

*Institute of Virology*  
*Director: Prof. Dr. Dr. h. c. F. Bürki*  
*and University Field Station*  
*Director: Dr. H. Szekeley*  
*of the Veterinary University, A-1030 Vienna, Austria*

## **Reduction of Rotavirus-, Coronavirus- and *E. coli*-Associated Calf-Diarrheas in a Large-Size Dairy Herd by Means of Dam Vaccination with a Triple-Vaccine\***

F. BÜRKI, K. MÖSTL, E. SPIEGL, E. HORVATH and H. SZEKELY

Address of authors: Prof. Dr. F. BÜRKI, Institute of Virology,  
Veterinary University Vienna, Linke Bahngasse 11, A-1030 Vienna, Austria

*With 2 figures and 5 tables*

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### **Summary**

Enzootic diarrhea of calves has formed a serious problem and caused losses for years in the dairy herd kept at Vienna Veterinary University's Field Station. In previous investigations bovine coronavirus had been determined as the most pathogenic enteric agent, bovine rotavirus as secondary causal virus, whereas enteropathogenic *E. coli* and Cryptosporidia were present but insignificant causally. Two earlier systematic immunoprophylactic trials to reduce the incidence and severity of diarrheas of newborn calves failed. Vaccinating pregnant dams twice with a trivalent commercial vaccine containing live attenuated rotavirus and coronavirus plus K 99 + antigen of *E. coli* (Scourguard 3 [R]) mediated most remarkable results. These were recorded clinically and were assessed by performing daily ELISAs on calf fecal samples up to day 14 after birth for both rotavirus and coronavirus antigens.

Shedding of enteropathogenic *E. coli* and of cryptosporidia were also controlled. Furthermore, antibody titres against rotavirus and coronavirus were determined in cows' blood serum, colostrum and whole milk as well as in calf sera, including control data from unvaccinated cows and their offspring. In addition, a number of calves originating from vaccinated or unvaccinated dams were perorally challenged with rotavirus and coronavirus, again daily screening their fecal samples for virus-shedding by ELISA. Very remarkable protective effects of dam vaccination were found clinically as well as regarding the number and shortened duration of calf shedding of rotavirus. None of 242 fecal samples contained coronavirus and none of 80 contained enteropathogenic *E. coli*. It is emphasized that calves must be fed milk of their vaccinated dams for a full 14 days after birth, so as to extend the continuous lactogenic immunity over the period of maximal susceptibility to the 3 enteropathogenic agents incorporated in the vaccine.

**Key words:** Coronavirus, Rotavirus, Calf-diarrhea, Vaccination

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## Introduction

Diarrheas belong to the most-feared diseases encountered in newborn calves. Enteropathogenic *E. coli* have been known for decades as potential etiology. Some 15 years ago bovine rotavirus and coronavirus were detected, and their pathogenicity was established in subsequent years. Thereafter, several approaches were undertaken to mediate immunoprotection against these enteropathogenic agents. As outlined in the discussion, peroral vaccination of newborn calves or cofeeding of first-day colostrum of unvaccinated cows failed under field conditions. Equivocal results have been published about the effect of dam vaccination with multicomponent vaccines, performed with the intention of stimulating as well as prolonging lactogenic immunity of their calves.

This report gives virological, serological, and clinical data established with dam vaccination in a large dairy herd, using a triple-vaccine, containing live rotavirus, live coronavirus, and inactivated K 99 + *E. coli*. Our investigation differs from others published in that it was performed in a herd with enzootic calf diarrhoea, whose pluricausal etiology had been followed for years, and in which two former immunoprotective trials had failed. Furthermore, to the best of our knowledge, it is the first large-size investigation, in which daily examination by rotavirus-ELISA and coronavirus-ELISA till the 14th day of life of each calf was performed as additional parameters to evaluate the effect of dam vaccination. The beneficial effect, which became apparent clinically, was impressively supported by our virological and bacteriological findings.

In order to further assess potency of lactogenic immunity, a number of calves of vaccinated dams, together with control calves of unvaccinated mothers, were challenged with rotavirus and coronavirus field isolates. Again, daily fecal ELISAs were performed to support clinical findings. These challenge-tests gave only limited information since, due to the small numbers of control calves available, we were unable to fully evaluate the test virus suspensions used.

The Discussion deals with the question of how far the favourable results obtained in our particular herd may have general application and to what extent vaccination of dams could be further improved.

