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VACCINATION OF COWS WITH A COMBINED ROTAVIRUS/ ENTEROTOXIGENIC «E. COLI» K99 VACCINE TO PROTECT NEWBORN CALVES AGAINST DIARRHOEA

W. Eichhorn[1], P.A. Bachmann[1], G. Baljer[1], P. Plank[2] and P. Schneider[2]

ABSTRACT

The protective effect of a combined rotavirus/enterotoxigenic *E. coli* K99 vaccine against naturally occurring neonatal diarrhoea was evaluated in two dairy and two beef herds. Before vaccination, frequency of diarrhoea in these herds was 46% up to 60% with losses of 7% to 20%. After vaccination of pregnant cows and heifers mild diarrhoea occurred in 14% to 20% of their calves, one out of 144 calves died with enteric symptoms. In 13 out of 26 calves of nonvaccinated controls where diarrhoea was noted, one of them died. K99-positive enterotoxigenic *E. coli* were not detected in any of the faeces tested. Rotavirus was found in one of the beef herds.

Neonatal diarrhoea is still one of the most serious problems in calf-breeding, especially in its modern form with large numbers of animals. The economic losses due to diarrhoeal disease during the first two weeks of life are enormous. They result from the loss of calves, retarded growth rates and — last but not least — from the cost of the treatment of affected animals.

A number of infectious agents can be recovered from the faeces of such diseased calves, including viruses, e.g. rota-, corona- or parvoviruses, bacteria like enterotoxigenic *E. coli* and protozooa, e.g. cryptosporidia (8). Recent results indicate that mixed infections with two or more of these agents are the rule rather than the exception. Furthermore, it can be supposed that the severe forms of diarrhoeal disease seen in the field are always caused by mixed infections. Evidence from experimental infections confirms this hypothesis. The clinical course of the disease after mixed infection with rotavirus and enterotoxigenic *E. coli* or coronavirus is more severe and results in higher mortality rates than monoinfections usually do. For instance, it has been shown that colostrum-deprived calves survived an infection with 10^8 enterotoxigenic *E. coli* whereas after mixed infection with rotavirus and the same doses *E. coli*, all of the animals died (15). Further evidence comes from other investigations (21, 17) in colostrum-deprived and conventional calves. The results indicate that adhesion, colonization and growth of enterotoxigenic *E. coli* in the small intestine is enhanced by a preceeding or simultaneous infection with rotaviruses. Moreover it seems that such mixed infections cause severe disease even in older calves. Taken together, these data

indicate a probable synergism of the pathogens involved, although up to now a possible interference cannot be excluded. However, for the development of an effective immunoprophylaxis, it can be deduced that only combined vaccines containing two or more of the pathogens described will be successful.

Former recommendations for the prophylaxis of neonatal enteric disease included the improvement of sanitary conditions, feeding of preserved colostrum for at least one week and active local immunization or newborn calves (3). This strategy, however, proved to be unsatisfactory. Early publications on the effect of a commercially produced rotaviral vaccine seemed to be promising (19), but following studies could not confirm these results (1, 9, 12).

On the other hand, oral immunization against enterotoxigenic *E. coli* is reported to be very effective (7). One of the draw-backs of this kind of vaccine is its strict O-antigen specifity. Considering the broad spectrum of O-antigens of enterotoxigenic *E. coli*, it is obvious that a specific vaccine must be prepared for each herd.

Finally, it seems to be impossible to achieve any amelioration of the hygienic conditions in the majority of the farms and most of the farmers usually neglect the advice to preserve colostrum and feed it to the calves for at least one week.

Because of this failure of the hitherto recommended prophylactic measures, interest is now focussed on passive immunization of calves by vaccinations of their dams (3, 14, 16, 22).

We have developed a combined rotavirus/enterotoxigenic *E. coli* K99 vaccine. This report describes its effects in the field.

