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INTESTINAL ANTIBODY RESPONSE AFTER VACCINATION AND INFECTION WITH ROTAVIRUS OF CALVES FED COLOSTRUM WITH OR WITHOUT ROTAVIRUS ANTIBODY

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ABSTRACT

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The intestinal and systemic antibody response of calves vaccinated and/or challenged with rotavirus was studied employing isotype-specific ELISAs for the detection of IgG₁, IgG₂, IgM and IgA antibodies to rotavirus. Monoclonal antibodies to bovine immunoglobulin isotypes of proven specificity were used as conjugated or catching antibody.

Five days after oral inoculation (dpi) of a 5-day-old gnotobiotic calf with rotavirus, IgM rotavirus antibodies were excreted in faeces, followed 5 days later by IgA rotavirus antibodies. The increase in IgM rotavirus antibody titre coincided with the inability to detect further rotavirus excretion. Faeces IgM and IgA rotavirus antibody titres fell to low levels within 3 weeks post infection. IgG₁ and IgG₂ rotavirus antibodies were not detected in faecal samples. In serum, antibodies to rotavirus of all four isotypes were detected, starting with IgM at 5 dpi.

Two SPF-calves, which were fed colostrum free of rotavirus antibodies, were vaccinated with a modified live rotavirus vaccine and challenged with virulent rotavirus 6 days later. Upon vaccination, the calves showed an antibody response similar to the response of the infected gnotobiotic calf. Intestinal IgM rotavirus antibodies were excreted before or on the day of challenge and appeared to be associated with protection against challenge infection with virulent virus and rotavirus-induced diarrhoea. In 3 control calves, which were challenged only, the antibody patterns also resembled that of the gnotobiotic calf and again the appearance of IgM rotavirus antibodies coincided with the end of the rotavirus detection period.

Two other groups of 3 SPF-calves were treated similarly, but the calves were fed colostrum with rotavirus antibodies during the first 48 h of life. These calves excreted passively acquired IgG₁ and IgG₂ rotavirus antibodies in their faeces from 2 to 6 days after birth. After vaccination, no IgM or IgA antibody activity in serum or faeces was detectable. Upon challenge, all calves developed diarrhoea and excreted rotavirus. Seven to 10 days after challenge low levels of IgM rotavirus antibody were detected for a short period.

These data indicate that the intestinal antibody response of young calves to an enteric viral infection is associated with the excretion of IgM antibodies, immediately followed by IgA antibodies. This response is absent or diminished in calves with passively acquired specific antibodies which may explain the failure to induce a protective intestinal immune response by oral vaccination with modified live rotavirus of calves fed colostrum containing rotavirus antibodies.

INTRODUCTION

Rotavirus infections occur worldwide and are an important cause of enteric disease in many species (Holmes, 1979). In young farm animals the morbidity and mortality can be high, causing considerable damage. In cattle, vaccination against rotavirus-induced diarrhoea has been attempted but the results were usually disappointing (Mebus et al., 1973; De Leeuw et al., 1980b; Snodgrass et al., 1980; Snodgrass et al., 1982; Bürki et al., 1983) or still need confirmation under field conditions (Saif et al., 1983; Saif et al., 1984). Part of the problem may be the empirical basis on which such vaccines and vaccination methods have to be developed, due to an incomplete understanding of the mucosal immune system. Particularly in cattle, only limited information is available and some of the results reported appear to be conflicting. For instance, whereas in most species IgA is the predominant immunoglobulin (Ig)-isotype* in secretions (Tomasi and Zigelbaum, 1963; Heremans, 1974), IgG₁ has been found to be the major isotype in the milk of cows and in the intestinal contents of 2-week-old calves (Newby and Bourne, 1976; Butler, 1983). IgA levels increased with age but IgG₁ was still the major isotype in the intestine of adult cattle. Newby and Bourne (1976) also demonstrated the presence of a high percentage of IgG₁ containing cells in the small intestine. In contrast, Porter et al. (1972) reported the predominance of IgM (and in some animals of IgG₂) in intestinal loop secretions of pre-ruminant calves. IgM-containing cells indeed predominate in the intestine of young calves but they appeared to be outnumbered by IgA-containing cells in older calves and adult cattle (Allen and Porter, 1975). This latter observation is in agreement with the results of Cripps et al. (1974) who found IgA to be the major isotype in intestinal loop secretions of adult sheep. As illustrated by these conflicting results, the mucosal immune system of cattle needs further study. This is particularly important as there is little doubt that resistance to enteric infection is largely independent of circulating antibody but relies on passively or actively acquired immunity in the gut (Mebus et al., 1973; Snodgrass and Wells, 1978a).

Few papers relate specifically to local rotavirus immunity in the calf. Hess et al. (1981) reported that specific antibodies to rotavirus were present in bovine intestinal fluid obtained via jejunal fistulae and in faeces of calves orally infected with rotavirus. In general, IgG₁ was the predominant isotype in these samples, but this isotype was not shown to be directly associated with anti-rotavirus activity. Vonderfecht and Osburn (1982) presented evidence that rotavirus inoculation of neonatal calves resulted in the appearance of large numbers of IgA rotavirus antibody producing cells in the mucosa of the proximal small intestine.

* If appropriate the word "isotype" includes also subisotypes IgG₁ and IgG₂.

