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Incidence of diarrhoea and of rotavirus- and coronavirus-shedding in calves, whose dams had been vaccinated with an experimental oil-adjuvanted vaccine containing bovine rotavirus and bovine coronavirus

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Summary

Dam-vaccination with an experimental oil-adjuvanted vaccine containing inactivated bovine rotavirus and coronavirus was practised. Fifty pregnant cows of a consistently field-infected Brown Swiss breeding herd were partly vaccinated, partly left as unvaccinated controls during one breeding season. The antibody titres of blood sera of all cows were determined previous to and following vaccination, as well as following parturition. Also, antibodies were determined in first-day colostrum and later milk of cows, as well as in sera of calves when 4 to 6 days old. Calves were exclusively fed colostrum and milk of their proper dams during their first 2 weeks of life. In the same period 35 calves were examined for development of diarrhoea and excretion of rotavirus and/or coronavirus when kept under natural holding conditions. Sixteen other calves were perorally test-infected with virulent rotavirus, 3 days later additionally with virulent coronavirus, and thereafter controlled in the same manner.

The experimental vaccine boosted preexisting antibody titres (present in all cows as a result of former field infections by these 2 viruses), but merely at an insufficient degree. Geometric mean titres in colostrum of vaccinated cows were less boosted than expected from the literature and titres of milk were remarkably low. Nevertheless, immediately following parturition even severe experimental test-infections were kept under control by colostral antibodies. So were the less severe natural field infections for the first week of life of calves. In their second week of life, however, lactogenic immunity was of insufficient potency to consistently protect calves kept under natural conditions against the degree of rotavirus and coronavirus exposure actually present on these premises.

Key words: Rotavirus, coronavirus, calf, diarrhoea, dam-vaccination

Introduction

Severe outbreaks of diarrhoea among calves of the Vienna Veterinary University's Field Station had been observed for years, rota- and coronaviruses being the dominant causative agents (11, 15, 16). These studies had disclosed that the greatest infection with both viruses occurred during the first and the second weeks of life of newborn calves.

Several reports in the literature document that cow herds in different countries possess antibodies resulting from previous infections by either or both of these viruses (for literature see 3). Already, however, it has been shown that passively acquired maternal antibodies, once they have been resorbed into the blood stream, are unable to exert a protective effect in newborn calves (10, 19). SNODGRASS and WELLS (17), later followed by several other authors, demonstrated that, on the other hand, rota- and coronavirus infections were efficiently influenced by lactogenic antibodies, i. e. maternal antibodies present inside the intestinal tract of newborn calves.

In order for lactogenic immunity to be effective, maternal antibodies
– must be present in a high percentage of cows of an infected herd;
– must be contained in sufficiently large quantities in cows' colostrum and milk; and
– must be given daily over the entire period in which the calves show the highest susceptibility to enteric infections by these two viral agents (3).

In breeding herds, in which these enteric viruses are established, most of the cows secrete specific antibodies in their colostrum; however, their titres vary widely and they decline to very low values within 2 to 4 days after parturition (7, 13, 19). Under natural holding conditions, i. e. without specific vaccinations, maternal antibody levels are not high enough to mediate lactogenic protection to their offspring for the most susceptible period of 2 weeks after birth. Therefore, scientists as well as the pharmaceutical industry have made attempts to boost and prolong lacteal antibody secretion with vaccines of different composition (for literature see 3). During one calving season Scourguard 3 (R) which contains live attenuated bovine rotavirus and coronavirus as well as inactivated *E. coli* K 99 and aluminum hydroxyde as adjuvant, was used in pregnant cows of our breeding herd. Systematic vaccination of dams resulted in a significant reduction of calf diarrhoea and excretion of rotavirus during an entire calving season and coronavirus was apparently fully suppressed (3).

Remarkably enough, this beneficial effect could not be linked to elevated antibody levels in the blood or colostral sera of vaccinated cows. This observation was in accord with that of other authors, who like us, reported on commercial vaccines adjuvanted with aluminum hydroxyde (12, 13, 14). Other authors reported higher antibody titres of vaccinated dams (4, 5, 8, 10, 18), apparently as a consequence of the respective adjuvants used.

With regard to the direct influence of an undisturbed digestion during the first 2 weeks of life on a newborn calf's health, and on the owner's economics, it was decided that Norden-Europe, Brussels, should produce a non-commercial, oil-adjuvanted vaccine of inactivated bovine rotavirus and coronavirus for experimental use in the same herd which our group had surveyed for the 5 preceding years.

Material and Methods

Vaccine and Vaccination

The experimental vaccine was injected intramuscularly in 2 ml amounts into pregnant cows.

