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Rotavirus infections in calves: efficacy of oral vaccination in endemically infected herds

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A commercially available modified live reovirus-like (rotavirus) vaccine proved innocuous in calves deprived of colostrum and protected one out of three calves against challenge 72 h after vaccination. The vaccine was evaluated in two dairy herds in the 1977 calf season. No significant differences were observed in the incidence rates or severity of undifferentiated neonatal calf diarrhoea or rotavirus-associated late diarrhoea between calves given a placebo (76) and vaccinated (74) calves in these herds. Samples of colostrum contained specific antibodies against rotavirus and neutralisation of the vaccine virus by colostral antibody in the intestinal tract was considered the major reason for the failure of the vaccine to reduce the incidence of neonatal diarrhoea in these herds.

FOLLOWING CELL CULTURE propagation of a bovine rotavirus isolate (Mebus *et al* 1971) a modified live virus vaccine was developed. Favourable results were reported from pre-licensing field trials in which all calves in a herd were vaccinated (Mebus *et al* 1972, 1973). In most herds the incidence and severity of neonatal calf diarrhoea were lower than in previous years or in the period before vaccination was started. After the commercial introduction of the vaccine in 1973, a random survey of its use in the field gave satisfactory results (Twiehaus *et al* 1975). However, beneficial effects from vaccination could not be shown when double blind field trials were conducted (Acres and Radostits 1976). This was confirmed by Thurber *et al* (1977), who used an experimental oral vaccine containing both the attenuated rotavirus isolate and an attenuated bovine coronavirus. They found, however, that neonatal calf diarrhoea morbidity and mortality rates were less in vaccinated than in control calves when the results of uninterrupted vaccination were compared with disease rates during a preceding or subsequent period. Thurber *et al* (1977) consider that the double blind field trial is not a suitable test design for evaluating a modified live virus oral vaccine since calves given a placebo may spread disease to vaccinated calves preventing or overwhelming a protective response from vaccination.

Our first objective was to evaluate the rotavirus vaccine on well managed dairy farms where rotaviruses were endemic. Pilot experiments were carried out in calves deprived of colostrum to examine the effectiveness of the vaccine against challenge with a Dutch rotavirus isolate. In 1977 a field trial was carried out which was deliberately kept small to allow thorough clinical examination and monitoring of rotavirus excretion in the faeces of diseased and healthy calves. Sequential vaccination was used during the major part of the study.

Materials and methods

Vaccination and challenge of calves deprived of colostrum

All experimental calves were Friesians and originated from the institute's closed herd. They were delivered by caesarean section and individually housed in an isolation room. Milk substitute was fed three times daily for the first four days and then twice daily.

Calves 1 to 7 were vaccinated orally within 6 h of birth, in accordance with the instructions of the vaccine manufacturer (Scourvax-reo 1; Norden). Calves 8 and 9 were left unvaccinated.

Calves were challenged by the oral administration of a Dutch rotavirus isolate purified from faeces (de Leeuw *et al* 1977). Calves 1 to 3 were not challenged; calf 4 was challenged 24 h after vaccination; calves 5 to 7 three days after vaccination.

Calves 8 and 9 were challenged at one and six days old, respectively.

All calves were examined at each feeding. At the same time rectal temperatures were taken and faecal samples were obtained. Blood samples were taken on day 1 and again four weeks later.

Description of herds

Herd A consisted of 180 milking cows. Calf management, housing and clinical history have been described by de Leeuw *et al* (1980). Herd C belonged to a government research station and had 270 milking cows. Management and hygienic conditions in this herd were comparable to those in herd A. The calving season lasted from January to May. All calves were individually housed in wooden boxes placed side by side. The boxes were cleaned and disinfected before new calves came in. Male calves were usually sold within the first few days.

Within 1 h of birth 500 ml of colostrum was fed to each calf. After that colostrum was given by means of a continuously filled nipple-bucket for three to four days. Each day the contents of the buckets were replaced with fresh colostrum. Care was taken that each calf consumed at least 4 litres daily. From either day 3 or day 4 to day 8 pooled cow milk was fed and after day 8 milk substitute.

Rotavirus infections in calves in this herd had been diagnosed frequently in previous years. During the calving season neonatal calf diarrhoea (NCD) morbidity approached 80 per cent; mortality remained below 10 per cent.