## Material and Methods

### *Vaccine and vaccination*

Scourguard 3 (R) of Smith Kline was used, containing live attenuated bovine rotavirus and coronavirus in lyophilized form, which are dissolved in 2 ml of inactivated *E. coli* K 99 + suspension immediately before use. The suspension contains aluminum hydroxide as adjuvant, and was injected twice in 2 ml quantities intramuscularly, 6—8 and 2—3 weeks before calving.

### *Animals*

Thirty cows of the Brown Swiss herd of the University Field Station, which contained 220 cattle in total, and their calves were used in the 1983/84 calving season. At the beginning, all pregnant cows were vaccinated (26), so as to suppress potential excessive virus excretion by calves of unvaccinated dams. During the last third of the experiment, every second cow only was vaccinated, so as to provide control calves of matched ages for the challenge infections.

Every calf included in the experiment was born at full term, healthy, and supplied exclusively with colostrum and milk respectively of its mother for the whole observation period lasting 14 days. Calves were housed singly or as pairs. For challenge infection they were moved to isolation boxes.

### *Evaluation of vaccination success*

A careful clinical record of calves' health, weight gain and consistency of fecal matter was conducted (24). Feces were examined daily for rotavirus excretion by Rotazyme (R) — ELISA of Abbott and for coronavirus by coronavirus-ELISA according to ELLENS et al. (8), sporadically also for enteropathogenic *E. coli* (80 samples in all) as well as for Cryptosporidia.

To procure serological data, blood and colostrum/milk specimens were taken from cows and their calves at times recorded in Table 4. Antibody contents were determined by the seroneutralisation test, using the Compton strain of bovine rotavirus, and by the HI-test (27), using the L 9 — strain of

bovine coronavirus. The rotavirus was propagated on monkey MA-104 cells, the coronavirus on fetal calf thyroid cells.

Milk samples were first defatted by centrifugation for 15 minutes followed by 2 hours centrifugation at 50,000 g. Blood sera as well as milk were inactivated for 30 minutes at 56°C and stored at -20°C. All titres obtained were computed to GMT's and are shown in Table 4.

#### *Challenge infections*

Six calves of vaccinated and 4 of unvaccinated cows were infected perorally in groups when 2 to 17 days old with virulent rotavirus and 3 days later with coronavirus. Both virus suspensions were kindly supplied by Prof. Dr. H. WETZEL of Smith Kline/München. Bovine rotavirus contained 7.5 log<sub>10</sub> TCD<sub>50</sub> of strain "Italy" 81/36 which had caused anorexia, diarrhea and dehydration in newborn calves (5). It was kept frozen at -20°C until used, when it was rapidly thawed at 37°C.

Bovine coronavirus used for test infection was strain "Holland" EV 800 SP and was transported on dry ice, stored at -70°C and thawed immediately before use. In our laboratory it contained 5120 rat cell hemagglutinating units per ml and its viability was secured by its induction of immunofluorescence in calf thyroid cells.

To prevent neutralization of challenge viruses by ingested maternal antibodies present in the calves' daily milk supply, on every challenge day the milk was entirely replaced by GGES (Glucose / Glycine / Electrolyte Solution) according to BYWATER and WOODE [4]).

For challenge, initially a dose of 2 ml of each test virus suspension, spaced 3 days apart, was given and mother's milk was fed thereafter. Challenge conditions were gradually rendered more severe when it became apparent that spurious or no viral "takes" occurred under the conditions we had started with. Each virus inoculum was then raised 5-fold to 10 ml, and in control calves the normal mother's milk was replaced by pooled boiled milk, so as to eliminate even small amounts of maternal antibodies, known to be present in all animals from previous work on these premises (14). Lastly, additional dietary stress (3) was induced by pre- and postfeeding control calves with milk replacer.

The exact virus dosage and diet of each test calf are shown in Fig. 2.

## Results

### *Unchallenged calves*

#### *Clinical observations*

As shown in Table 1 diarrhea was rarely observed. It became manifest in 6 only of the 22 calves (27.3 %, Table 3) of vaccinated dams, was present only between days 6 and 12, and was of short duration. Fig. 1 shows that, when it did occur, diarrhea was mostly of the severe watery form. We deem it most remarkable that of 242 fecal samples only 11 (4.5 %) were judged as diarrhoic (Table 3).