Animals

Fifty pregnant cows of the Brown Swiss herd of the University Field Station were used. They gave birth to 48 healthy calves during the observation period. Ten cows received one dose of oil-adjuvanted vaccine 9–32 days before parturition (group A), 19 cows were vaccinated twice, i. e. 49–92 days and 8–28 days a. p. (group B) and 21 cows served as unvaccinated controls (group C). Care was taken to intersperse control cows with vaccinated cows, so as to eliminate the known seasonal fluctuation of spells of rotavirus and coronavirus in naturally infected herds (2, 15). Furthermore, to have calves of matched age groups when performing oral test infections.

All calves were fed in a feeding bucket exclusively colostrum and milk of their proper dams for a period of 14 days after birth, in quantities of 6–8 litres per day divided into 3 feeds during the first 3 days of life and two daily from the 4th day onwards. Calves were housed singly or as pairs, all under the same roof. Animals used for challenge infections were later moved to isolation boxes.

Clinical observations and examination of faeces

Clinical observations of every calf, especially the consistency of the faeces, were recorded daily for a period of 2 weeks. Between the 4th and 13th day of life a total of 5 faecal samples were collected from each calf and examined for rota- and coronavirus excretion by ELISA of either virus, using a double antibody sandwich-blocking assay (6).

Periodically, calf faeces were also examined for shedding of enteropathogenic *E. coli* and Cryptosporidia.

Serological examinations

Sera of cows were taken before vaccination (see Tables 3 and 4) and 4—6 days after parturition. Colostrum was sampled at the first milking and milk 10—14 days later. Sera of calves were taken 4—6 days after birth.

Milk samples were first defatted by centrifugation for 15 minutes followed by 2 hours' centrifugation at 50,000 g. Blood- and milk sera were inactivated for 30 minutes at 56 °C and stored at −20 °C.

Antibody contents against rota- and coronavirus were determined by rota- and coronavirus-blocking-ELISAs, respectively, using a positive faecal sample of the respective viruses as standard antigens and the sera to be tested, serially diluted, for blocking. Titres are recorded as the reciprocal value of the highest dilution showing > 50 % blocking of the standard antigen. All titres were computed to GMTs.

Challenge infections

Viruses used for challenge infections. For rotavirus challenge a cell-culture harvest of strain 81/36 F (Italy) provided by Prof. Zygraich, Norden-Europe, was inoculated perorally in amounts of 5 to 12 ml. The higher doses of rotavirus were given to calves 31, 32, 43, 44, 45, 46, 49 and 50 after it had become apparent that challenge with 5 ml supplied only little information.

For coronavirus challenge an inoculum had been produced by ourselves in a newborn colostrum-deprived calf that had received a virulent Dutch coronavirus strain, kindly provided by Dr. P. W. De Leeuw, Lelystad. This particular calf had developed severe diarrhoea within 24 hours after inoculation. Its diarrhoeic faeces had been collected and tested, ELISAs confirming the presence of coronavirus and the absence of rotavirus, respectively, and bacterial cultures were negative. These faeces were stored frozen at −70 °C in small aliquots to serve later as challenge material. Challenge viruses were rapidly thawed immediately prior to their application.

Table 1. Occurrence of diarrhoea, average weight gain and shedding of rota- and coronavirus in 35 unchallenged calves during their first 14 days of life

Calves born to dams with different vaccination status	calves developing diarrhoea		diarrhoeic faeces		average weight gain	calves shedding rotavirus		rotavirus ⊕ faecal samples / nr faecal samples tested		calves shedding coronavirus		coronavirus ⊕ faecal samples / nr faecal samples tested	
	n	%	n	%	(kg)	n	%	n	%	n	%	n	%
dams vaccinated once or twice (groups A and B) n = 18	8	44.4	24	13.3	3.8	5	27.8	8/90	8.8	1	5.6	2/90	2.2
dams left unvaccinated (group C) n = 17	7	41.2	15	8.8	4.7	5	29.4	10/85	11.7	2	11.8	3/85	3.5
total	15	42.9	39	11.1		10	28.6	18/175	10.3	3	8.6	5/175	2.9

Performance of challenge infections

The 16 calves listed in Table 2 aged 2—19 days were consecutively testinfected perorally with virulent rotavirus and 3 days later with virulent coronavirus. Seven of them stemmed from mothers of group A, 5 from group B and 4 from unvaccinated cows of group C. Groups were formed primarily according to expected time of birth, to match as far as possible the ages of the calves. Where possible, a calf of the control group C was included in a challenge test.

Two feeds before challenge infections the calves received milk replacer in order to prevent neutralization of challenge viruses by passively acquired antibodies present in the gut. Before as well as after inoculation all calves strictly received milk of their proper dams. Every challenge calf, even when infected at a relatively late age (Table 2), received its dams milk until 7 days p.i. with coronavirus. Faecal samples were taken daily and examined for excretion of rota- and coronavirus.

