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FIELD TRIAL EVALUATION OF AN INACTIVATED ROTAVIRUS VACCINE AGAINST NEONATAL DIARRHEA OF CALVES

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Key words: Rotavirus vaccine - Neonatal calf diarrhea - Field trials.

Field trials were conducted using an inactivated rotavirus vaccine for prevention of calf neonatal diarrhea.

For the trials, 458 pregnant cows from 26 herds were involved. In each herd, cows which had been inseminated within a period of two months were selected and randomly subdivided in two groups. Cows in one group (248 head in total) were vaccinated 6 weeks before calving and again 4 weeks later; cows in the other group (210 head in total) were left as unvaccinated controls. At calving, colostrum was collected from each cow and stored at —30°C until used for feeding calves.

The newborn calves, beginning the second day of life and for the next 7-10 consecutive days, each was fed a daily supplement of 400 ml of colostrum from its dam.

The diarrhea occurred in 86 (40.9%) calves that had received colostrum from unvaccinated dams (normal colostrum), and in 7 (2.8%) calves which were fed colostrum from vaccinated dams (immune colostrum). The disease was very severe in the normal colostrum-fed calves and 52 of them died. Those calves which survived the disease underwent a significant loss of condition. By contrast, the 7 immune colostrum-fed calves displayed a rather mild enteric condition, and all recovered without any sequela being observed.

INTRODUCTION

Since 1969 (5) rotavirus has been incriminated as one of the most common pathogenic agents causing neonatal diarrhea in calves.

Attempts to prevent rotavirus-associated diarrhea were made since 1973. The first approach consisted of vaccinating newborn calves with an oral modified live vaccine (6). The use of the vaccine apparently decreased calf morbidity and mortality (9, 10). However, more recent field studies failed to substantiate the efficacy of the vaccine (1, 4). The failure was generally attributed to the colostral antibodies that eventually interferred with active immunization of the newborn calf (4).

The second attempt was to passively immunize the calf by stimulating the dam, through vaccination, to secrete antibody in the colostrum and milk (7, 8, 11). There was evidence that feeding immune colostrum from cows vaccinated with inactivated calf rotavirus vaccine, delayed the onset of diarrhea and reduced its incidence, duration and severity in a naturally occurring outbreak of the disease (8).

Also, under experimental conditions it was proved that passive immunity does effectively protect the newborn calf from rotavirus diarrhea (3).

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This paper reports the results of field trials conducted with the aim of verifying whether the administration of an inactivated rotavirus vaccine to pregnant cows would protect calves to naturally occurring diarrhea in herds with a history of regular recurrence of the disease.

MATERIALS AND METHODS

Vaccine. - Strain 81/36F of bovine rotavirus (2) grown in an embryonic rhesus monkey kidney cell line (MA-104) was used as antigen. The virus was at its 18th passage and had a titer of $10^{6.74}$, median tissue culture infectious doses ($TCID_{50}$)/ 0.2 ml. The infectivity of the virus was inactivated by overnight incubation at 4°C with 0.5% formaldehyde. One portion was then emulsified in an equal volume of Freund's incomplete adjuvant and drown in volumes of 2.0 ml in disposable plastic syringes. The remainder of the suspension was distributed in vials in volumes of 10.0 ml. The two vaccine preparations were stored at 4°C until used.

Herds. - The trials were conducted in 26 dairy herds with a history of neonatal diarrhea in the last 5 years. The herds were located in two provinces (Bari and Taranto) of the Puglia region. Circulation of rotavirus in the selected herds was revealed by virus isolation from diarrheic calves, and also by detection of neutralizing antibody to the virus in serum samples obtained from cows.

Field trial design. - The field trials were carried out during the 1984-1985 calving season under the supervision of a cooperating veterinarian. In each herd the cows which had been inseminated within a period of two months were selected for the trials. As depicted in Table 1, the selected cows in each herd were randomly subdivided into two groups. Cows in one group (248 head in total) were vaccinated, whereas cows in the other group (210 head in total) served as unvaccinated controls. Vaccination was started approximately 6 weeks before calving. At this time each cow received 2.0 ml of the emulsified antigen vaccine preparation subcutaneously in the dewlap. A second injection of 10.0 ml of single antigen suspension was given by the same procedure 2 weeks before calving.

A pool of colostrum from the first and second milkings after calving was obtained from each vaccinated or control cow. The pool was dispended in 400 ml containers and stored at —30°C. The newborn calves were raised according to the existing management practices of the herd, with the exception that, beginning the second day after birth, and for the next consecutive 7-10 days, each calf was fed a supplement of 400 ml of colostrum obtained from its dam, as described above.

TABLE 1.
Field trials to evaluate the efficacy of a rotavirus * inactivated vaccine against calf diarrhea.