Table 1  
Daily incidence of diarrhea of 22 calves of vaccinated cows

day	pulpy feces	watery feces	total of diarrhoic feces	percentage of diarrhoic feces
4	0	0	0	0
5	0	0	0	0
6	1	0	1	4.5
7	0	1	1	4.5
8	0	2	2	9.1
9	0	1	1	4.5
10	0	1	1	4.5
11	1	2	3	13.6
12	1	1	2	9.1
13	0	0	0	0
14	0	0	0	0
	3	8	11	

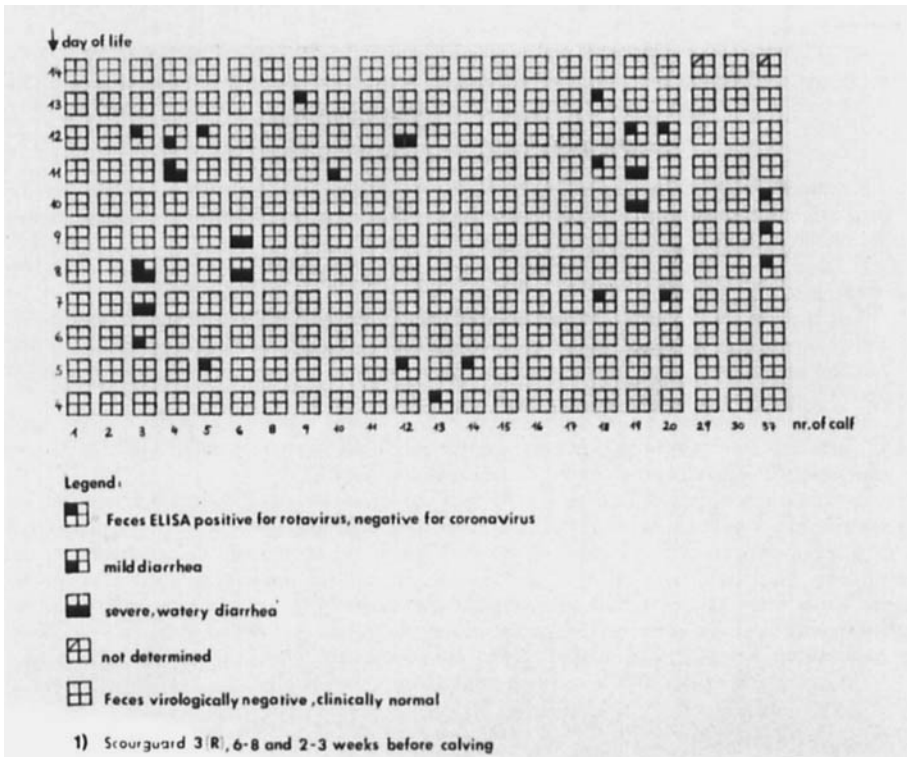


Fig. 1. 22 calves born to 2 × immunized cows.  
Correlation of rotavirus- and coronavirus-excretion with diarrhea on days 4 to 14 after birth

As the control calves of this calving season were invariably used for challenge experiments (pre-tests and main tests), no data from them are available for direct comparison and so, we evaluate below the clinical (and virological) benefit of dam vaccination by comparing Table 5 with data from the same premises and the previous winter, when no active immunization was practised.

Replacement of milk by GGES for a day or two sufficed to stop diarrhea in the 6 calves which developed it; in no instance was antibiotic treatment necessary. Sixteen calves (72.7%) remained healthy during their first 14 days of life (Fig. 1). The average weight gain per calf from day 4 to 14 was 7.7 kg, whereas it was only 4.9 kg in the previous calving season (13).

#### *Fecal virus excretion*

**Rotavirus:** Systematic daily ELISA testing revealed, that 11 of 22 calves of vaccinated mothers (50%) excreted rotavirus, but only for very short periods, accounting for a mere 7.5% (18 out of 240 samples tested) (Table 3). As Table 2 shows, rotavirus shedding occurred 8 times more frequently in clinically silent than in clinically manifest form. This shows that, as a rule, lactogenic immunity was able to keep the widespread rotavirus infection under control, contrary to earlier observations on the same premises (14, 20).

**Coronavirus:** As Fig. 1 and Table 3 show, coronavirus was not found in any of 240 fecal samples from calves of vaccinated dams. This remarkable suppression of coronavirus, which in the previous calving season had accounted for the most severe diarrheas (14), will be further discussed in the Discussion.