Results

Clinical observations and virus excretion in faeces of 35 unchallenged calves

Occurrence of diarrhoea in 35 unchallenged calves between their 4th and 13th day of life, shedding of rota- and coronavirus during this period and their mean weight gain are shown in Table 1. All calves of vaccinated dams, irrespective of whether vaccinated once or twice, are presented together because of the small total number of calves of group A. (Most calves of dams belonging to group A were used for challenge infections).

A total of 15 calves (42.9%) developed diarrhoea. Diarrhoeic faeces were observed on 39 observation days (11.1%). No statistical difference was observed between calves of vaccinated dams and those of control cows. Diarrhoea occurred between the 5th and 13th day of life and lasted for 1—7 days, as specified individually in Fig. 1.
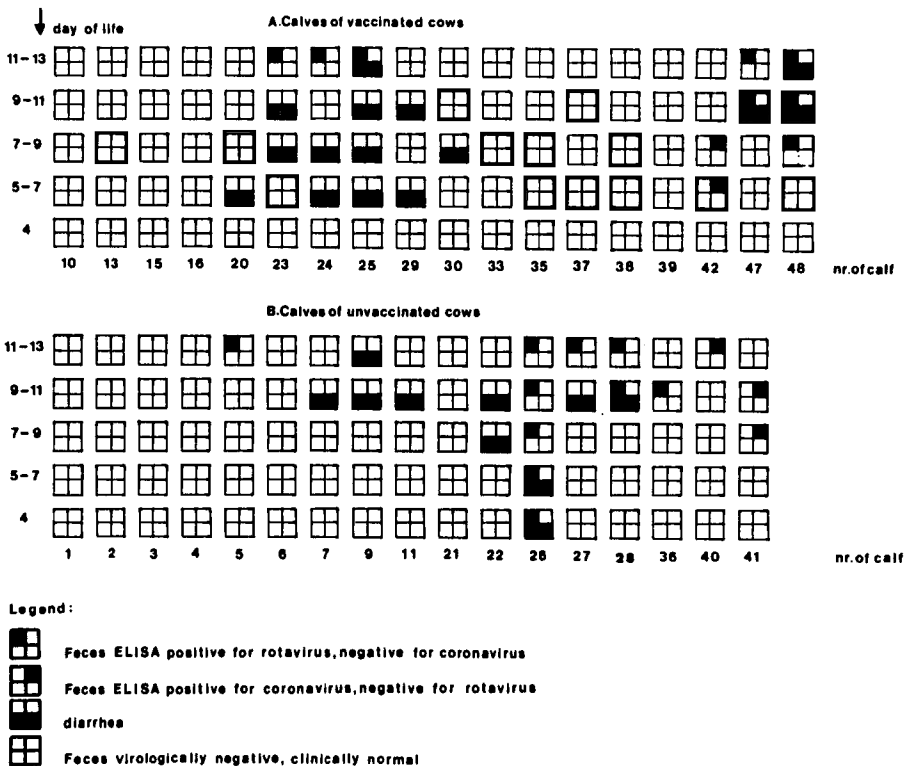

Fig. 1. Graphical presentation of rotavirus and coronavirus shedding in faeces of 35 calves correlated with diarrhoea, on days 4 to 13 after birth

Rotavirus was excreted in the faeces of 10 animals (28.6 %) with a total of 18 positive samples out of 175 tested samples (10.3 %). Coronavirus was excreted in the faeces of 3 calves (8.6 %), a total of 5 were positive out of 175 faecal samples (2.9 %) tested.

Correlation between virus excretion and occurrence of diarrhoea is shown in Fig. 1. Rota- as well as coronavirus excretion occurred in calves of vaccinated as well as unvaccinated cows. Excretion of rotavirus was mostly correlated with diarrhoea of varying duration (in 8 calves) and only 2 animals shed rotavirus without clinical signs. In calf 26, born of an unvaccinated dam, rotavirus excretion started earlier and persisted longer than in any other calf. Apart from this animal, no difference was observed that could have been linked with performance or omission of vaccination.

On the other hand, coronavirus excretion was not accompanied by diarrhoea, as all 5 positive faecal samples were of normal consistency.

All faeces tested for enteropathogenic *E. coli* were negative, 3 out of 28 tested contained cryptosporidia, without apparent relationship to diarrhoea.