Our approach to the study of the immune response in calves infected with rotavirus involved first the development of isotype-specific ELISAs for the detection of rotavirus antibody in serum and secretions. The specificity of the anti-immunoglobulin reagents employed in such tests is of crucial importance and has to be proven in the same assay in which they will be used (Butler, 1980; Townsend et al., 1982). With conventional antisera against bovine immunoglobulin (BIg) isotypes that appeared to be specific in agar gel precipitation techniques we observed marked cross-reactions in sensitive ELISAs. To circumvent these problems we produced monoclonal antibodies (MCA) to BIg-isotypes and characterized them in ELISAs (Van Zaane and IJzerman, 1984). MCAs specific for IgG₁, IgG₂ and IgA were obtained, while MCAs against IgM showed a weak cross-reaction with IgA. The MCAs were applied in isotype-specific ELISAs for the detection of IgG₁, IgG₂, IgM and IgA rotavirus antibody. Evidence was presented that these tests were isotype-specific, except for the IgM test, which showed at most 3% cross-reactivity with IgA rotavirus antibodies.

During the development of the tests, an inherent problem in isotype-specific ELISAs, i.e. the competition effects that occur (Chantler and Diment, 1981; Townsend et al., 1982; Van Zaane and IJzerman, 1984), was also studied. When isotype-specific reagents are used as conjugates, competition between isotypes for the limited amount of antigen (inter-isotype competition) can prevent the detection of an isotype with a relatively low antibody concentration or affinity. Another disadvantage of the ELISA is the possible interference of rheumatoid factors. Both problems can be circumvented by applying isotype-specific reagents as catching antibodies. In this case, however, competition within a particular isotype for the limited amount of catching antibody can occur (intra-isotype competition). Based on the evaluation of these competition effects optimum ELISAs were selected (Van Zaane and IJzerman, 1984).

In this paper we report the results of preliminary studies on humoral aspects of the intestinal immune response of calves after oral vaccination and/or infection with rotavirus and the influence that passively acquired rotavirus antibody may have.

MATERIALS AND METHODS

Experimental animals

In all experiments Friesian calves were used. A colostrum-deprived gnotobiotic calf was reared in a positive-pressure isolation unit. It was fed condensed milk (Nutricia, Zoetermeer, the Netherlands) during the whole experiment. The calf was free of bovine virus diarrhoea (BVD) virus and it remained free of bacteria and serologically negative for bovine coronavirus and BVD-virus.

The SPF-calves used formed part of another study described in detail elsewhere (De Leeuw and Tiessink, 1984). The experimental design is outlined in Table I.

TABLE I

Experimental design

Group	Calf.no.	Rotavirus Ab in colostrum	Vaccination	Challenge	Fig.no.
A	28,33,58	-	-	+	3
B*	35,62	-	+	+	4
C	235,236,237	+	-	+	5
D*	241,242,243	+	+	+	6

* Groups B and D in this paper correspond with groups B₂ and D₁, respectively, in the paper of De Leeuw and Tiessink (1984). For further explanation see the materials and methods section.

Briefly, calves in groups A and B received bovine colostrum free of antibodies to rotavirus, whereas calves in groups C and D were fed "positive" colostrum, i.e. with antibodies to rotavirus. Groups B and D were vaccinated orally 1 hr after the first colostrum feeding. All groups were challenged on day 6 after birth with virulent rotavirus. At that time the peak of rotavirus infection normally occurs in the field (De Leeuw et al., 1980a) and colostrum antibodies are probably no longer present in the gut. Calves used in experimental groups A and B were born to mothers in the institute's SPF-herd, which is serologically negative among others for rotavirus and bovine coronavirus. Calves in experimental groups C and D were from cows obtained through a market. Calves were obtained by caesarian section and reared in isolation. The day of birth is taken as day 0. Calves used in the same experimental group were housed in individual boxes in one isolation room. They always arrived on the same day. Calves were fed 8 litres of colostrum, divided over 4 meals, during the first 2 days. Thereafter a commercial milk substitute was given, 2 times 2 litres daily. Colostrum without antibodies against rotavirus and bovine coronavirus was obtained from cows in the institute's SPF-herd; "positive" colostrum was obtained from cows in a dairy herd on a government farm, where rotavirus and bovine coronavirus infections were endemic (De Leeuw et al., 1980a). The latter colostrum pool had an ELISA-blocking titre (log₁₀) against rotavirus of 2.7 and against bovine coronavirus of 2.4 (De Leeuw and Tiessink, 1984). Calves were examined twice daily at which times faecal samples were collected from the rectum. The consistency of the faeces was noted as normal, (bright) yellow but with largely normal consistency, semi-liquid or liquid. No treatment was given or dietary measures taken when diarrhoea developed. The clinical and virological examinations have been described in detail by De Leeuw and Tiessink (1984).

Viruses and vaccination

The gnotobiotic calf was infected with a Dutch virulent rotavirus field strain at 5

days of age. Three and 4 days later, faecal samples containing rotavirus were obtained, pooled and stored in small quantities at -70°C . This was used as the challenge inoculum for calves in experimental groups A-D, and it had a titre of 10^5 tissue culture infective doses 50 per cent (TCID_{50}) per ml. These calves were simultaneously challenged with virulent bovine coronavirus obtained from another experimentally infected gnotobiotic calf. Both viruses were given orally.

The vaccine used was Scourvax[®]-2* containing both attenuated bovine rotavirus and bovine coronavirus. Each calf received 2 ml of the reconstituted vaccine orally as specified by the manufacturer. The rotavirus titre of the vaccine in our hands was $10^{6.2}$ $\text{TCID}_{50}/\text{ml}$. Vaccination was always performed one hour after the first colostrum feeding; the calves were then 5 to 8 hours old.

Test samples

Within one hour after collection, faecal samples were cooled to 4°C , homogenized the same day with 4 volumes of PBS containing 0.05% w/v Tween 80 and subsequently stored at -20°C . Before testing, samples were thawed and centrifuged for 10 min at $250 \times g$. The supernatants were taken as "undiluted" test samples. No specific measures were taken to inhibit proteolytic activity, but it was shown that incubation of purified IgG₁ rotavirus antibodies with a fresh faecal extract for 24 h at 37°C did not reduce the antibody titre. Therefore, proteolytic degradation of Ig after collection of faecal samples was not considered a problem.