Design of field trial

The modified live rotavirus vaccine, supplied in desiccated form with 4 ml of sterile diluent, had a titre of $10^{4\cdot8}$ $TCID_{50}$ per ml. A placebo of phosphate buffered saline was bottled in volumes of 4 ml and presented as a new experimental vaccine; the experiment was presented as a comparative field trial. Both vaccine and placebo were stored on the farms at 4°C. Vaccination was carried out by the animal attendants or the farm managers by oral administration as soon as possible after birth, usually within 1 h, according to the instructions of the manufacturer of the rotavirus vaccine. The day of birth in this study is regarded as day 0.

The vaccine trial in herd A lasted from January to June 1977. Until the first week of March all calves housed in one section of the calf house were vaccinated (period 1), those housed in the other section received the placebo (period 2). For the next month all newborn calves were vaccinated (period 3); for the last two months all calves received the placebo (period 4).

In herd C the vaccine trial lasted from February to May 1977. The four months were divided into five three-week periods. All calves born in periods 1, 3 and 5 were given the placebo; calves born in periods 2 and 4 were vaccinated.

In both herds clinical examination was carried out daily for three weeks after birth by someone who was not familiar with the vaccination scheme. The consistency of faeces was noted as normal, semi-liquid or liquid for which 0, 1 and 2 points were given, respectively. Diarrhoea was defined as the excretion of semi-liquid or liquid faeces. Faecal samples were obtained from the rectum of all female calves on either days 2 and 4 or days 3 and 5 and additional samples were taken when diarrhoea developed later. During March this scheme was expanded by taking samples routinely after day 5. Blood samples were taken on day 3 and again one month later. In addition, colostrum samples from the first feeding were obtained from 54 calves in herd A and from 81 calves in herd C.

Treatment and examination of samples

Faecal samples were collected, stored and treated as described by de Leeuw *et al* (1980). For the first two months of the field trial all faecal extracts were examined for the presence of rotaviruses by electron microscopy (EM) (de Leeuw *et al* 1977). Thereafter enzyme-linked immunosorbent assay (ELISA) and immunoelectroosmophoresis (IEOP) were used in parallel. Both methods have been described elsewhere (Ellens and de Leeuw 1977; Ellens *et al* 1978b). Clinical data and laboratory results were not linked before completion of all tests.

Serum and colostrum samples were stored at −20°C and later titrated for antibodies against rotavirus by complement fixation (CF) (de Leeuw *et al* 1977) and ELISA (Ellens *et al* 1978a), respectively. Serum and colostrum titres are expressed as described by de Leeuw *et al* (1980).

Results

Experiments in calves deprived of colostrum

No adverse reactions were observed after vaccination of calves 1 to 3. The colour of the faeces of calves 1 and 2 became bright yellow a few days after vaccination but the consistency of the faeces of all three calves remained normal. Rotaviruses were demonstrated by EM in the faeces of these animals for at least two days (Fig 1).

Calf 4, challenged 24 h after vaccination, produced liquid faeces on days 3 and 4 and remained diarrhoeic for two more days. Rotaviruses were detected in the faeces from day 3 to day 10.