Outcome of peroral challenge infections

Occurrence of diarrhoea in 16 doubly challenge-infected calves and shedding of rota- and coronavirus are shown in Table 2. Irrespective of the vaccination status of the dams, a high dose of virulent rotavirus was usually required to induce viral takes, manifested by faecal rotavirus shedding. Calf No. 16 of a dam vaccinated once was an exception of this rule, but as Fig. 2 shows, viral excretion was proved on a single day only and remained subclinical. Among the 6 calves receiving a challenge dose of 12 ml of rotavirus, 5 animals excreted rotavirus (Table 2) for periods of 1−6 days (Fig. 2). Invariably these heavily infected calves developed diarrhoea that lasted for 4−10 days. As Fig. 2 shows, calves of vaccinated cows as well as of controls developed diarrhoea and shed rotavirus.
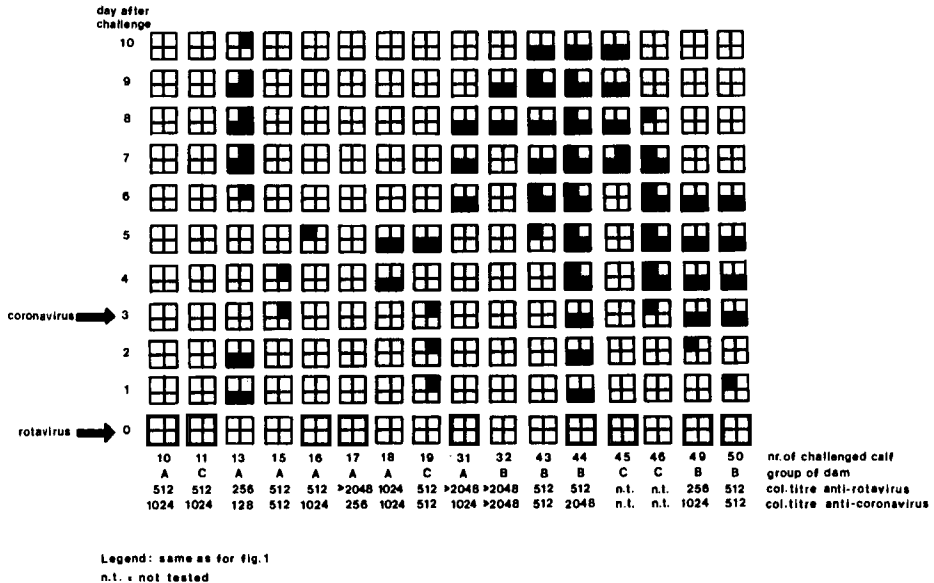

Fig. 2. Graphical presentation of rotavirus and coronavirus shedding and diarrhoea in 16 calves following virulent test-infection with either virus, correlated with antibody titres of their dams' colostrum

Table 2. Occurrence of diarrhoea and shedding of rota- and coronavirus observed in 16 perorally challenged calves

Calf no.	challenge age in days 1)	group of dam 2)	challenge dose rotavirus	coronavirus	diarrhoea	shedding of rotavirus 3)	coronavirus 3)
10	17	A			−	−	−
11	15	C			−	−	−
13	13	A	5 ml	2 ml	+++	−	+++
15	19	A			−	−	++
16	18	A			−	+	−
17	11	A	5 ml	4 ml	−	−	−
18	9	A			++	−	−
19	7	C			+	−	++
31	4	A	10 ml	4 ml	++	−	−
32	2	B			++	−	−
43	10	B	12 ml	4 ml	+++	++	−
44	8	B			+++	+++	−
45	12	C			+++	−	+
46	12	C			+++	+++	−
49	10	B	12 ml	4 ml	+++	+	−
50	8	B			+++	+	−

1) day of rotavirus challenge, invariably followed by additional coronavirus challenge 3 days later
2) group A ... cows vaccinated once
   group B ... cows vaccinated twice
   group C ... cows unvaccinated controls
3) Shedding of rota- and coronavirus was determined quantitatively by measuring the absorption (450 nm) with Titertek Multiskan (Fa. Flow). Degree and duration of shedding are expressed as plus symbols to make perception easier.

Coronavirus was found in the faeces of 4 calves (Table 2) for periods of 1—5 days (Fig. 2), being accompanied by diarrhoea in 3 of them. The fourth coronavirus shedder did not show clinical signs. There was no difference between calves of vaccinated cows or controls following coronavirus challenges.

Serological examinations

The GMTs of antibodies of cows and unchallenged calves against rota- and coronavirus in blood- and milksera are shown in Tables 3 and 4, respectively. Prevaccination titres in sera of cows taken 10—12 weeks ante partum were remarkably uniform. One (and more so 2) vaccinations of pregnant cows boosted their antibody titres against rota- and coronavirus, whereas those of unvaccinated controls showed a tendency to fall. (For explanation of the latter observation, see Discussion).