Isotype-specific ELISAs

The isotype-specific ELISAs for the detection of antibodies to rotavirus have been described in detail elsewhere (Van Zaane and IJzerman, 1984). MCAs to bovine Ig-isotypes G₁, G₂, M and A were used as conjugate in the indirect double antibody sandwich assay (IDAS) or as catching antibody in the antibody capture assay (ACA) (Fig. 1). Based on an analysis of inter- and intra-isotype competition effects in IDAS and ACA, respectively, and a direct comparison of both tests, the IDAS was selected for the detection of IgG₁ and IgG₂ rotavirus antibodies whereas the ACA was the test of choice for the detection of IgM and IgA rotavirus antibodies. The assays were carried out as follows: The polystyrene ELISA-plates (Dynatech, M129A) were coated with a predetermined optimal concentration of antibody (swine anti-rotavirus Ig (IDAS) or MCA-anti-BIgM or BIgA (ACA)) in 50 mM NaHCO_3 , pH 9.6, for 16 hr at 37°C . All subsequent reagents, except the substrate, were diluted in ELISA-buffer (0.5 M NaCl, 10 mM PO_4^{3-} -pH 7.2, 0.05% w/v Tween 80) and were added at a predetermined optimal concentration in a volume of 100 μl . After each incubation step, plates were washed ten times with 0.05% w/v Tween 80 in demineralized water.

* Scourvax[®]-2, Smith-Kline, Norden Laboratories, Lincoln, Nebraska, USA.

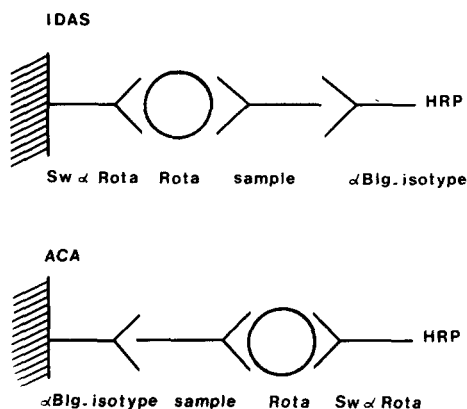


Fig. 1. Schematic diagram of ELISAs used. IDAS: Indirect double antibody sandwich assay; ACA: Antibody capture assay; Sw α Rota: Swine anti-rotavirus Ig; Rota: rotavirus; α BIg-isotype: monoclonal antibody against bovine immunoglobulin isotype G₁, G₂, M or A; HRP: horseradish peroxidase.

For the IDAS, coated plates were incubated with a faecal sample containing rotavirus (obtained from experimentally infected SPF-calves) for 1.5 hr at 37°C and subsequently with two-fold dilutions of test samples (starting at 1/20) in ELISA-buffer, containing 10% v/v horse serum, for 2 hr at 4°C. Finally, MCA-anti-BIg-isotype conjugated with horseradish peroxidase (HRP) (Wilson and Nakane, 1978) was added and incubated for 1 hr at 37°C.

For the ACA, coated plates were incubated with two-fold dilutions of test samples (starting at 1/20) in ELISA-buffer for 2 hr at 37°C. Incubation with rotavirus was as for the IDAS. Swine-anti-rotavirus Ig conjugated with HRP was incubated for 1 hr at 37°C. Recrystallized 5-aminosalicylic acid (Ellens and Gielkens, 1980) was used as substrate. Three to 16 hr after addition of the substrate, absorbance was measured in a Titertek Multiscan (Flow) at 450 nm. The reciprocal of the highest dilution of a test sample yielding an $E_{450} > 0.150$ (2-3x background E_{450}) is taken as titre. As a control, each test sample was also tested in a 1/20 dilution against a faecal sample free of rotavirus. For this, a pool of faeces from conventional calves was used (see Fig. 1, step 2 and 3 for the IDAS and ACA, respectively). All samples were negative in this test.

RESULTS

Response of the gnotobiotic calf

A germ-free calf was infected orally with rotavirus on the fifth day of life. Rotavirus antibodies of all isotypes were detected in its serum starting with IgM 5 days post inoculation (dpi). In faeces only IgM (from 5 dpi on) and IgA rotavirus antibodies (from 10 dpi on) were found to be present (Fig. 2). Rotavirus was detected in the faeces by ELISA from 2-6 dpi.