MATERIALS AND METHODS

1) *Vaccine*

a) Rotavirus

The bovine rotavirus isolate Munich V 1005/78 was grown in Ma 104 cells in the presence of trypsin (50 µg/ml) and EDTA (20 µg/ml) in roller bottles (4). When about 90% of the cell showed a cytopathic effect — usually after 24 hours — the virus was harvested by freezing, thawing and low speed centrifugation. The supernatants exhibited a $TCID_{50}$ of at least $10^{6.5}$ and in the rotavirus antigen ELISA titres between 1:32 and 1:64. They were mixed with 1% of a monospecific hyperimmune serum against bovine rotavirus and left at 4°C overnight. After this treatment no activity could be detected in the rotavirus antigen ELISA, whereas in the rotavirus antibody ELISA, titres between 1:16 and 1:32 were determined. In preceeding experiments we have shown that antibody complexed rotavirus together with adjuvants elicits higher antibody titres in the milk of vaccinated animals than uncomplexed rotavirus together with the same adjuvants (Figure 1).

b) E. coli

The *E. coli* K99 pilus-antigen was prepared according to a described method (18) with minor modifications. The *E. coli* strains 0 101 K99 was incubated for 16 hours on minca-plates, suspended in saline and homogenized. After high speed centrifugation (17,000 rpm, 30 min) merthiolate (0.01%) was added to the supernatant. The K99 antigen was titrated by hemagglutination with horse erythrocytes in the presence of 1% mannose at 4°C.

One vaccine dose, composed of 512 hemagglutinating units of the K99 antigen and 10 ml of the rotavirus antigen-antibody complexes, was mixed with 5 ml aluminium hydroxid — prediluted 1:8 — and left overnight at 4°C. Finally, 4 ml adjuvants PD (Philip Duphar, Amsterdam) were added and after vigorous shaking the vaccine was ready for use.

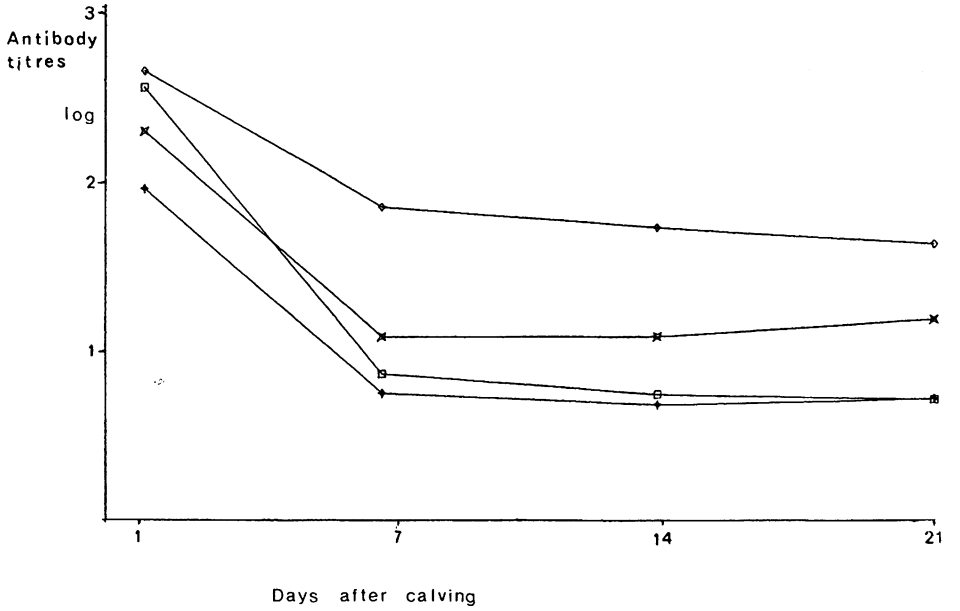

Fig. 1. Rotavirus antibody titres (as measured in the ELISA) in colostrum and milk from cows and heifers vaccinated with different preparations. □ Control animals (n = 10). + Adjuvants PD (n = 9). * Rotavirus and adjuvants (n = 3). ◊ Antibody complexed rotavirus and adjuvants (n = 23).