First-day colostrum of vaccinated dams showed significantly higher titres than of unvaccinated controls. As expected, 2 vaccine doses induced higher colostrum titres against coronavirus (Table 4). For the erratic observation, that the GMT against rotavirus in colostrum of group B was lower than of group A (Table 3) we have no scientific explanation. It seems to result from a technical bias, as the GMTs in sera of calves, corresponding to the resorbed part of maternal antibodies, are entirely in accord with expectation, i. e. they were highest in group B, median in group A, and lowest in control group C against either virus.

Table 3. GMTs of cows and their respective calves against rotavirus, resulting from different vaccination procedures

group	prevaccination sera of cows 12–10 weeks a.p.*	vaccination performed	sera of cows 3–2 weeks a.p.	vaccination performed	first-day colostrum	sera of cows 4–6 days p.p.	sera of calves 4–6 days p.p.	milk 10–14 days p.p.
A	⎫	no	48.5 (n = 10)	first	1647.8 (n = 10)	128.0 (n = 10)	181.0 (n = 8)	2.1 (n = 10)
B	⎬ 52.9 (n = 29)	first	80.6 (n = 18)	second	1018.3 (n = 17)	191.2 (n = 19)	275.4 (n = 19)	2.9 (n = 18)
C	⎭	no	35.6 (n = 13)	none	576.2 (n = 17)	25.1 (n = 17)	61.1 (n = 18)	1.6 (n = 18)

* Ante partum only 29 of the 50 cows could be sampled so long ahead, as in the others advanced pregnancy impeded this first sampling. As prevaccination titres varied little, all sera taken at this time were averaged in one and the same GMT.

Remarkably, and as outlined in the Discussion, apparently critically low antibody titres were found in milk examined 10 to 14 days following parturition. Two doses of vaccine had not even doubled the respective GMT against rotavirus (Table 3), as compared to the control group, whereas boosting was 2.7 fold against coronavirus (Table 4).

Discussion

The experimental oil-adjuvanted vaccine used in this research project was very well tolerated, exerting no adverse local nor systemic reaction in any of the vaccinated cows. The vaccine proved immunogenic against rotavirus (Table 3) as well as against coronavirus (Table 4).

The blood sera of unvaccinated cows gave invariably low titres and those of group C remained constant against coronavirus and fell insignificantly against rotavirus within the relatively short pre- to postparturition period. This fall is in line with the physiological enrichment of maternal antibodies in a cow's colostrum during a few weeks pre- to post parturition (1).

As could have been expected, two doses of vaccine induced higher titres in blood serum, colostrum and milk of vaccinated cows than a single one. We do not have a scientific explanation as to why GMTs against rotavirus (Table 3) in colostrum form an exception to this rule. It is very unlikely that we have lost some class of colostral antibody during their laboratory processing (9), and anti-coronavirus titres recorded in Table 4 were determined on the same samples and showed no irregularity.

Table 4. GMTs of cows and their respective calves against coronavirus resulting from different vaccination procedures

group	prevaccination sera of cows 12–10 weeks a.p.*	vaccination performed	sera of cows 3–2 weeks a.p.	vaccination performed	first-day colostrum	sera of dams 4–6 days p.p.	sera of calves 4–6 days p.p.	milk 10–14 days p.p.
A	⎫	no	29.9 (n = 10)	first	774.2 (n = 10)	207.0 (n = 10)	127.6 (n = 8)	3.7 (n = 10)
B	⎬ 51.6 (n = 29)	first	173.7 (n = 18)	second	980.3 (n = 17)	407.8 (n = 19)	330.5 (n = 19)	6.3 (n = 18)
C	⎭	no	51.7 (n = 13)	none	266.7 (n = 17)	42.6 (n = 17)	54.9 (n = 18)	2.3 (n = 18)

*) Ante partum same footnote as Table 3.

As Tables 3 and 4 show, calves of vaccinated cows possessed significantly higher antibody titres in their blood sera than those of the unvaccinated control group, but they obtained little benefit from resorbed antibodies, as known from the literature (see 3) as well as documented by Table 2, the challenge results are discussed below. Again, the literature as well as Table 2 show that maternal antibodies must be persistently supplied until age resistance is built up to exert a protective effect against colonization of a calf's mucosa by rota- and coronaviruses.

However, as shown in Tables 3 and 4, respectively, anti-rotavirus titred merely at best 1.8 fold after double vaccination over the GMTs of controls, and anti-coronavirus titred 2.7 fold in milk taken 10 to 14 days after parturition.

Rapid falls in lacteal antibodies post partum had already been reported before dam vaccination was possible (7, 13, 19) and accounted for ensuing susceptibility to intestinal virus infections. In fact, in our spontaneously infected University cattle herd, before performing dam vaccination, maternal antibody secretion sufficed merely for passive protection of newborn calves during their first 4 days of life (11). On the other hand, an extremely rich supply of maternal antibodies given for the first ten days after birth made calves fully refractory to experimental infection with a virulent rotavirus (4).