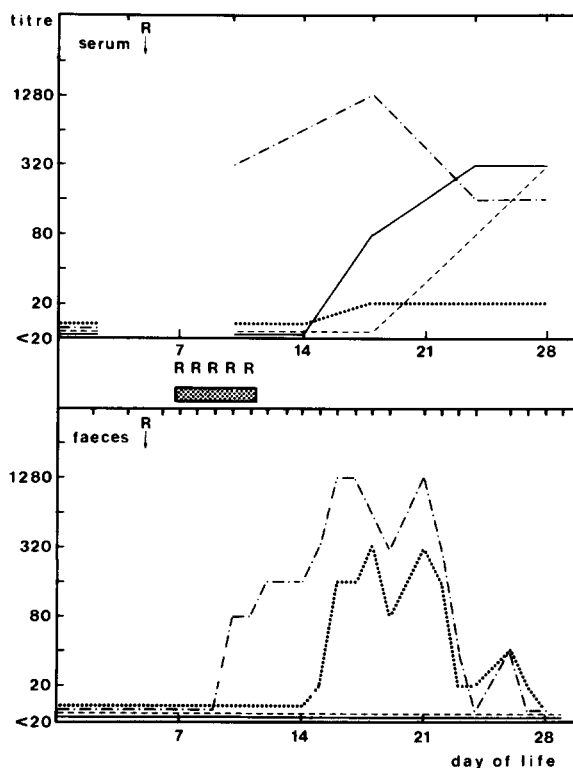





Fig. 2. Systemic and intestinal antibody response of a gnotobiotic calf after oral infection with rotavirus. Rotavirus antibody titres in serum (upper panel) and faeces (lower panel) were determined by isotype-specific ELISAs: IgG₁—; IgG₂ ---; IgM -.-.-; IgA R with an arrow underneath in upper and lower panel indicates the time of oral infection (5th day of life). Time of sampling is given by small bars at the upper edge of each panel. The middle panel summarizes rotavirus excretion as detected by ELISA (R). The severity of diarrhoea is indicated in the middle panel of fig. 2-6 as follows:  (bright) yellow, but with normal consistency;  semi-liquid faeces;  liquid faeces.

Vaccination experiment

The vaccinated calves of group B as well as the controls, which were challenged only (group A), showed a systemic and intestinal immune response similar to that observed in the gnotobiotic calf. In faeces of group A calves IgM rotavirus antibodies were detected starting on 4-6 dpi, followed by IgA rotavirus antibodies 2-6 days later. Antibody titres fell to low levels within 3 weeks post infection (Fig. 3). In group B calves IgM rotavirus antibodies were detected for the first time 5-6 days post vaccination (dpv) (Fig. 4). In

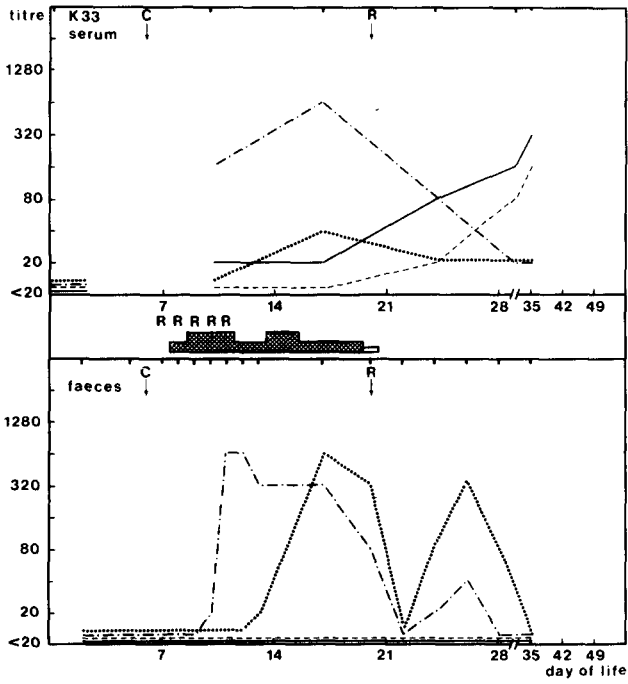
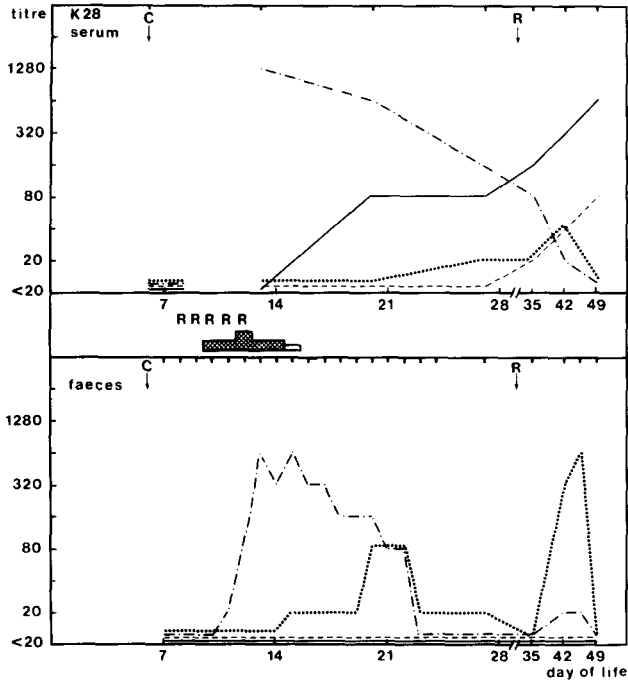


Fig. 3 (Group A)

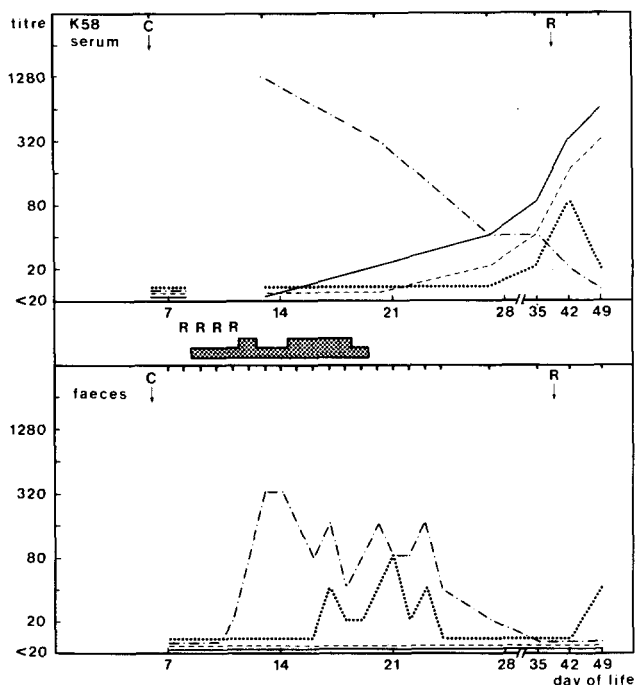


Fig. 3. Systemic and intestinal antibody response of group A calves. Calves were fed colostrum free of rotavirus antibody and were orally infected with virulent rotavirus on day 6 (C). Time of reinfection with rotavirus is indicated by R with an arrow underneath in the upper and lower panel. The calf number is shown in the upper left hand corner. For further explanation see Fig. 2.

other words, antibodies to rotavirus were excreted before or on the day of challenge and, therefore, must have been induced by the vaccine virus. IgG₁ or IgG₂ rotavirus antibodies were not detected in any of the faecal samples of the calves from groups A and B.