2) *Animals*

Pregnant cows and heifers — 35 in the two dairy herds and 135 in the two beef herds — were vaccinated twice. They were immunized first at the end of lactation, i.e. about six weeks before the calculated date of parturition and boostered as soon as possible after calving, i.e. in the first two days. The placebo was given to every fifth animal. Between 1979 and 1981 rotavirus and/or enterotoxigenic *E. coli* had been detected in all four herds.

3) *Isolation procedures*

Faecal material was collected from both healthy and diseased animals. The samples were tested for rotaviral antigen in the ELISA (2) and inoculated onto Ma 104 cells after treatment with acetylated trypsin as described by others (4, 23).

Hemagglutinating faecal samples were tested for the presence of bovine coronaviruses by hemagglutination-inhibition using specific antisera.

Bacteriological examinations were performed according to standard procedures. *E. coli* isolates with hemagglutinating activity in the presence of mannose were tested for K99 antigen (8) and enterotoxin in the baby mouse test (11).

RESULTS

Table I summarizes the clinical data from the dairy herds A and B in winter 1980/81 and in winter 1981/82 when the cows were immunized. Vaccination reduced the percentage of calves with diarrhoea from 50% up to 60% to 15.4% and 20%, respectively. In contrast, five out of seven control calves displayed

signs of disease. The one animal from herd B that died with enteric symptoms had been very weak from birth and had ingested only small amounts of colostrum and milk. The other animals — both vaccinates and controls — showed only soft faeces whereas severe forms of the disease with liquid faeces and dehydration were not seen.

Table I. Frequency of diarrhoea and deaths due to enteric disease in winter 1980/81 and 1981/82 in the dairy herds A and B

	Herd A			Herd B		
	Calves born	Diarrhoea	Calves died	Calves born	Diarrhoea	Calves died
1980/81	20	10 (50%)	4 (20%)	30	24 (60%)	4 (13%)
1981/82						
Vaccinates	13	2 (15.4%)	0	15	3 (20%)	1 (6.6%)
Controls	4	2 (50%)	0	3	2 (66%)	0

In none out of 39 faecal samples from herd A could rotavirus or rotavirus antigen be detected. Coronavirus was found for three days in the faeces of one affected animal. In herd B, however, four out of five diarrhoeic calves shed rotavirus in their faeces for up to four days as demonstrated by ELISA and virus isolation in cell culture. Except for the calf that died on day eleven, virus shedding was first seen on day six when the calves were no longer fed with the milk of their dams.

In both herds, hemagglutination *E. coli* were detected in about one half of the samples but none of the isolates possessed K99 antigen or enterotoxin. Shedding of *E. coli* did not correlate with the disease.

In the two beef herds C and D there also had been a high incidence of neonatal diarrhoea in the years before vaccination. During the winter of 1979/80 54% of the calves suffered from the disease and 7.2% died. In the winter of 1980/81 53% of the calves from herd C and 46% in herd D were affected with enteric disease and 7.7% respectively 4.9% of the calves were lost. Vaccination of the dams reduced the frequency of diarrhoea to 14% in herd C and 17.5% in herd D (Table II). All the calves from vaccinated mothers survived whereas one

Table II. Frequency of diarrhoea and deaths due to enteric disease during winter 1980/81 and 1981/82 in the beef herds C and D

	Herd C			Herd D		
	Calves born	Diarrhoea	Calves died	Calves born	Diarrhoea	Calves died
1980/81	78	41 (53%)	6 (7.7%)	61	28 (46%)	3 (4.9%)
1981/82						
Vaccinates	64	9 (14%)	0	52	9 (17.3%)	0
Controls	13	7 (53.8%)	1 (7.7%)	6	2 (33%)	0

of the control calves died. Although the occurrence of diarrhoea in the control group was much higher than in vaccinated animals, in both groups only a mild and transient diarrhoea, with the exception for the calf that died, was noted.