As Fig. 1 of this paper demonstrates, in the calving season in which the experimental oil-adjuvanted vaccine was used, lactogenic immunity was of intermediate duration and degree. Viral shedding was suppressed during the entire first week of life. Only 2 out of 48 calves shed virus during this period. Calf 26 of the unvaccinated group shed rotavirus for at least 9 days and was clinically ill. Calf 42 (born to a vaccinated mother) excreted coronavirus for a short period in clinically silent form. In their second week of life, however, irrespective of the vaccination status of their dams, a number of calves excreted either one of the viruses we examined for, but in no case both.

Evidently then, lactogenic immunity was too weak to mediate intestinal protection from the 7th day after birth onwards. With all probability, the amount of rotavirus and of coronavirus field strains shed during this particular calving season was low, as we may deduct from Table 5, in which shedder rates of former calving seasons are shown for comparison. According to Table 1 calves of unvaccinated dams had only a marginally

Table 5. Incidence of diarrhoea and virus excretion in calves reared during 3 successive calving seasons on the same premises, but from unvaccinated or vaccinated dams, respectively

parameter evaluated	mother-cows unvaccinated	mother-cows vaccinated with Scourguard 3®	mother-cows vaccinated with oil-adjuvanted experimental vaccine
part of calves developing diarrhoea	28/ 30 = 93.3 %	6/ 22 = 27.3 %	15/ 35 = 42.9 %
days with diarrhoea per calf group	126/294 = 42.9 %	11/242 = 4.5 %	39/350 = 11.1 %
part of calves excreting rotavirus	22/ 30 = 73.3 %	11/ 22 = 50.0 %	10/ 35 = 28.6 %
days with rotavirus-excretion per calf group	74/288 = 25.6 %	18/240 = 7.5 %	*
part of calves excreting coronavirus	12/ 30 = 40.0 %	0/ 22 = 0.0 %	3/ 35 = 8.6 %
days with coronavirus-excretion per calf group	37/286 = 12.9 %	0/242 = 0.0 %	*

* In the presently described calving-season calf faeces were only examined every second day for virus shedding. Accordingly, no direct comparison is possible with results from the 2 previous calving-seasons, where fecal samples were examined daily.

higher virus excretion record (and an even better weight gain) than calves of vaccinated dams. Thanks to vaccination of two-third of the dams and to ensuing relative protection of their offspring, "infection pressure" on these premises of rotavirus and coronavirus was certainly far lower than in previous calving seasons, when no dam vaccination had been practised (Table 5). Indirectly then, all calves benefitted from vaccination. Under the condition that viral exposure must have been of low order, calves of vaccinated dams should have been protected against field-infection in their second week of life too. Since this was not the case in 6 out of 18 calves in Fig. 1 allows the direct conclusion that antiviral titres recorded in milk were in fact too low to be immuno-protective. An indirect conclusion that dam vaccination should have mediated stronger and longerlasting lactogenic immunity is supported by findings of other authors. After use of their experimental vaccines, colostrum titres against rotavirus exceeded 32-fold (4) and 200-fold (18) those of their respective control colostra. SNODGRASS et al. (18) were able to demonstrate elevated antibody titres in milk for at least 28 days after calving and, EICHHORN (5) and HESS (8) for 21 days, respectively.

In our experiments test-infection challenged calves were certainly exposed to much higher virus doses than the unchallenged calves. As Fig. 2 shows, in several challenged calves lactogenic immunity was almost immediately broken. (By mischief, calf no. 19 apparently had contracted coronavirus already by contact.) As a rule, this happened in calves that were challenged at a relatively late age (Table 2) and had received relatively low maternal antibody supply (Fig. 2). This unfortunate effect is best represented by calf no. 13. In contrast, calves nos. 31 and 32 withstood a very severe double-challenge because they became test-infected at the youngest age, before the titres in milk had fallen.

Fig. 2 allows us to conclude that a high level of lactogenic immunity has a greater impact to prevent "takes" than a high virus dose has to produce them. With SNODGRASS et al. (18) we agree, however, that very high virus doses overwhelmed lactogenic immunity.

Not only are titres and protection results published by different authors difficult to compare. Even our team cannot directly compare its titres reported here with those determined by ourselves on the same premises in former years. We presently titrate antibodies by the modern ELISA, whereas formerly a serum neutralization assay was used for rotavirus and a haemagglutination-inhibition assay for coronavirus, respectively (3).