In group A, rotavirus was excreted between 1 and 6 dpi. In group B vaccine virus was detected 2-5 dpv but after the challenge no rotavirus was found by ELISA or by electron microscopy (Figs. 3 and 4).

Calves fed "positive" colostrum (groups C and D) excreted IgG₁ and IgG₂ rotavirus antibody in their faeces from 2 to 6 days after birth. Maternally derived IgG₁ and IgG₂ rotavirus antibodies were also found in serum in high titre (Figs. 5 and 6). After the challenge, only one calf in group C (no. 236) showed a systemic and an intestinal IgM rotavirus antibody response. This response developed later than that observed in the group A calves. Furthermore, only relatively low titres were found which decreased

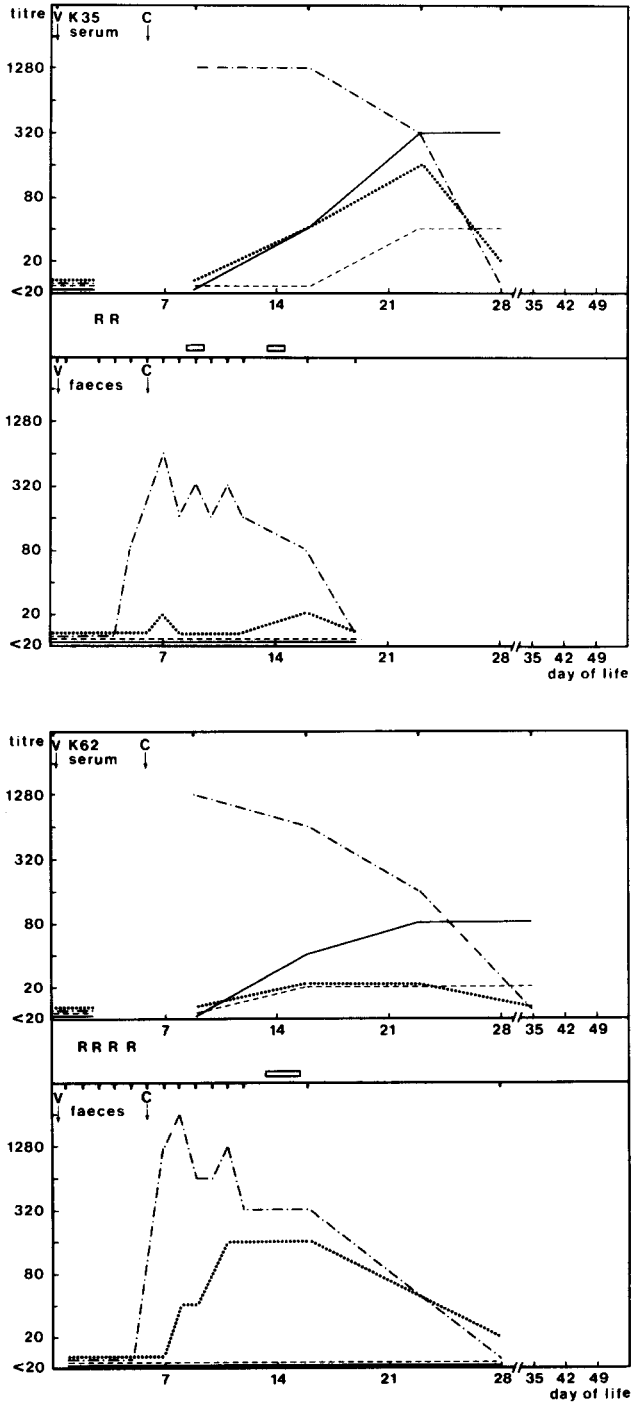


Fig. 4. (Group B).

rapidly. No IgA rotavirus antibodies were detected. Rotavirus excretion was observed in only two calves, nos. 236 and 237, for 3 and 1 day(s), respectively (Fig. 5).

The vaccinated calves of group D were not protected against the rotavirus challenge 6 dpv. Rotavirus excretion was found 1-8 days after the challenge, but not between the time of vaccination and challenge. No evidence was obtained for an intestinal or a systemic IgM or IgA antibody response after vaccination. Only relatively late after challenge and excretion of rotavirus, a moderate IgM rotavirus antibody response was observed both in serum and in faeces (Fig. 6).

Two other calves fed positive colostrum and challenged 8 days after vaccination reacted similarly (not shown).