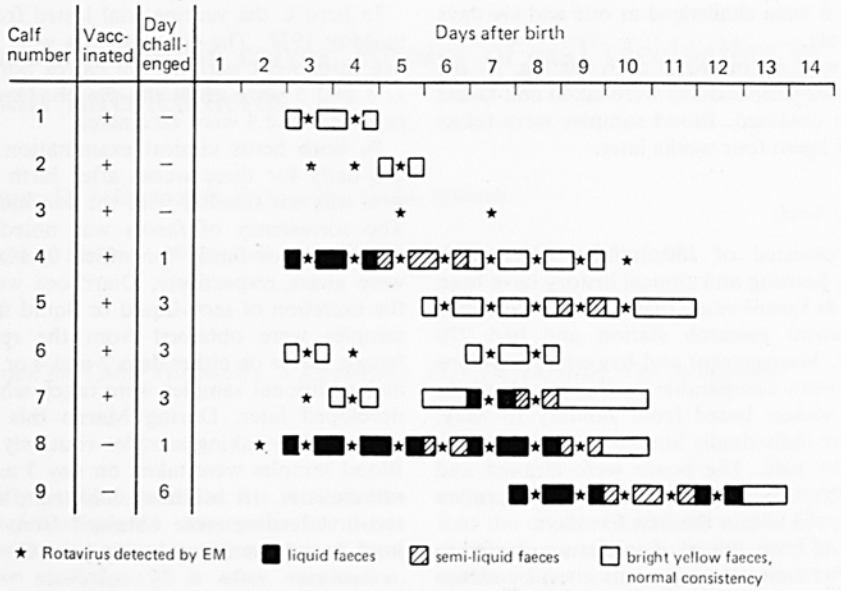

FIG 1: Vaccination experiments in calves deprived of colostrum

Calf 6, challenged 72 h after vaccination, remained healthy. Calves 5 and 7, also challenged 72 h after vaccination, developed diarrhoea but were less severely affected than calf 4 and the two control calves 8 and 9. Rotaviruses were detected in the faeces of calves 6 and 7 prior to challenge and in the faeces of calves 5, 6 and 7 after challenge (Fig 1).

Calves that developed diarrhoea appeared depressed and sometimes refused to drink but all recovered without supportive therapy other than normal dietary measures.

Antibodies against rotavirus were not detected by CF in the first-day sera of these calves. Four weeks later CF titres ranged from 16 (calf 1) to more than 128 (calves 4 and 7).

Field trial

Herd A. Two clinical stages could be distinguished: a mild and short-lasting 'early diarrhoea' during the first three days of life and a more severe 'late diarrhoea' between the third and 14th day. In general the clinical and virological findings were similar to those observed in the same herd during the corresponding period of the preceding year (de Leeuw *et al* 1980). Diarrhoea was considered to be associated with rotavirus if it started within two days or less of the first appearance of rotavirus in the faeces.

Rotavirus excretion was detected in 20 vaccinated calves and in 19 calves given the placebo. Rotavirus-positive samples were obtained after an average of 4·9 (±1·8) and 4·8 (±2·6) days, respectively. During the first two months of the vaccine trial the incidence of rotavirus-associated diarrhoea appeared to be lower in vaccinated calves (period 1) than in calves given the placebo (period 2) (Fig 2). However, this difference was not found to be significant by the χ^2 test. During March, when all calves were vaccinated, the incidence rate of rotavirus-associated diarrhoea remained at the highest level observed previously but the severity increased (Table 1).

TABLE 1: Severity of undifferentiated and rotavirus-associated diarrhoea in groups of vaccinated calves and calves given a placebo

Farm	Category	Period number 1	2	3	4	5	Total
A	Vaccinated	1·7 / 4·3		4·1 / 5·6			3·0 / 5·0
	Placebo	—	2·3 / 4·4		1·1 / 1·9		1·5 / 2·9
C	Vaccinated		1·3 / 2·6		0·5 / 1·1		1·0 / 2·1
	Placebo	0·8 / 1·5		1·2 / 3·1		1·4 / 1·8	1·1 / 2·1

Severity of diarrhoea defined as the total point score (daily observations: liquid faeces 2 points, semi-liquid faeces 1 point) divided by the total number of calves observed. Numerator: rotavirus-associated diarrhoea; denominator: undifferentiated diarrhoea

Thereafter both the incidence rates of diarrhoea and the severity decreased. Over the whole period of the vaccine trial the incidence rates of early and late diarrhoea were higher in vaccinated calves (periods 1 and 3) than in calves given the placebo (periods 2 and 4) ; the incidence rates of rotavirus-associated diarrhoea in both categories were

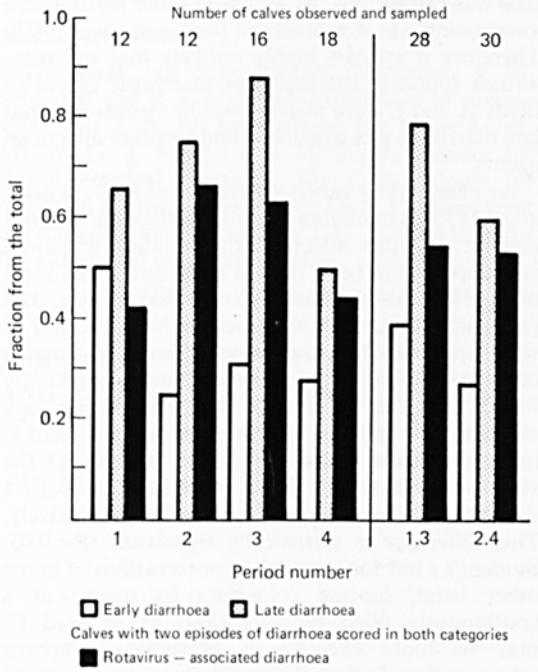

FIG 2: Incidence rate of neonatal calf diarrhoea in vaccinated calves and calves given a placebo, herd A

approximately the same (Fig 2). The severity of rotavirus-associated diarrhoea was higher in vaccinated calves than in those given the placebo (Table 1).