We have, however, kept constant over the years in our laboratory the clinical evaluation of newborn calf diarrhoea and the determination by ELISAs of virus shedding. This allowed us to compare the prophylactic effect of the experimental oil-adjuvanted bivalent vaccine used now with that of the commercially produced trivalent product Scourguard 3 (R) in the previous calving season (3), and lastly with the diarrhoeic situation encountered in the preceding calving seasons, when no dam vaccination was practised on these premises (11). As Table 5 shows, diarrhoea was extremely severe before dam vaccination was initiated, affecting 93.3 % of calves in their first two weeks of life, of which 73.3 % excreted rotavirus and 40 % coronavirus, respectively.

The prophylactic use of two doses of Scourguard 3 vaccine (R) had been followed by a dramatic reduction of the incidence of diarrhoea and coronavirus shedding, whereas rotavirus was still excreted by 50 % of the calves, although over a significantly reduced period.

The presently used oil-adjuvanted vaccine gave protection under natural holding conditions similar to Scourguard 3 (R). Fewer calves excreted rotavirus now, whereas, protection against coronavirus proved less effective. In fact, we had expected a better protection rate for the oil-adjuvanted vaccine, but as shown in Tables 3 and 4, its boosting effect on colostral and milk antibody levels remained below values we had expected from the literature.

This investigation was performed under natural holding conditions, but with pertinent scientific exactness, but unanswered questions remain. On the one hand we are astonished that, irrespective of severe challenge, no calf showing a 'take' of rotavirus challenge was successfully superinfected by coronavirus 3 days later. On the other hand,

we still encounter so many instances where diarrhoea could not be assigned to any of the frequently described causal agents. Lastly, as a non-specific parameter, but as a consequence of chance allotment to groups, we had to record the fact that calves of the unvaccinated control group showed a higher average weight gain than those of the vaccinated groups.

Acknowledgements

Zusammenfassung

Häufigkeit von Durchfällen und Rotavirus- bzw. Coronavirusausscheidung bei Kälbern, deren Mutterkühe mittels eines diese beiden Viren enthaltenden, Mineralöl-adjuvierten Versuchsimpfstoffes geimpft worden waren

Es wird über Mutterkuh-Schutzimpfung mittels einer Versuchsvakzine, welche inaktivierte bovine Rotaviren und Coronaviren in Mineralöladjuvans enthielt, berichtet. Von 50 trächtigen Brown Swiss Kühen eines seit Jahren an einer stallständigen Infektion durch diese Viren leidenden Zuchtbestandes wurde während einer Abkalbesaison ein Teil schutzgeimpft, ein Teil als ungeimpfte Kontrollen belassen. Die Antikörpertiter im Blutserum aller Kühe wurden vor wie nach der Impfung und nach dem Abkalben bestimmt. Außerdem wurde der Antikörpergehalt von Ersttagskolostrum, reifer Milch, wie auch im Serum 4 bis 6 Tage alter Kälber gemessen. Während der ersten 14 Lebenstage wurde den Kälbern ausschließlich Kolostrum bzw. Milch ihrer eigenen Mütter verfüttert. Während dieser 14 Tage wurden von 35 unter natürlichen Stallbedingungen gehaltenen das Auftreten von Durchfall registriert als auch die Ausscheidung von Rotavirus und Coronavirus bestimmt. Sechzehn Kälber wurden experimentell peroral nacheinander zunächst mit virulentem Rotavirus, 3 Tage später zusätzlich mit virulentem Coronavirus infiziert und in gleicher Weise kontrolliert. Die Ölversuchsvakzine vermochte in sämtlichen Kühen deren präexistente, auf früheren Stallinfektionen beruhende Antikörpertiter zu steigern, indessen nur in ungenügendem Ausmaß. Die geometrischen Mitteltiter wiesen im Kolostrum schutzgeimpfter Kühe einen geringeren Boostereffekt auf, als nach der Literatur zu erwarten war, und die Titer reifer Milch waren auffallend niedrig. Immerhin vermochten kolostrale Antikörper unmittelbar nach der Geburt erfolgende schwere Testinfektionen unter Kontrolle zu halten. Desgleichen genügte die induzierte lactogene Immunität, um die graduell schwächere Stallinfektion während der ersten Lebenswoche der Kälber zu unterdrücken. Jedoch reichte die erzielte lactogene Immunität nicht aus, um die natürlich gehaltenen Kälber auch in ihrer 2. Lebenswoche vor Rotavirusinfektion wie Coronavirusinfektion des Schweregrades, wie er im Bestand aktuell vorherrschte, verläßlich zu schützen.