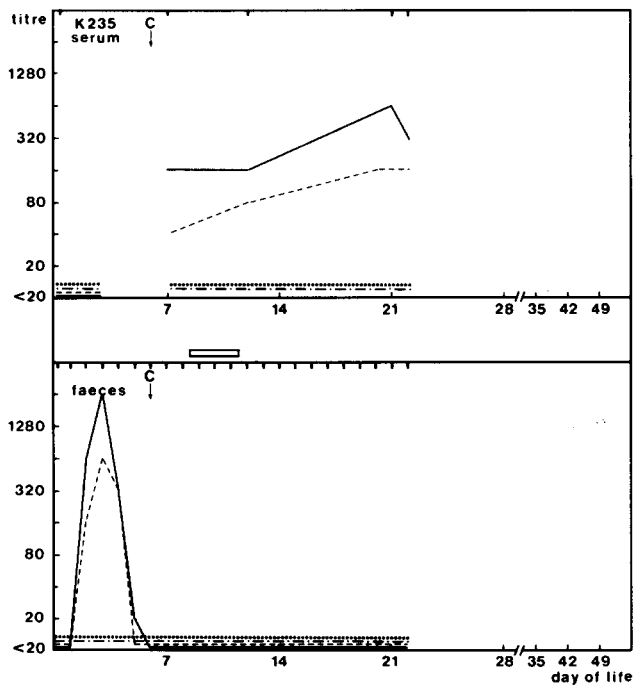


Fig. 5. Systemic and intestinal antibody response of group C calves. Calves were fed colostrum containing rotavirus antibody and were orally infected with virulent rotavirus on day 6. For further details see Figs. 2 and 3. (Continued next page).

Fig. 4. Systemic and intestinal antibody response of group B calves. Calves were treated as described in the legend of Fig. 3. However, they were vaccinated with modified live rotavirus on the day of birth (V).

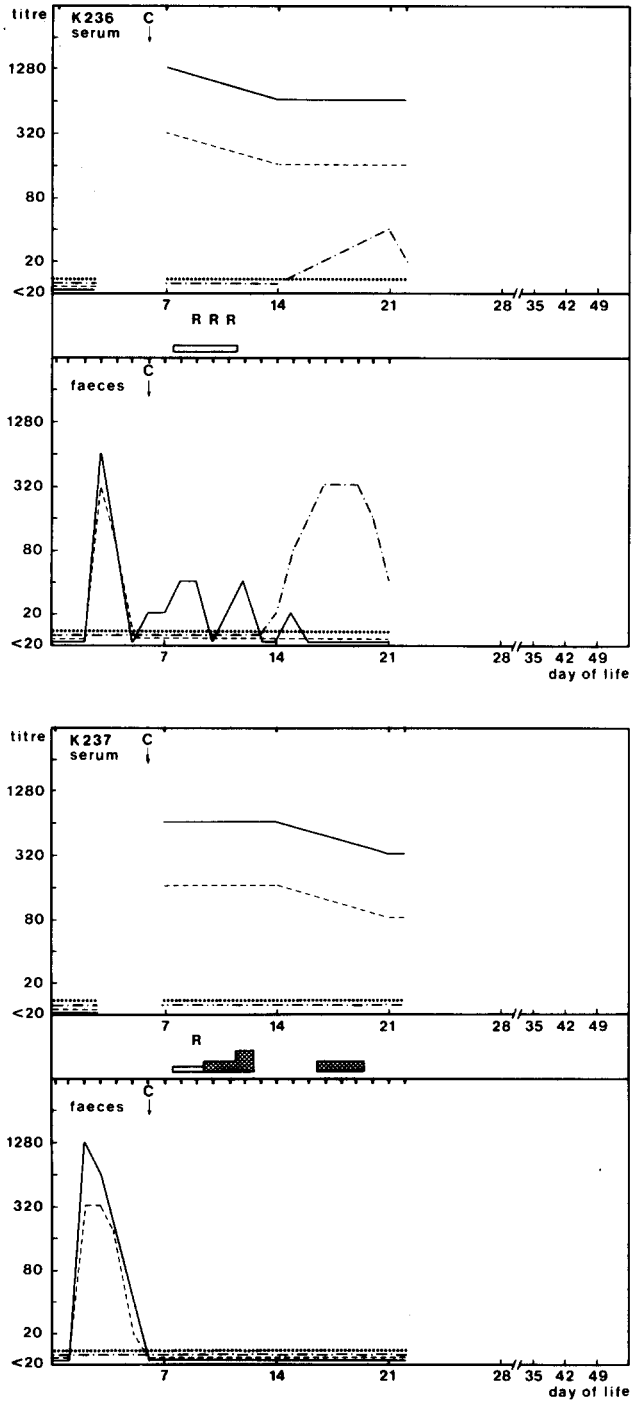


Fig. 5. (Continued Group C).

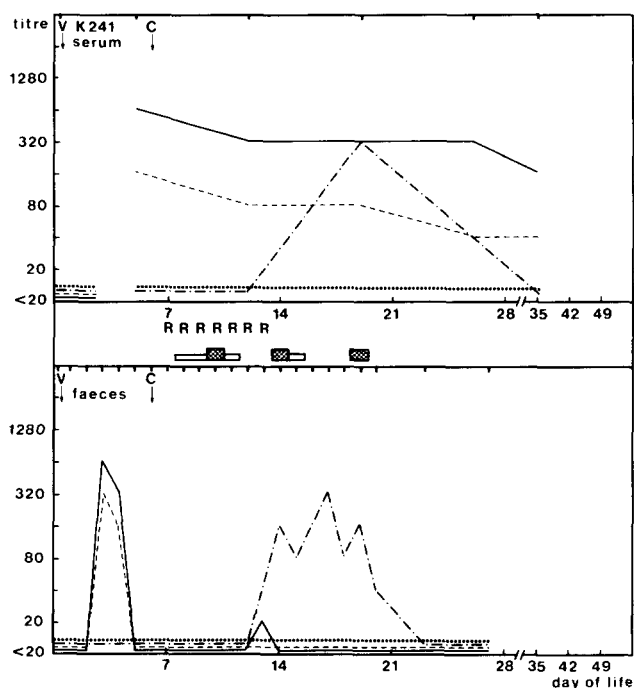


Fig. 6. Systemic and intestinal antibody response of group D calves. Calves were treated as described in the legend of Fig. 5. However, they were vaccinated with modified live rotavirus on the day of birth (V). (Continued next page).

