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Central Veterinary Institute, Department of Virology,
15 Edelhertweg, 8219 PH Lelystad, The Netherlands

Laboratory Experiments on Oral Vaccination of Calves against Rotavirus or Coronavirus induced Diarrhoea

By P. W. DE LEEUW and J. W. A. TIESSINK

Address of authors: Central Veterinary Institute, Department of Virology,
15 Edelhertweg, 8219 PH Lelystad, The Netherlands

With 2 figures and one table

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Introduction

The efficacy of oral vaccination of calves with a modified live virus vaccine against rotavirus and/or coronavirus induced diarrhoea has been the subject of considerable dispute in the literature. Early reports by MEBUS and others (MEBUS et al., 1972, 1973) suggested the effectiveness of a rotavirus vaccine under experimental conditions as well as in the field. However, this was not confirmed by the results of double blind field trials (ACRES and RADOSTITS, 1976). THURBER et al. (1977) considered that the double blind experimental design, or rather a 50 per cent vaccination, was not a suitable method to test an oral vaccine against diarrhoea caused by enteric viruses. By this method they were unable to show protection after vaccination with a bivalent rotavirus / bovine coronavirus vaccine, which, however, appeared to be present when sequential vaccination was used. We employed both experimental approaches when testing a monovalent rotavirus vaccine in calves from endemically infected Dutch dairy herds (DE LEEUW et al., 1980 b). Positive results with respect to the incidence or severity of undifferentiated or rotavirus-associated diarrhoea could not be shown with either method. Recently similar findings were reported by BÜRKI et al. (1983). Since in SPF-calves deprived of colostrum some protection against challenge was already found after three days, it was speculated that neutralization of the vaccine virus by specific antibodies in colostrum could be a major reason for the failure of the vaccine under field conditions (DE LEEUW et al., 1980 b).

In the present report the results of laboratory experiments are described that were designed to test the above neutralization or "colostrum-barrier" hypothesis.

Material and Methods

Experimental animals

Origin of calves. All calves were Friesians. Those used in experiments A, B 1 and B 2 were born to mothers in the Institute's SPF-herd, which is serologically negative for rotavirus and bovine coronavirus. The other calves were from cows obtained through a market. They were delivered by caesarian section and reared in isolation. The day of birth is taken as day 0 in this study.

Housing. Calves used in the same experiment were housed in individual wooden boxes placed side by side in one isolation room. They always arrived on the same day. The three calves of experiment B 1 were not housed completely isolated, but in a room adjacent to the one where the calves of experiment A were housed. They arrived four days after the challenge of group A calves. The

rooms were connected by a corridor; there was an air pressure gradient from the rooms to the corridor. Personnel was instructed to take care of the youngest calves first and to use separate clothes and utensils.

Feeding. Calves were fed eight litres of colostrum, divided over four meals, during the first two days. Thereafter a commercial milk substitute was given, three litres twice daily. Colostrum without antibodies against rotavirus and bovine coronavirus was obtained from cows in the Institute's SPF-herd and "positive" colostrum from cows in a large dairy herd of a government farm where rotavirus and bovine coronavirus infections were endemic (DE LEEUW et al., 1980 a). Excess first-day colostrum of individual cows was frozen at − 20 °C and later thawed, pooled and frozen again in quantities of approximately four litres. Total storage time was between two and twelve months.

Surveillance. Calves were examined twice daily, at which time rectal temperatures were taken and faecal samples were collected. They were weighed daily before the second feeding. The consistency of the faeces was noted as normal, (bright) yellow but with largely normal consistency, semi-liquid or liquid. No treatment was given or dietary measures taken when diarrhoea developed.