*Table 2*  
Daily percentage correlation of diarrhea and rotavirus-ELISA in 22 calves of vaccinated mothers

day	diarrhea +	ELISA +	diarrhea -	ELISA +	diarrhea +	ELISA -
4		0		4.5		0
5		0		13.6		0
6		0		0		4.5
7		0		9.1		4.5
8		4.5		4.5		4.5
9		0		4.5		4.5
10		0		4.5		4.5
11		4.5		4.5		9.1
12		0		18.2		9.1
13		0		9.1		0
14		0		0		0
percentages summarized		9.0		72.5		40.7

*Table 3*  
Incidence of diarrhea and of excretion of rotavirus and coronavirus in 22 calves of vaccinated dams

	diarrhea	rotavirus excretion	coronavirus excretion
calves	6/22 = 27.3 %	11/22 = 50.0 %	0/22 = 0 %
fecal specimens	11/242 = 4.5 %	18/240 = 7.5 %	0/242 = 0 %

#### *Antiviral antibodies*

Titres against rotavirus as well as coronavirus showed wide individual variation in the cow sera, colostrum and milk, as well as in calf sera. The GMT's, computed separately for vaccinated and unvaccinated cows and for the respective groups of calves, are given in Table 4. Little or no differences of GMT's were seen between the respective groups.

#### *Search for other potential enteropathogenic agents*

This search was restricted to occasional testing of calf feces. Most remarkable was the finding, that not one of 80 fecal samples tested contained enteropathogenic *E. coli*, contrary to the previous winter when no vaccination had been performed (see Discussion). Likewise, none of the 10 challenged calves excreted enteropathogenic *E. coli*.

Large numbers of *Clostridium fesceri* were found in fecal samples of 3 calves, one being diarrhoic, the other two asymptomatic.

No pathogenic agent was found in calves 6 and 12 when these experienced short spells of severe diarrhea.

#### *Effectiveness of dam vaccination in comparison with diarrhea and virus excretion scores of the previous winter*

As all calves stemming from unvaccinated mothers of the 1983/84 calving season were used for experimental challenge infections, no direct evaluation of the success of dam vaccination was possible in this season. But in Table 5 diarrhea scores and virus excretions are plotted for two successive winters on the same premises with the same control procedures.

Table 4  
GMT — Titres of cows and calves against rotavirus and coronavirus

specimens	rotavirus		coronavirus	
	vaccinated cows	unvaccinated controls	vaccinated cows	unvaccinated controls
cow sera before vaccination	/	158.0	/	67.9
cow sera 4-6 days after calving	134.0	139.5	121.5	45.2
first-day colostrum sera	870.0	512.0	1123.0	860.5
10 days' milk sera	5.5	5.3	2.4	2.8
calf sera 4-6 days after birth	192.9	228.0	144.7	161.2
calf sera 21-28 days after birth	131.3	101.5	84.8	71.7

Table 5  
Incidence of diarrhea and virus excretion in calves reared in two successive calving seasons, but from unvaccinated or vaccinated dams, respectively

	dams unvaccinated winter 1982/83		dams vaccinated 2 × winter 1983/84
	calves daily coted with first-day colostrum till day 13	calves fed mother's milk for 7 d, then milk-replacer (= normal diet)	calves fed mother's milk for 14 d
No. of calves developing diarrhea	20/22 = 90.9 %	8/8 = 100 %	6/22 = 27.3 %
Days with diarrhea per calf group	90/214 = 42.1 %	36/80 = 45 %	11/242 = 4.5 %
No. of calves excreting rotavirus	16/22 = 72.7 %	6/8 = 75 %	11/22 = 50 %
Days with rotavirus-excretion per calf group	54/212 = 25.5 %	20/76 = 26.3 %	18/240 = 7.5 %
No. of calves excreting coronavirus	9/22 = 40.9 %	3/8 = 37.5 %	0/22 = 0 %
Days with coronavirus-excretion per calf group	30/219 = 13.7 %	8/77 = 10.4 %	0/242 = 0 %

In the winter of 1982/83 no dam vaccination was practised, but 22 calves were coted first-day colostrum (determined to be relatively rich in antiviral antibodies) for 13 days. Eight calves were given the normal diet and served as controls. Diarrhea occurred in dramatic form, predominantly caused by enteric coronavirus infections (14). Data of this are given in Table 5. In addition, enteropathogenic *E. coli* were found in 13 of 64 (20.3 %) fecal specimens but these bacteriological findings are not included in Table 5, as no pathogenic activity could be attributed to the infection.