Herd No.	Pregnant cows	
	Vaccinated No.	Unvaccinated No.
1	15	12
2	7	5
3	41	37
4	9	7
5	6	3
6	3	6
7	4	6
8	6	1
9	5	4
10	7	3
11	13	11
12	5	4
13	8	8
14	4	6
15	17	10
16	5	1
17	13	10
18	14	6
19	10	14
20	6	6
21	18	14
22	6	4
23	8	18
24	3	3
25	5	5
26	10	6
Total	248	210

* 81/36F bovine rotavirus strain (2).

TABLE 2.
Neutralizing antibody to 81/36F strain of bovine rotavirus in the colostrum of vaccinated * and unvaccinated cows in six randomly selected herds.

Herd No.	Antibody in the colostrum of:			
	Vaccinated cows		Unvaccinated cows	
	Samples No.	Titer +	Samples No.	Titer +
2	5	496.00	5	48.00
3	10	492.00	10	44.00
11	5	224.00	5	20.00
17	5	224.00	5	40.00
19	7	840.00	7	63.00
21	8	475.00	8	77.00
Total	40	458.50	40	48.75

* 81/36F bovine rotavirus, inactivated vaccine.
\+ Average reciprocal value.

TABLE 3. — The incidence of diarrhea and mortality associated with diarrhea, in calves fed with colostrum from their vaccinated * or unvaccinated dams.

Herd No.	Calves							
	Fed with immune colostrum △				Fed with normal colostrum +			
	Diarrhea		Died		Diarrhea		Died	
	No.◇	%	No.○	%	No.◇	%	No.○	%
1	2/15	13.3			6/12	50.0	4/12	33.3
2	0/7	0			2/5	40.0	1/5	20.0
3	0/41	0			9/37	24.3	5/37	15.5
4	0/9	0			0/7	0	0/7	0
5	0/6	0			2/3	66.6	1/3	33.3
6	0/3	0			3/6	50.0	1/6	16.6
7	0/4	0			3/6	50.0	0/6	0
8	0/6	0			1/1	100.0	1/1	100.0
9	0/5	0			0/4	0	0/4	0
10	0/7	0			2/3	66.6	2/3	66.6
11	2/13	15.3			8/11	72.7	6/11	54.5
12	0/5	0			2/4	50.0	1/4	25.0
13	0/8	0	>0	>0	3/8	37.5	1/8	12.5
14	0/4	0			2/6	33.3	1/6	16.6
15	1/17	5.8			4/10	40.0	4/10	40.0
16	0/5	0			1/1	100.0	1/1	100.0
17	0/13	0			5/10	50.0	4/10	40.0
18	0/14	0			3/6	50.0	1/6	16.6
19	2/10	20.0			9/14	64.2	8/14	57.1
20	0/6	0			3/6	50.0	1/6	16.6
21	0/18	0			3/14	21.4	0/14	0
22	0/6	0			2/4	50.0	1/4	25.0
23	0/8	0			5/18	27.7	3/18	16.6
24	0/3	0			3/3	100.0	3/3	100.0
25	0/5	0			2/5	40.0	1/5	20.0
26	0/10	0			3/6	50.0	1/6	16.6
Total	7/248	2.8	0	0	86/210	40.9	52/210	24.7

* 81/36F bovine rotavirus, inactivated vaccine.
△ From vaccinated cows.
\+ From unvaccinated cows.
◇ No. of calves with diarrhea/No. of calves considered.
○ No. of calves which died/No. of calves considered.

The calves were observed for 30 days for the appearance of neonatal diarrhea. When diarrhea occurred, fecal swabbings were taken and cultured for virus.

Serologic tests. - Tests for the presence of neutralizing antibody to 81/36F bovine rotavirus were performed on 80 colostrum samples obtained from cows of six randomly selected herds among the 26 considered for the vaccination trials. In each of the selected herds an equal number of samples were taken from the vaccinated cows and from the control cows. Tests were conducted as previously described (3). In brief, the colostrum was centrifuged at 5,700 g for 30 minutes and the whey obtained from each sample was filtered through 0.45 μm (a.d.p.) acrodisc membrane filters (Gellman Sciences, Ann Arbor, Michigan). Serial 2-fold dilutions in minimum essential medium (MEM) (Microbiological Associates, Bethesda, MD) of each filtered whey sample were mixed with 100 $TCID_{50}$ of virus in 96-well microtiter plates. The plates were held for 90 minutes at room temperature (22°C) and then 20,000 MA-104 cells, suspended in MEM containing 0.5% fetal bovine serum and 5 μg/ml of trypsin, were added to each well in a volume of 0.05 ml. Titers were expressed as the highest dilution giving complete neutralization.

Virus isolation. - Attempts to recover rotavirus were made from 34 fecal swabbings obtained from diarrheic calves in 12 herds. Of these, 7 were

from calves born from vaccinated cows whereas the remainder 27 samples were collected from calves delivered by unvaccinated dams. For virus isolation MA-104 cell cultures were used as described elsewhere (2). When virus was isolated, its identity was determined by neutralization tests carried out with antiserum to 81/36F bovine rotavirus prepared in rabbit (2).