Herd C. The clinical picture of NCD and the virological findings in herd C were comparable to those observed in herd A. Both early and late diarrhoea could be distinguished but late diarrhoea was less severe, occurred less frequently and the onset was later than in herd A.

Rotaviruses were shed by 22 calves given the placebo and by 23 vaccinated calves and were first detected after an average of 7·2 (±2·5) and 7·5 (±2·2) days, respectively.

The incidence rates of early diarrhoea, of late diarrhoea and of the rotavirus-associated diarrhoea were approximately the same in vaccinated calves (periods 2 and 4) and in calves given the placebo

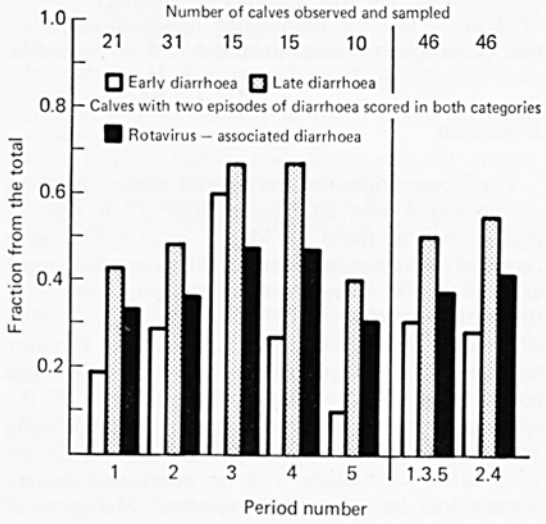

FIG 3: Incidence rate of neonatal calf diarrhoea in vaccinated calves and calves given a placebo, herd C

(periods 1, 3 and 5) (Fig 3). The severity of diarrhoea in these groups was also comparable (Fig 3). The results obtained in the successive periods show an increasing diarrhoea incidence in periods 1 to 3, a peak in periods 3 and 4, and a decrease in period 5 as expected at the end of the calving season (Fig 3).

Serology

All calves, except for one calf in herd C, had circulating antibodies against rotavirus detectable by CF on the third day of life. The titres ranged from 4 to more than 128. No significant differences were found between the geometric mean serum titres of vaccinated calves and calves given the placebo, either in the same herd, or between the two herds. There was no relationship between the CF titre of individual calves and the development of rotavirus-associated diarrhoea. Only the sero-negative calf and three other calves with low titres on day 3 had significantly higher, at least a fourfold, CF titres one month after birth. The titres of all other calves remained the same or decreased during this period.

Colostrum samples were titrated with ELISA starting at a dilution of 1 : 100. Only samples from colostrum fed to two calves in herd A and six calves in herd C were negative at this dilution. The titres of the other samples ranged from 100 to 6400. Only minor differences were found between the geometric mean ELISA titres of colostrum samples fed either to vaccinated calves or to calves given the placebo on the same farm. However, the geometric mean ELISA titre of colostrum samples obtained in herd C

($2{\cdot}8 \pm 0{\cdot}4$, $n=81$) was significantly higher ($P<0{\cdot}05$, Student's t test for unmatched observations) than that of samples obtained in herd A ($2{\cdot}5 \pm 0{\cdot}3$, $n=54$).

Discussion

The observations on vaccinated calves deprived of colostrum and challenged after 72 h (Fig 1) partly confirm those of Mebus *et al* (1973) who reported resistance in 19 out of 20 calves challenged after 48 to 72 h. They are at variance, however, with those of Woode *et al* (1978) who found protection seven days after vaccination but not after three or five days. It was speculated that such differences could result from variation in virulence of the challenge virus used by different authors (Woode *et al* 1978). Protection against homologous challenge 96 h after vaccination with an attenuated bovine coronavirus has also been reported (Mebus *et al* 1976). Since neutralising antibodies against this virus in washings of isolated loops of the lower ileum were not found before six to eight days after inoculation, Mebus *et al* (1976) suggested that initial resistance against infection with virulent virus was due to an interference phenomenon and later resistance to the presence of local antibody. Evidence for the presence of interferon in the intestinal loops was not presented.

