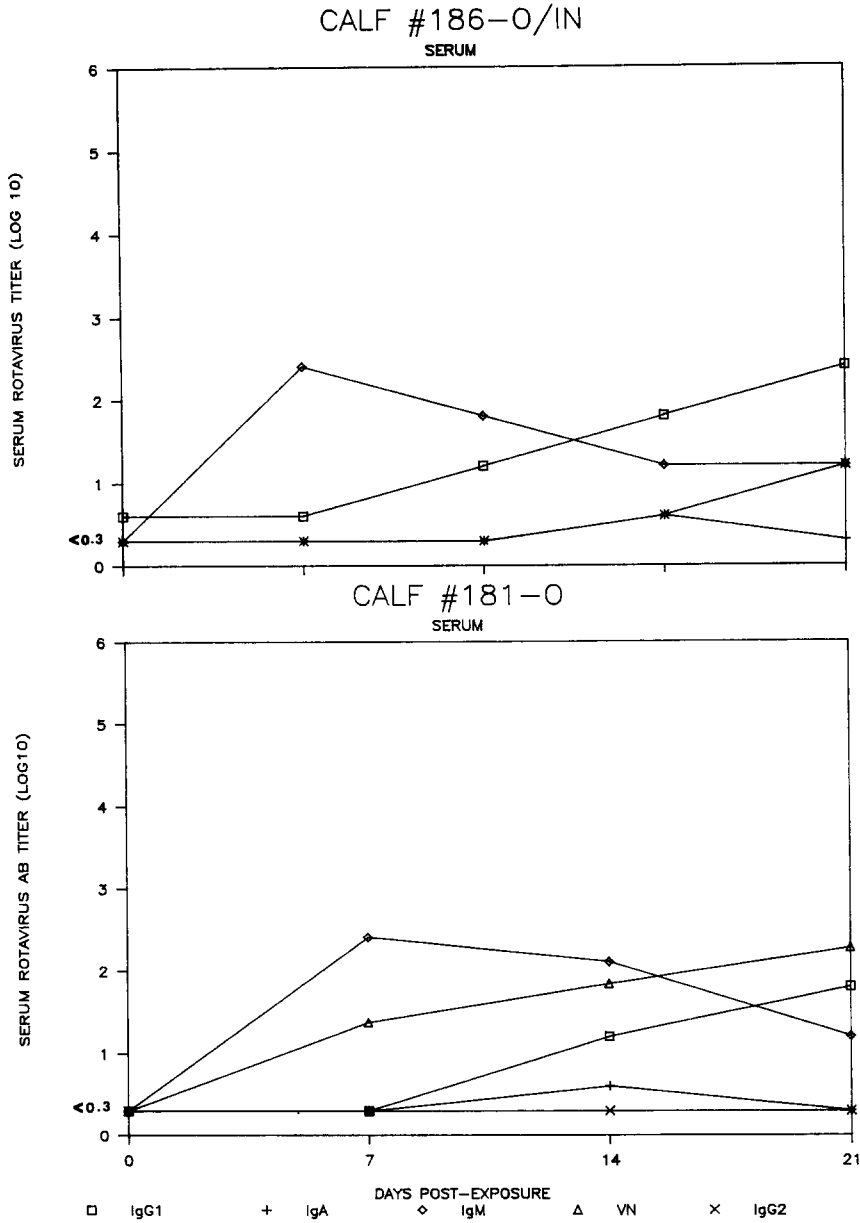


Figure 6. Rotavirus antibody isotype-specific responses detected using ELISA, and VN tests on sera from calves #186 (upper panel) and #181 (lower panel) inoculated with rotavirus.

antibodies in the one calf are uncertain, but may relate to intestinal inflammation with transudation of serum IgG<sub>1</sub> antibodies under such conditions.