Comparison of these data shows that dam vaccination in 1983/84 induced a dramatic improvement. Most impressive (Table 5) was the total disappearance of enteric coronavirus infection; likewise, in none of 80 fecal samples tested could enteropathogenic *E. coli* be found. Rotavirus infection was greatly reduced, regarding both the number of affected calves and the shedding periods.

Clinical findings clearly ran parallel with microbiological findings. Diarrhea cases were much reduced (3.4 fold) in number and in affected days (9.5 fold). Furthermore, no antibiotics had to be given and no calf was lost among the offspring of vaccinated dams, whereas in the previous calving season, when no vaccination was performed, intensive dietetic and antibiotic treatment was necessary, despite which 2 diarrhoic calves died (14).

#### *Rotavirus and coronavirus challenges*

Pretesting in control calves of challenge inocula available was limited by the fact that few calves were available for the purpose. This forced us to perform test infections without

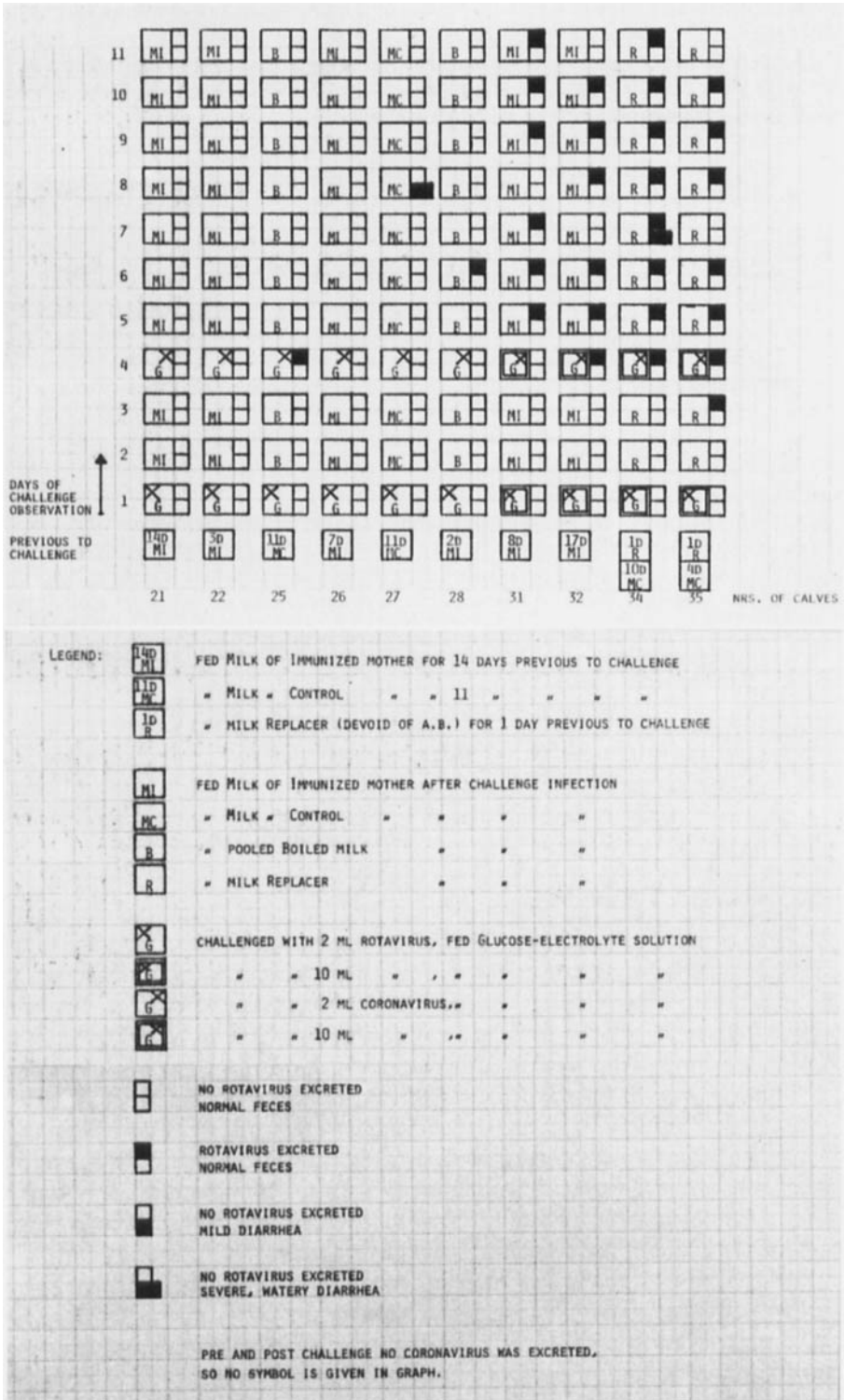


Fig.2. 10 Calves perorally challenged for immunity with rotavirus plus coronavirus. Feeding regimen, diarrhea, virus excretion in feces

knowing the ideal dosage of each virus used. By rapidly examining with the ELISA-test fecal samples of calves involved in the protection tests we were, however, able to make more severe the test conditions from one group of calves to the next by raising by a factor of 5 each virus dose as well as subjecting the control calves to dietary stress. Details are given under "Materials and Methods" and in Fig. 2.

As Fig. 2 shows, no test condition chosen turned out to be of ideal severity, and so all challenges performed are evaluated in summarized form only.

Rotavirus given at low dosage was only excreted by two of 6 calves, and for a single day only. Both these calves were post-fed with boiled milk, and thus received an antibody-free diet.

Rotavirus at high dosage gave long-lasting "takes" in all 4 calves subjected to it (Fig. 2), but according to a gradual excretion index computed with the Rotazyme (R) chart, lactogenic immunity depressed this severe challenge to values of 3 and 9, respectively, in calves 31 and 32, both fed milk of vaccinated dams. On the other hand, excretion indices were 23 in calf 34 and 16 in calf 35, which calves were from unvaccinated cows and had been fed antibody-free milk replacer. These indices show that lactogenic immunity was capable of modifying challenge infection in both calves of vaccinated cows.