Response to reinoculation

Calves in group A were reinoculated orally with rotavirus 2 to 4 weeks after primary infection. The rotavirus isolate originated from another herd than the first one. The inoculum also contained Cryptosporidium. All 3 calves developed diarrhoea and excreted Cryptosporidium oocysts in their faeces. Although no virus excretion could be detected, a marked rotavirus antibody response followed in two calves (nos. 28 and 33). In this case, IgA rotavirus antibody was the predominant antibody found in faeces; it was excreted in high titre 4-14 days after reinfection (Fig. 3). IgM rotavirus antibodies were also excreted but at low titre. Again, no IgG₁ or IgG₂ rotavirus antibodies were detected in the faeces. The changes of the isotype-specific antibody titres in serum are difficult to interpret as they may still be related to the primary response. It appears, however, that after reinoculation the IgG₁ and IgG₂ titres did increase and in nos. 28 and 58 also the IgA titre.

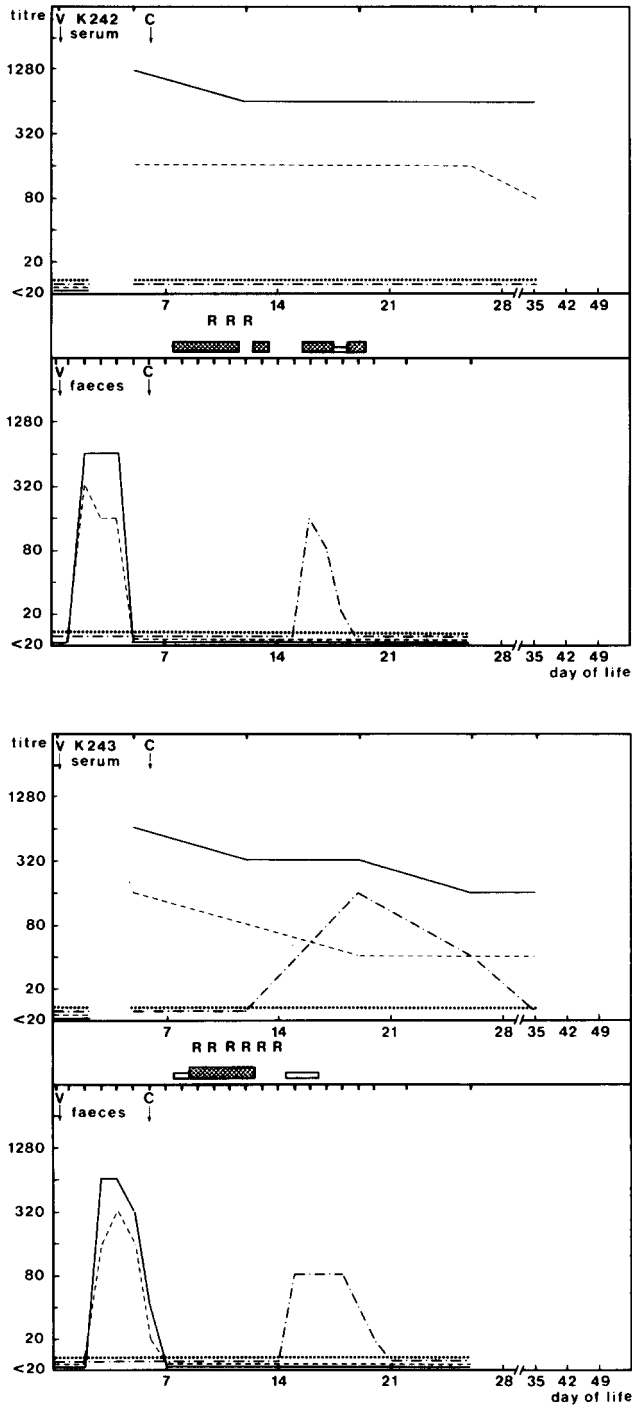


Fig. 6. (Continued Group D).

DISCUSSION

We have shown that the intestinal immune response of SPF-calves after oral infection with virulent or attenuated rotavirus is characterized by a rapid excretion of IgM rotavirus antibodies in faeces starting 4-6 dpi followed 2-6 days later by the excretion of IgA rotavirus antibodies. A similar pattern was observed after infection of a gnotobiotic calf. In both cases antibody excretion persisted for 2-3 weeks.

Reinoculation of 3 SPF-calves resulted, in at least 2 of them, in the excretion of IgA rotavirus antibodies and, at low titres, of IgM rotavirus antibodies for at least 1 week. This isotype-switch of the predominant rotavirus antibody might be characteristic for a secondary antibody response of primed cells. Recently, evidence for the existence of a memory response of the mucosal immune system in rabbits (Keren et al., 1982), rats (Andrew and Hall, 1982) and man (Wright et al., 1983) has been presented. On the other hand, Allen and Porter (1975) were unable to show the existence of a memory response in the intestine of calves, using dead bacteria. Also, it cannot be excluded that the isotype-switch mentioned before simply reflects age-related changes of the primary intestinal response. This possibility and the existence of mucosal memory in calves are subject of further study.