In none of the faecal samples — neither from vaccinated nor control animals — was rotavirus or coronavirus detected. *E. coli* with haemagglutinating activity were isolated from the faeces of both healthy and affected animals, but none of them possessed K99 antigen or enterotoxin.

DISCUSSION

The development of the combined rotavirus/*E. coli* K99 vaccine is based on two recent events: first there is a series of papers demonstrating the possibility of prolonging the secretion of rotavirus antibodies in the milk of cows by parenteral immunization (6, 14, 22). Secondly, it has been shown that colostrum from cows vaccinated with enterotoxigenic K99 positive *E. coli* protects newborn calves from experimental infection (6, 10, 20).

The protective effect of passive immunization against enteric disease by vaccination of the dams is strictly dependent upon the continuous feeding of milk from vaccinated animals for at least fourteen days. This is evident from the situation in herd B, where the calves were fed with their mothers' milk for five days only. After vaccination of the dams diarrhoea was still noted in 20% of the calves. In four out of five affected animals, shedding of rotavirus from day six onwards was demonstrated.

In contrast, in the dairy herd B, where the calves were fed with their mothers' milk for fourteen days, and in the two beef herds, where the calves suckled their dams for months, the incidence of diarrhoea was lower (between 15 and 17%) and none of the faecal samples contained rotaviruses. As coronaviruses were detected only in the faeces of one animal and the *E. coli* isolates possessed neither the K99 antigen nor enterotoxin, the possible importance of non-infectious factors as a cause of mild transient diarrhoea should be taken into account (13).

It is not surprising that in these herds neither rotaviruses nor enterotoxigenic *E. coli* could be recovered from the faeces of the control calves. In most of the herds affected with high frequency of neonatal diarrhoea, the severe forms of the disease are generally first seen some weeks after the calving period has begun. It can be supposed that during these weeks enteric pathogens are accumulating. If a certain threshold is exceeded the clinical course becomes severe. This accumulation of pathogens can be prevented by passive immunization. As a result, the so called controls will not be infected either. Therefore the data from such controls must be handled with care. In our opinion, passive immunization of calves against enteric disease by vaccination of their dams with combined vaccines is a reliable way to reduce the incidence of neonatal diarrhoea.

A larger field trial with a vaccine containing also corona- and parvovirus is currently in progress.

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DISCUSSION

KAPIKIAN: Did you compare the titers achieved with the antibody complexed rotavirus with a rotavirus preparation inactivated by conventional methods such as formalin or heating?

By using «inactivated» rotavirus antigen as you have, to induce rotavirus antibody in cows, is of particular interest in view of a recent report by Myers & Snodgrass in which they evaluated the ability of a US approved live, modified bovine (NCDV) rotavirus vaccine to induce colostral or milk antibodies in pregnant cows. This vaccine was initially approved for oral administration to calves, later also approved as a combined vaccine with live, modified bovine coronavirus for oral administration to calves and more recently also approved for administration by the IM route to the pregnant cow. The authors reported that a comparison of GMT in colostrum and 3 day milk in vaccinated and non-vaccinated cows were not significantly different. They recommended the use of inactivated antigen for stimulating colostral or milk antibody in the cow for several reasons including the possible inhibition of viral replication by naturally occurring antibody in the cow.

EICHHORN: It does not make any difference how you inactivate the virus; when you complex the virus it elicits higher titers than non-complexed virus. In our next field trial we shall use inactivated, antibody complexed virus.

ALLEN: What kind of adjuvant did you use?

EICHHORN: We used aluminium hydoxide and a polymer adjuvant. No side reactions were seen in any of the animals.

RIJKE: Do you have an explanation for the immunogenicity of your rotavirus antibody complexes?

In EM we have looked at these complexes, they look like big aggregates. That could specifically stimulate the immune response as has been described in other animals.

EICHHORN: When you look at the antibody complexed virus in the EM you see large clusters of virus, which are possibly more immunogenic than non-complexed virus. It is not known whether there are other immunologic mechanisms in cattle as described for laboratory animals.