IMMUNIZATION AGAINST BOVINE ROTAVIRAL INFECTION

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Key words: Rotavirus - Immunization - Calves

Calves fed with colostrum from cows vaccinated with an inactivated rotavirus vaccine were refractory to experimental infection with strain 81/36F of bovine rotavirus.

In the field study, 458 pregnant cows from 26 herds were involved. In each herd, cows were selected and randomly subdivided in two groups. Cows in one group (248 head in total) were vaccinated, whereas 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 days, were each 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. 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 (8) there were increasing indications that rotaviruses play an important role in the causation of enteric conditions in young animals and man (7, 10). Attempts to control rotaviral infections in calves were made by vaccinating the newborn, since a tissue culture attenuated vaccine was available (9). However, the efficacy of this vaccine under field conditions was questionable (1, 6). The failure was generally ascribed to the maternally-derived antibody and/or to the fact that the infection in the calf occurs very early in the life, long before the eventual development of a protective immunity in the newborn. An alternative approach was to passively immunize the calf by stimulating the dam, through vaccination, to secrete antibody in the colostrum and milk (11) for a substantial period after calving.

This paper summarizes the results of our studies (2, 3, 4) in which the efficacy of colostrum from vaccinated cows in protecting calves against experimentally induced or naturally occurring rotavirus infection, was tested.

PROCEDURES

1. Protection of calves fed immune colostrum against experimentally induced rotavirus infection

Virus and vaccine preparation – Cytopathic strain
81/36F of bovine rotavirus (5), propagated in MA-104 cell cultures, was used at the 18th passage for vaccination of the cows and the related serologic test, and the 7th for the challenge exposure of calves. Titers of the virus were $10^4$ or $10^8$ median tissue cultures infectious doses (TCID$_{50}$/0.2 ml, respectively for the 18th and the 7th passage level.

For the preparation of the vaccine, cultures infected MA-104 were subjected to 1 freeze-thaw cycle when about 75% of the cells showed cytopathic effects (CPE). The infectious material was inactivated by overnight incubation at 4°C with 0.5% formaldehyde. One portion of the inactivated viral suspension was distributed in vials in volumes of 10 ml and stored at -30°C. The remainder of the suspension was centrifuged at 30,000 r.p.m. for two hours in a Spinco centrifuge, using R35 rotor. The pellet was resuspended in minimum essential medium (MEM) in 1/50th the original volume, and an equal volume of this viral material and incomplete Freund adjuvant was emulsified. The emulsion, in volumes of 2 ml, was drawn into disposable plastic syringes and stored at 4°C.

**Cows** - Of 23 pregnant Frisian cows, 14 were vaccinated with the rotavirus for the production of immune colostrum, whereas the nine served as unvaccinated controls for the normal colostrum. Vaccination was started 6 weeks before calving when each cow received 2 ml of the 50X emulsified vaccine subcutaneously in the dewlap. A second injection of 1X vaccine (10 ml) was given by the same procedure 2 weeks before calving. The cows were bled prior to vaccination and at calving time.

Colostrum from the first and second milkings after calving was obtained from vaccinated and non-vaccinated cows. Pools of either the immune or normal colostrum were made and stored at -30°C until fed to calves. In addition, samples of milk for serology were taken from the vaccinated cows 10 days after calving.

**Calves** - Twenty-six Frisian calves, from 8 to 12 hours old, born from rotavirus unvaccinated cows, were subdivided into 3 treatment groups: namely immune colostrum or group A with 10 calves; normal colostrum or group B with 8 calves and control or group C with 8 calves. The colostrum supplement was fed to the calves by substituting 200 ml of milk with 200 ml of appropriate colostrum pool, twice daily. Colostrum treatment of calves (groups A and B) was started 24 hours after their arrival at the Center, and was continued daily for the next 9 days. Soon after the second colostrum-supplemented meal, all calves, i.e., the colostrum treated calves and the controls, were inoculated with 81/36F bovine rotavirus, each calf receiving 30 ml of the culture orally. The calves remained on experiment for 30 days. During this time they were observed twice daily, with particular attention to the character of the feces. Rectal swabbing for the recovery of virus were taken from all the calves at the time of infection (time 0), and again on post infection days (PID) 2, 4, 8, 10 and 12. In addition, blood samples for serology were obtained from each calf on PID 30.

**Virus isolation** - Attempts to recover virus from rectal swabbings were made in MA-104 cell cultures as described previously (5). When virus was isolated, its identity was determined by neutralization tests carried out with antisera (5) specific for 81/36F bovine rotavirus.

**Serologic tests** - Tests for the presence of neutralizing antibody to 81/36F bovine rotavirus were carried out on sera obtained from the cows and the experimental calves, and also on colostrum and milk samples. The sera were inactivated at 56°C for 30 minutes, the colostrum and the milk were centrifuged at 5,700 g for 30 minutes, and the whey was collected from each sample. Serial two-fold dilutions in MEM of each serum or whey sample were mixed with 100 TCID$_{50}$ of virus in 96-well microtiter plates, using 1 well for each serum/whey mixture. The plates were held for 90 minutes at room temperature (22°C) and then 2X10$^4$ 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. Readings were made on the 3rd day, when the CPE were complete in the virus control cultures. The titer of each serum/whey sample was expressed as the highest dilution neutralizing the virus.

2. Protection of calves fed immune colostrum against naturally occurring rotavirus infection

**Herds** - The trials were conducted in 26 dairy herds with a history of neonatal diarrhea in the last five years. 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** - In each herd the cows which had been inseminated within a period of two months were selected for the trials. The selected cows in each herd were randomly subdivided into two groups. Cows in one group (248 head in total) were vaccinated according to the procedure mentioned above, whereas cows in the other group (210 head in total) served as unvaccinated controls.

A pool of colostrum from the first and second milkings after calving was obtained from each vaccinated or control cow. The pool was dispensed 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 7-10 days, each calf was fed a supplement of 400 ml of colostrum obtained from its dam. The calves were observed for 30 days for the appearance of diarrhea. When diarrhea occurred, fecal swabbing were taken and cultured for virus.

**Serologic tests** - Tests for the presence of neutralizing antibody to 81/36F bovine rotavirus were