RESULTS

Cows. - The 458 cows constituting these trials, both the vaccinees (248 head) and those which were left as untreated controls (210 head), did not display any signs of disease during their pregnancy and all calved normally, giving birth to healthy calves. The only observation was the appearance in most of the vaccinated cows of a nodule at the inoculation site 24-48 hours after injection of the antigen conjugated with Freund's incomplete adjuvant. The nodules were variable in size (from 2 to 5 cm in diameter), warm, and painful to the touch. They persisted for 5-6 days and regressed without any residual alteration in the skin.

The whey prepared from the colostrum of cows from six randomly selected herds, had an average neutralizing titer to 81/36F bovine rotavirus of 1:458.50 or 1:48.75, respectively, for vaccinated cows or for untreated controls (Table 2).

Newborn calves. - As stated under « Materials and Methods », the newborn calves were fed from the 2nd day after birth, and daily for the next 7-10 days with colostrum collected from their dams. From Table 3 it can be seen that diarrhea occurred in 86 of the 210 (40.9%) calves which were delivered by unvaccinated cows (control calves) in 24 herds out the 26 selected for the trials. Diarrhea was also observed in 7 calves of the 248 (2.8%) born from vaccinated cows (immune calves). The latter were from 4 herds included in the 24 mentioned above. In the control calves diarrhea usually appeared within 24-72 hours from birth and was consistently associated to a marked dehydration which in 52 calves terminated with death 3 to 15 days after the onset of diarrhea. In the calves which survived, the diarrhea lasted from 10 to 15 days; however, the calves recovered very slowly and their general condition was still very poor several weeks after recovery.

The enteric syndrome which was observed in the 7 calves, which were born from vaccinated cows, was generally mild compared to that of the control calves. The diarrhea developed within 3 days from birth and lasted from 3-4 days. The diarrheic calves did not show any other sign of disease, and all recovered in about one week without any significant loss in their condition.

TABLE 4.
Isolation of rotavirus from diarrheic calves fed with colostrum from their vaccinated * or unvaccinated dams.

Herd No.	Isolation of rotavirus from feces of calves: Fed with immune colostrum^ — Fecal samples No. (Positive/examined°)	Fed with normal colostrum+ — Fecal samples No. (Positive/examined◇)
1	0/2	NT
3	NA	1/5
6	NA	2/3
11	0/2	1/2
14	NA	0/2
15	0/1	NT
17	NA	2/4
19	0/2	2/3
20	NA	2/3
21	NA	1/3
23	NA	0/1
26	NA	0/1
Total	0/7	11/27

* 81/36F bovine rotavirus, inactivated vaccine.
^ From vaccinated cows.
+ From unvaccinated cows.
° All fecal samples of diarrheic calves (7 samples) in this group have been available for testing.
◇ Among 86 diarrheic calves in this group, fecal samples of 27 of them have been available for testing.
NA = not applicable (no cases of diarrhea occurred).
NT = not tested.

The results of the attempts to recover virus from rectal swabbings of diarrheic calves are depicted in Table 4. Virus was not isolated from any of the 7 diarrheic calves born from vaccinated cows. By contrast, rotavirus was isolated from 11 of the 27 fecal swabbings that were collected from the control calves.

DISCUSSION

The results of these field trials show that by feeding newborn calves with colostrum of their dams previously vaccinated with an inactivated bovine rotavirus, we reduced significantly the incidence of neonatal diarrhea. This is substantiated by two main findings obtained in this study. First, the occurrence of diarrhea was observed in the 40.9% of the control calves, with the involvement of 24 herds of the 26 that were selected for the trials. By contrast, only 2.8% of the calves that were fed the immune colostrum, had clinical signs of the disease, and diarrheic calves were seen in 4 herds only. Second, the disease which was observed in the 'immune calves' was rather

mild and none died. In these calves the diarrhea was of a short duration (3-4 days), there was no significant loss of condition, and virus was not isolated from their feces. On the contrary, among the control calves, diarrhea lasted much longer (10-15 days) and 52 of the 86 affected calves died. Finally, among 27 fecal swabbings obtained from these calves, 11 were positive for rotavirus.

The results of this study seem to strengthen both the opinion that the rotaviruses have an important etiological role in the neonatal diarrhea of calves, and the suggestion that passive immunity would represent a logical way to prevent the disease (3, 8). As far as the latter aspect is concerned, the aim might be attained by the way indicated in these tests and also suggested by others (3, 7, 11), i.e.: a. vaccination of the dams with an inactivated vaccine and, b. feeding newborn calves throughout the period of greatest risk (the first 7-10 days of their life) with colostrum of their dams as part of the diet.

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