Rotavirus excretion in the faeces of vaccinated calves deprived of colostrum has, to our knowledge, not been reported. Woode *et al* (1978) did not observe rotavirus excretion in the faeces of vaccinated gnotobiotic calves; after challenge virus excretion was found only in calves that developed diarrhoea. In our study rotavirus excretion was found in all three vaccinated calves that were not challenged and in two other calves before challenge (Fig 1). Rotavirus excretion was also found in vaccinated calves after they had been challenged, including calf 6 that remained healthy. The virus excretion patterns of calves 6 and 7, indicate that the virus found on days 7 and 8 was virulent virus (Fig 1). If this were true, it would imply that vaccinated calves that are protected from disease may still shed large quantities of virulent virus. However, a definite conclusion cannot be drawn since we were unable to distinguish vaccine virus from virulent virus (unpublished results).

No beneficial effects of vaccination were found in herds A and C when all data for vaccinated calves and calves given the placebo were compared (Figs 2 and 3, Table 1). Although the incidence rate of rotavirus-associated diarrhoea in vaccinated calves in herd A from January to March was lower than that in the control calves during the same period, this difference was not significant. The other differences in the incidence rates of early, late and rotavirus-associated diarrhoea in periods 3 and 4 in herd A and in the succeeding periods in herd C, appear to represent normal seasonal variation. In herd A for instance, a similar clinical picture was observed in 1976. In addition, the association between late diarrhoea and rotavirus excretion as observed during the vaccine trial in herds A and C was also found in herd A before and after the vaccine trial was carried out, as well as in other herds where vaccination was not practised (de Leeuw *et al* 1980). Therefore it appears highly unlikely that the rotaviruses found in the faeces of diarrhoeic calves in herds A and C were merely vaccine viruses and that late diarrhoea was usually caused by other infectious agents.

As observed by others (Mebus *et al* 1973; Woode *et al* 1975), circulating specific antibodies did not prevent rotavirus infection. Examination of paired sera appeared to be of limited diagnostic value since only a few calves showed an increase in titre. The mean maternal antibody titres in herds A and C were approximately the same although the mean colostral antibody titre in herd C was significantly higher than that in herd A. This observation may explain why rotavirus infections in calves in herd C in general were found later than in herd A: the virus was first detected after an average of $7{\cdot}3 \pm 2{\cdot}4$ days ($n=45$) and $4{\cdot}9 \pm 2{\cdot}2$ days ($n=39$), respectively. This difference is statistically significant ($P<0{\cdot}05$, Student's t test for unmatched observations). On the other hand, feeding colostrum by means of a continuously filled nipple-bucket, as in herd C, may be more effective in preventing rotavirus infection than feeding it twice daily as was practised in herd A (de Leeuw *et al* 1980).

The failure of the rotavirus vaccine to produce any beneficial effect under the conditions prevailing in herds A and C could be due to several factors. The results obtained with calves deprived of colostrum (Fig 1) show that after vaccination at least a few days are needed before immunity is fully developed. Under field conditions one may expect that some calves will become infected with virulent rotavirus shortly after birth, which in that case cannot be protected by vaccination. However, the majority of calves appears to be protected against virulent rotavirus infection during the first day of life by specific colostral antibodies present in the intestinal tract (de Leeuw *et al* 1980). In this study nearly all samples of colostrum examined had ELISA titres against rotavirus of more than 100. In addition the antibodies present in colostrum samples obtained in herds A and C neutralised the vaccine virus (Ellens *et al* 1978a). Therefore, in our opinion, neutralisation of the vaccine virus by colostral antibody is the most likely explanation

for the failure of the vaccine under field conditions. In fact, in well managed dairy herds it is illogical to carry out oral vaccination against rotavirus soon after birth since colostrum is fed at this time. When rotavirus infections have been causing problems for some time, colostrum may be expected to contain specific antibodies which in particular occur in high quantities shortly after parturition (Ellens *et al* 1978a; Woode *et al* 1975).

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