The absence of IgG₁ and IgG₂ rotavirus antibodies in faeces (except for a few days after ingestion of colostrum containing rotavirus antibodies) is striking in view of the observations of Newby and Bourne (1976). These authors reported IgG₁ to be the predominant isotype in the intestinal content of calves. This isotype appeared to be partly serum-derived. Consequently, IgG₁ rotavirus antibodies would be expected to be present in the faeces after infection. It is conceivable that IgG₁ or IgG₂ rotavirus antibodies secreted in the intestinal lumen are degraded by proteolytic enzymes during passage through the gut. However, incubation of purified IgG₁ containing rotavirus antibody activity with fresh faeces for 24 hr at 37°C did not reduce the IgG₁ rotavirus antibody titre. Therefore, no indication was obtained that the absence of IgG₁ rotavirus antibodies in faeces is due to proteolytic degradation, but the possibility that antibodies were degraded during passage in the intestinal tract is not excluded. It should also be noted that differences in sensitivity of the tests (which are difficult to assess) might influence the relative importance of Ig-isotypes. For instance, a low relative sensitivity of the IgG₁-IDAS might explain the absence of IgG₁ rotavirus antibody activity in faeces. (The detection of IgG rotavirus antibodies in faeces after the ingestion of "positive" colostrum can be explained by the large amount of antibody administered). On the other hand, results of other groups (Porter et al., 1972; Cripps et al., 1974; Allen and Porter, 1975) demonstrated, in accordance with our results, a major role for IgM and IgA in the intestine of cattle (see introduction). The predominance of IgA rotavirus antibodies in faeces late after infection or after reinoculation of rotavirus described in this paper is furthermore in agreement with the results of Vonderfecht and Osburn (1982). They demonstrated that 4 weeks after rotavirus inoculation in Thiry-Vella loops

of calves the majority of the cells that produced rotavirus antibodies contained IgA and only a few per cent stained for IgG and IgM. Our results compare also rather well with those obtained in unweaned piglets. Evidence has been presented that their intestinal lamina propria contains equal numbers or more of IgM producing cells as compared to IgA producing cells (Allen and Porter, 1973; Brown and Bourne, 1976). In addition, rotavirus infections in pigs were associated with the excretion of IgM rotavirus antibodies which were found, however, in concert with an excess of IgA rotavirus antibodies (Corthier and Franz, 1981). It has been suggested earlier that IgM might play an important role in (the early phase of) the mucosal immune response (Porter et al., 1972; Allen and Porter, 1973; Brandtzaeg, 1975). Differences in the results mentioned above may be due to a variety of factors, i.e. species, differences in methodology (techniques, isotype-specific reagents), parameters measured (immunoglobulin concentration, antibodies, plasma-cells) and samples examined (secretions of intestinal loops, intestinal content obtained post mortem, faeces).

Final proof for the local production of IgM rotavirus antibodies in the bovine intestine awaits further studies using double immunofluorescence tests on gut tissue sections (Vonderfecht and Osburn, 1982). Similarly, the relevance of IgG in the intestine should be studied further using monoclonal isotype-specific reagents and hopefully, this will shed some light on this controversial issue (Morgan et al., 1980).

Another important aspect of this study is the evaluation of the immune response after oral application of a modified live rotavirus vaccine in young calves. The results obtained with group A and B calves, fed colostrum without specific antibodies, suggest that IgM is the main antibody-isotype responsible for recovery from and early protection against viruses causing enteric disease. In the field, nearly all adult cattle possess rotavirus antibodies and can transfer these via colostrum and milk to their offspring. Evidence has been presented that effective oral vaccination of calves against rotavirus-induced diarrhoea is hindered by the presence of these lacteal antibodies (De Leeuw et al., 1980b; Bürki et al., 1983; De Leeuw and Tiessink, 1984). Our results with group C and D calves (Figs. 5 and 6) further substantiate this view. These calves excreted antibodies in their faeces up to 6 days after birth. The presence of these antibodies in the intestine most likely prevented the normal multiplication of the vaccine rotavirus. It appears from studies of transmissible gastroenteritis virus infections in swine that an intestinal immune response to a virus infection requires an extensive infection of the intestinal mucosa (Bohl and Saif, 1975). Together, this probably explains why no intestinal antibody response was observed after vaccination of group D calves (Fig. 6), comparable to that in group B calves (Fig. 4). However, a suppressing effect of serum antibodies on the mucosal antibody response cannot be excluded. This effect is suggested by the delayed and weak IgM-response after challenge of group C and D calves, which at that moment did not excrete detectable quantities of IgG rotavirus antibodies in their faeces. In some calves, the intestinal

response was even absent. The observations with group C and D calves also confirm that feeding of colostrum antibody for only a few days does not provide protection against challenge infection several days later. This requires continuous uptake of rotavirus antibodies (Bridger and Woode, 1975; Lecce et al., 1976; Snodgrass and Wells, 1978b).

Although our data suggest that IgM is the major antibody isotype involved in recovery from and early protection against enteric viral infection, the precise role of IgM and IgA has yet to be defined. In addition, other immune mechanisms have not been studied and a possible protective role for IgA and IgG at a later age or longer after an infection or a vaccination still remains possible.

In conclusion, our results indicate that the intestinal immune response of young calves to an enteric viral infection is associated, as in other species, with the excretion of IgA antibodies, which is preceded by the excretion of IgM antibodies. This response is absent or diminished in calves with passively acquired specific antibodies in serum and intestine. Oral vaccination with modified live rotavirus of calves fed colostrum with rotavirus antibodies did not induce a protective intestinal immune response. This is most likely explained by neutralization of the vaccine virus as suggested by De Leeuw and Tiessink (1984). In addition or alternatively, serum antibodies might suppress the development of intestinal immunity. Although oral vaccination rapidly induced a protective intestinal immune response in calves which were not fed colostrum with rotavirus antibodies, it is not sensible to omit or delay colostrum feeding. Therefore, alternative vaccination methods are needed to induce an active intestinal immune response or a persistent secretion of rotavirus antibodies in milk. To facilitate this, further study of the mucosal immune system of young and adult cattle is necessary.

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