To our knowledge, there are no published studies of isotype-specific responses in nasal secretions following oral or respiratory challenge with

rotavirus or coronavirus. The patterns of immune responses were similar in calves given coronavirus, which replicated in the upper respiratory tract, compared with calves given rotavirus which was not shown to replicate in the upper respiratory tract, either in the present study or others (Tallett et al., 1977; Heiber et al., 1978; Lewis et al., 1979; Stals et al., 1984). The major differences seen were in the higher magnitude of the nasal IgM and IgA antibody responses in the coronavirus-inoculated calves. In a similar study in children with rotavirus diarrhea, rotavirus IgA antibodies were detected in pharyngeal secretions at levels comparable to those in feces, even in the absence of detectable rotavirus shedding from pharyngeal secretions (Stals et al., 1984).

Several explanations are possible for occurrence of rotavirus antibodies in nasal secretions in the absence of nasal shedding of virus. First, O-inoculation of calves with rotavirus may stimulate an immune response in lymphoid tissue of the oropharynx such as the tonsils or salivary glands due to presence of viral antigen in the oral cavity. However previous attempts at direct stimulation of the oral cavity have yielded poor antibody responses or protection (Lehner et al., 1980), perhaps due to a paucity of IgA plasma cells in tonsils and salivary glands (Crabbe and Heremans, 1967; Cripps and Lascelles, 1976). A second possible explanation for occurrence of antibody responses of a similar magnitude and onset at a distant mucosal site (respiratory tract) after oral stimulation with rotavirus may be the existence of the common mucosal immune system (Bienenstock and Befus, 1980). An immunologic link between the intestinal and respiratory tracts has been shown previously (reviewed in Bienenstock and Befus, 1980; Husband, 1985) with either intestinally-derived IgA immunocytes, or possibly intestinal IgA antibodies secreted in serum, transported to the respiratory tract. Either mechanism could account for the predominance of IgA antibodies in nasal secretions of rotavirus-infected calves which failed to show nasal virus shedding. However the parallel rise seen in fecal and nasal IgA antibodies, but their delayed detected in serum, suggests the former explanation may be more likely. In addition, in ruminants the bulk of the IgA in respiratory secretions is locally produced and not serum-derived (Scicchitano et al., 1984). Although in this study we cannot exclude the possibility that rotavirus replicated in other tissues of the respiratory tract such as trachea or lung, this has not been observed in other species (Tallett et al., 1977; Heiber et al., 1977; Theil et al., 1978; Lewis et al., 1979; Stals et al., 1984) and absence of nasal shedding of virus under such conditions would be unlikely.

IgA antibodies in nasal secretions could play a role in protection against enteric viruses by contributing additional antibodies to saliva thereby neutralizing virus at the portal of entry. Others have reported that oral inoculation of rabbits, mice or man with respiratory viruses also led to IgA

responses in nasal secretions (Peri et al., 1982; Waldman et al., 1983). Studies in sheep have shown that the numbers of IgA antibody containing cells in the upper respiratory tract could be enhanced following intratracheal boosting of intraperitoneally primed animals (Scicchitano et al., 1984). Consequently the oral route may provide an effective route of immunization for stimulation of immunity against viruses which infect either the respiratory or intestinal tracts or both.

In an earlier report (Howard et al., 1986), replication of Mycoplasma bovis in the bovine respiratory tract elicited IgM and IgA responses in tracheo-bronchial washings, similar to responses in nasal secretions in the present study. However, these investigators also observed an IgG<sub>1</sub> and IgG<sub>2</sub> antibody response in such samples by 4 weeks postinoculation. No IgG<sub>1</sub> or IgG<sub>2</sub> nasal antibody responses were detected in virus-infected calves in the present study. These differences may relate to the invasiveness of M. bovis which causes extensive lung lesions not evident in coronavirus infections of the respiratory tract (McNulty et al., 1984; Reynolds et al., 1985; Howard et al., 1986; Saif et al., 1986). Such invasive lower respiratory tract infections might evoke IgG responses since IgG plasma cells predominate in the lower respiratory tract (compared with IgA cells in the upper tract, Morgan et al., 1981). Alternatively IgG antibodies may be partially serum-derived in response to the inflammation and damage produced by invasive infections of respiratory tissues.

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