References

1. BAUSE, I., und F. W. SCHMIDT, 1980: Zur Persistenz präzipitierender Antikörper im Blutserum leukoseinfizierter Rinder unter besonderer Berücksichtigung des Kalbezeitpunktes. Tierärztl. Umschau 35, 642—649.
2. BÜRKI, F., G. SCHUSSER, and H. SZEKELY, 1983: Clinical, virological and serological evaluation of the efficacy of peroral live rotavirus vaccination in calves kept under husbandry conditions. Zbl. Vet. Med. B 30, 237—250.
3. BÜRKI, F., K. MÖSTL, E. SPIEGL, E. HORVATH, and H. SZEKELY, 1986: Reduction of rotavirus-, coronavirus- and E. coli-associated calf-diarrhoeas in a large-size dairy herd by means of dam vaccination with a triple-vaccine. J. Vet. Med. B 33, 241—252.
4. CASTRUCCI, G., F. FRIGERI, M. FERRARI, V. CILLI, V. CALEFFI, F. ALDROVANDI, and A. NIGRELLI, 1984: The efficacy of colostrum from cows vaccinated with rotavirus in protecting calves to experimentally induced rotavirus infection. Comp. Immun. Microbiol. infect. Dis. 7, 11—18.

 5. EICHHORN, W., 1981: Verlängerung der Ausscheidung von Rotavirusantikörpern mit der Milch frischlaktierender Kühe durch parenterale Vakzination. Dissertation Tierärztl. Fakultät München.
 6. ELLENS, D. J., and P. W. DE LEEUW, 1977: Enzyme-linked immunosorbent assay for diagnosis of rotavirus infections in calves. J. Clin. Microbiol. **6**, 530—532.
 7. ELLENS, D. J., P. W. DE LEEUW, and P. J. STRAVER, 1978: The detection of rotavirus specific antibody in colostrum and milk by ELISA. Ann. Rech. Vét. **9**, 337—342.
 8. HESS, R. G., P. A. BACHMANN, W. EICHHORN, K. FRAHM und P. PLANK, 1982: Stimulierung der laktogenen Immunität des Rindes gegenüber Rotavirusinfektionen. Fortschritte Vet. Med. **35**, 103—108.
 9. HONKANEN-BUZALSKI, T., and M. SANDHOLM, 1981: Association of bovine secretory immunoglobulins with milk fat globule membranes. Comp. Immun. Microbiol. infect. Dis. **4**, 329—342.
10. MEBUS, C. A., R. G. WHITE, E. P. BASS, and M. J. TWIEHAUS, 1973: Immunity to neonatal calf diarrhoea virus. J. Amer. Vet. Med. Ass. **163**, 880—883.
11. MÖSTL, K., B. HINAIDY, CHR. LOIBL, H. SZEKELY, E. HORVATH und F. BÜRKI, 1984: Zur Entero-Pathogenität boviner Coronaviren unter Feldbedingungen. Zbl. Vet. Med. B **31**, 743—754.
12. MYERS, L. L., and D. R. SNODGRASS, 1982: Colostral and milk antibody titers in cows vaccinated with a modified live-rotavirus-coronavirus vaccine. J. Amer. Vet. Med. Ass. **181**, 486—488.
13. RODAK, L., L. A. BABIUK, and S. D. ACRES, 1982: Detection by radioimmunoassay and enzyme-linked immunosorbent assay of coronavirus antibodies in bovine serum and lacteal secretions. J. Clin. Microbiol. **16**, 34—40.
14. SAIF, L. J., L. SMITH, B. J. LANDMEIER, E. H. BOHL, K. W. THEIL, and D. A. TODHUNTER, 1984: Immune response of pregnant cows to bovine rotavirus immunization. Am. J. Vet. Res. **45**, 49—58.
15. SCHUSSER, G., B. HINAIDY, and F. BÜRKI, 1982: A follow-up study on bovine rotavirus dissemination among calves of a large dairy herd. Microbiol. **5**, 321—332.
16. SIBALIN, M., H. SZEKELY und F. BÜRKI, 1980: Rotavirusinfektionen in einem größeren Rinderbestand. Wien. Tierärztl. Mschr. **67**, 122—127.
17. SNODGRASS, D. R., and P. W. WELLS, 1978: Passive immunity in rotaviral infections. J. Amer. Vet. Med. Ass. **173**, 565—570.
18. SNODGRASS, D. R., K. J. FAHEY, P. W. WELLS, I. CAMPBELL, and A. WHITELAW, 1980: Passive immunity in calf rotavirus infections. Maternal vaccination increases and prolongs immunoglobulin G 1 antibody secretion in milk. Infect. Immun. **28**, 344—349.
19. WOODE, G. N., J. JONES, and J. BRIDGER, 1975: Levels of colostral antibodies against neonatal calf diarrhoea virus. Vet. Rec. **97**, 148—149.