In no calf could a "take" of the coronavirus challenge be demonstrated. Previous to its application the inoculum was found ELISA positive and hemagglutinated rat erythrocytes to high titre; later it was successfully checked for viability by immunofluorescence. We are unable to decide whether failure of "takes" was due to low virulence or to insufficient dosage. As Fig. 2 shows, there was rarely any clinical response to these challenge experiments. Although rotavirus excretion was demonstrated in 6 calves on a total of 29 days, only control calf 34 experienced on one day a severe watery diarrhea and this lasted only for one day. The etiology of diarrhea of control calf 27, also of one day's duration, remained undisclosed.

### Discussion

In comparison with the high prevalence and severity of diarrhea, as well as the appearance and duration of virus shedding observed in former years in this same herd, dam vaccination was followed by an impressive reduction of all the parameters of enteric infections in newborn calves. Was this improvement a chance observation? If not, to what extent was it attributable to the vaccination of the dams?

Calf diarrhea had occurred for years in enzootic form on these premises and had accounted at times for severe losses. Originally, our etiological search concentrated on rotavirus, which was shed with high frequency. The impact of lactogenic immunity on rotavirus shedding had already become apparent in our first systematic investigation, when no dam vaccination was performed. At that time the exclusively infection-induced maternal antibodies were able to reduce to a significant degree the excretion of fecal rotavirus in calves during their first 7 days of life, during which time they were fed exclusively their dams' colostrum. On days 8 to 14, when milk replacer, devoid of antibodies, was fed, rotavirus excretion levels rose significantly (20).

Before practising dam vaccination, we found on these same premises that maternal antibodies against coronavirus, although induced by previous infection and present in all cows, did not suffice to prevent severe enteric coronavirus infections in their calves (14). In fact, not even daily feeding of 200 ml of individual first-day colostrum up to day 13 was able to protect these calves against severe diarrhea (13). A careful systematic investigation disclosed that a remarkable correlation existed between the excretion of coronavirus and diarrhea, whereas only a loose correlation between rotavirus excretion and diarrhea, and no correlation at all between successful isolation of enteropathogenic *E. coli* and diarrhea in calves of these premises (14).

Two earlier efforts to reduce the incidence of virus-associated diarrhea in calves born on these premises and losses resulting therefrom had failed. Specifically, peroral live



rotavirus/coronavirus vaccination of newborn calves gave no protection, although we took great care to prevent immediate neutralization of the viruses by lactogenic immunity in the calves' gut (3). Also, feeding first-day colostrum of unvaccinated mothers, partly preserved in frozen form and partly with citric acid, had been unable to prevent severe diarrhea and viral shedding (13, 14). The data for this latter trial are included in Table 5 for comparison.

We refer to these earlier investigations for several reasons: Firstly to show that we had acquired a thorough knowledge of the incidence and etiology of diarrhea on these premises over a period of 4 years before starting dam vaccination. Secondly, to recall that two other systematic trials to reduce diarrheas in this particular herd had failed. Thirdly, to substantiate that dam vaccination with Scourguard 3 (R) as practised in this calving season, almost certainly accounts for the beneficial effects which were recorded. Statistically, we cannot prove this in the actual calving season, since all the control calves of unvaccinated dams were used for experimental test infections. The scientific proof has two flaws: maternal antibodies were not significantly raised in vaccinated dams and their offspring (Table 4) on one hand and challenge infections performed suffered from unavailability of enough control calves to ideally assess the two test virus suspensions.

With our failure to significantly raise preexisting serum and milk antibodies (originating from previous infection by rotavirus and coronavirus), we have confirmed earlier findings of others who used live-viruses for dam vaccination (15, 18, 19). Still other authors have shown, that oil-adjuvant (21) or complexing of virus with homologous antibody (7) is required to significantly stimulate preexisting antibody levels.

How then can the beneficial effects (both at clinical and microbiological levels) obtained in our present investigation be explained despite the fact that, according to Table 4, vaccination had little impact on antiviral titres?

We draw attention to three parameters: duration of feeding the dams' milk, quality of antibody class spectrum, and cellular immunity.

It is certain that prolongation of feeding dams' milk from 7 days in former years to 14 days now had a profound impact on colonization of a calf's gut by viruses. We have referred already in this paper to our original observation that stopping dams' milk after 7 days resulted in a significant flare-up of rotavirus shedding (20). This shows that, although anti-viral titres fall to low levels after the 3rd day (9, 21) extending the feeding-period of the milk prolongs lactogenic immunity. On the other hand, shortening the feeding of mothers' milk logically shortens the period of lactogenic protection. A large-size field trial published recently by Canadian authors (28) shows this impressively. These authors used the same triple-vaccine as we did in the present investigation, but withheld from the calves the protective antibodies stimulated in their dams 3 to 4 days after birth. It is not surprising that they judged as useless a vaccine which did so well in our hands.

By withholding the milk of immunized dams after the 3rd or 4th day of the calves' life lactogenic immunity is abolished when it would be most needed. Since meconium never contains rotavirus (6) and, since rotavirus as well as coronavirus-induced diarrheas started at or after the 4th day of life in our experience (3, 14), it is imperative to supply dams' milk up to the 14th day of life.

Immunoglobulin classes may be involved in the high protection rate observed in our investigation and is now under study (MÖSTL and GMEINER, unpublished results). Specifically, we have the IgA class in mind, which has only recently been shown as linked to fat globules in bovine milk (11). We postulate that vaccination of previously infected cows might have stimulated gut-active antibody secretion, similar to the observations made with Transmissible Gastroenteritis in pigs (2) and with *Vibrio cholerae* infection in man (25). Whether increased number of T-cells are formed in vaccinated cows, whose udders harbor such carriers of cellular immunity, homed-in after previous gut infection by virulent rotavirus and coronavirus, is not known. Current knowledge about cellular immunity in milk is scarce (16).

That our favourable results obtained by dam vaccination were not a mere chance observation is supported by the results obtained by other authors, who used the same (10),

or similar (1, 12) vaccines and fed milk of immunized dams for 14 days, as we did. The favourable results allow the conclusion that the strains included in present-day multicomponent vaccines are of correct antigenic composition. According to a very comprehensive survey made in USA 89% of bovine rotavirus isolates belong to bovine serotype I (29), which is included in Scourguard 3 (R) vaccine. Recently two other important discoveries were made in Scotland. First, there also the vast majority of field isolates (91%) belonged to bovine rotavirus serotype I. Second, monotypic vaccination boosted titres against all serotypes to which these cows had already been reactive. Both observations made by SNODGRASS *et al.* (23) conclude that single-type vaccination suffices to immunoprotect against all bovine rotaviruses.

Bovine coronavirus has recently been shown to be monotypic (17) and in a limited survey, enabled by the kind provision of anti-Breda 1 serum by Dr. G. N. WOODÉ, Ames/Iowa, our laboratory has not been able to prove the presence of this corona-like-virus in Austria (30).

On our University Field Station, whose microbial flora has been surveyed for many years (see 14), enteropathogenic *E. coli* and *Cryptosporidia* played no role in the etiology of calf diarrhea. H. WILLINGER (pers. comm. 1984) confirms, that enteropathogenic *E. coli* are encountered rarely with calf scours in Austria. It appears that this is a phenomenon linked with the small herd size prevalent in Austria (6 cows on the average), because in neighbouring Hungary, where large State Farms with 4000 cows are in operation, enteritis and death are frequently caused by enteropathogenic *E. coli* (B. NAGY, pers. comm. 1985). In consequence, the Scourguard 3 (R) vaccine used for dam vaccination is of adequate composition. Veterinarians must instruct farmers to feed milk of vaccinated cows for a full 2 weeks in order to provide their calves with optimal lactogenic immunity during their period of highest susceptibility to enteropathogenic agents. We trust that the use of a more potent adjuvant (1, 7, 22) would enhance the lactogenic immunity of calves of vaccinated dams.

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### Zusammenfassung

#### Reduktion der mit Rotavirus, Coronavirus und *E. coli* assoziierten Durchfälle neugeborener Kälber in einem großen Milchviehbestand durch Mutterkuh-Impfungen mittels eines trivalenten Impfstoffes

Enzootische Kälberdurchfälle bildeten seit Jahren ein ernsthaftes Problem und verursachten Verluste in der Rinderherde des Lehr- und Forschungsgutes der Veterinärmedizinischen Universität Wien. In früheren Erhebungen hatten wir ermittelt, daß bovines Coronavirus das Agens von höchster Pathogenität darstellte, daß bovines Rotavirus causal als zweitwichtigster Erreger fungierte, wogegen enteropathogene *E. coli* und *Cryptosporidien* wohl vorhanden aber ohne ursächliche Bedeutung für das Durchfallgeschehen waren. Zwei frühere systematische immunoprophylaktische Versuche, Häufigkeit wie Schweregrad der Durchfälle neugeborener Kälber zu reduzieren, hatten ihr Ziel nicht erreicht. Demgegenüber erbrachte nunmehr die zweimalige Schutzimpfung trächtiger Kühe mittels eines trivalenten Markimpfstoffs, welcher lebende attenuierte Rotaviren und Coronaviren plus K 99 + Antigen von *E. coli* enthält (Scourguard 3 [R]), sehr bemerkenswerte Ergebnisse. Diese wurden zum einen klinisch ermittelt, außerdem aber und wichtiger, durch Vornahme täglicher ELISAs auf Rotaviren wie Coronaviren an Kotproben bis zum 14. Lebenstag. Ferner wurde in Intervallen auf Ausscheidung enteropathogener *E. coli* und gelegentlich von *Cryptosporidien* untersucht. Weiter wurden Antikörpertiter gegen Rotaviren und Coronaviren im Blutserum, Kolostrum und reifer Milch geimpfter Kühe und dem Blutserum ihrer Kälber bestimmt. Kontrollwerte wurden an ungeimpften Mutterkühen und ihren Kälbern ermittelt. Zusätzlich wurden eine Anzahl Kälber geimpfter wie

andererseits ungeimpfter Kühe peroraler Testinfektion durch Rotavirus und Coronavirus unterworfen, wobei wiederum täglich Kotproben mittels ELISA auf Virusausscheidung verfolgt wurden. Es wurden sehr beachtenswerte Schutzwerte der Mutterkuhimpfungen in klinischer Hinsicht wie bezüglich Anzahl und verkürzter Dauer der Rotavirusausscheidung ermittelt. Coronaviren und enteropathogene *E. coli* verschwanden sogar zur Gänze, erstes an 242 insgesamt negativen, letzteres an 80 in toto negativen Kotproben beurteilt. Es wird betont, daß Kälbern geimpfter Kühe während voller 14 Tage deren Milch verabreicht werden muß, um ihnen während ihrer ganzen höchst empfänglichen Lebensphase kontinuierliche laktogene Immunität gegen die 3 in der Marktvakzine enthaltenen enteropathogenen Infektionserreger zu verleihen.

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