Laboratory methods

Virus titrations. Rotavirus titration was performed in MA 104 cells grown on coverslips in rolled Leighton tubes. Monolayers were inoculated in duplicate with 0.3 ml volumes of tenfold dilutions of a virus suspension prepared in maintenance medium (Hanks' BSS with 0.5 % LAH and antibiotics). No serum was used in the medium during virus titration; reading was by immunofluorescence after 24 and 48 hours (ELLENS et al., 1978 b). Bovine coronavirus was titrated in the same manner in a trachea cell line derived from a bovine foetus.

Faeces examination. Faecal extracts, approximately 20 per cent in PBS with 0.05 per cent Tween 80, were stored at − 20 °C. Each sample was examined by enzyme-linked immunosorbent assay (ELISA) for the presence of rotavirus (ELLENS and DE LEEUW, 1977), bovine coronavirus (ELLENS et al., 1978 c) and the K 99 antigen of *Escherichia coli (E. coli)* (ELLENS et al., 1979). Part of the faecal samples was also examined by electron microscopy (EM) (DE LEEUW et al., 1977) as indicated under Results.

Serology. Serum and colostrum samples were titrated for antibodies against rotavirus, bovine coronavirus and the K 99 antigen of *E. coli* by the 50 per cent blocking method of ELISA as described elsewhere (ELLENS and DE LEEUW, 1977; ELLENS et al., 1978 a, c; ELLENS et al., 1979).

Table 1

Experimental design

Experiment	Number of calves	Colostrum	Vaccination	Challenge on day 6	Challenge on day 8
A	3	−	−	+	
B 1	3 [1]	−	+	+	
B 2	2	−	+	+	
C	3	+	−	+	
D 1	3	+	+	+	
D 2	3	+	+		+

[1] Contrary to the other groups, these vaccinated calves were not housed completely isolated. For further explanation, see Material and Methods.

Experimental design, vaccination and challenge

The experiments are outlined in Table 1. The vaccine used was Scourvax-2* containing both attenuated bovine rotavirus and bovine coronavirus. It was supplied in a desiccated form. Each calf received two ml of the reconstituted vaccine orally as specified by the manufacturer. The rotavirus titre of the vaccine in our hands was $10^{6.2}$ and the coronavirus titre was 10^5 tissue culture infective doses 50 per cent per ml ($TCID_{50}$). Vaccination was always performed one hour after the first colostrum feeding; the calves were then five to eight hours old.

* Scourvax®-2, Smith Kline, Norden Laboratories, Lincoln, Nebraska, U.S.A., kindly supplied by Drs. C.H.L. KLAASSEN (Smith Kline, Netherlands) and N. ZYGRAICH (Smith Kline/R.I.T., Belgium).

The challenge inoculum consisted of four ml of virulent rotavirus containing gnotobiotic calf faeces, a similar volume of gnotobiotic calf faeces containing virulent bovine coronavirus, and seven ml of PBS. The inoculum was given orally. Both virus isolates were of Dutch origin. The gnotobiotic calf faecal samples used were obtained three to four days after experimental inoculation i.e. at the height of virus excretion. They were immediately frozen at − 70 °C and later thawed, homogenized and stored again in small quantities at the same temperature. In these faecal samples no viruses other than the one used to inoculate the gnotobiotic calf were detected by EM. The gnotobiotic calves were free of bovine virus diarrhea (BVD) virus and remained serologically negative for BVD-virus. The faeces used as challenge inoculum contained more than 10^{11} rotavirus particles per ml as estimated by EM (De Leeuw et al., 1977) and it had a titre of 10^5 $TCID_{50}$/ml. An infectious coronavirus titre could not be established, but it was estimated by EM that the inoculum contained 10^{12} coronavirus particles per ml.

Results

Calves receiving colostrum free of antibodies against rotavirus and coronavirus

The three control calves (exp. A) began excreting rotaviruses in their faeces 24 to 48 hours after challenge and developed diarrhoea one to two days later. Coronaviruses were detected in the faeces of all three but shedding started later than in the case of rotaviruses and did not last as long (Fig. 1 A). Diarrhoea in these calves lasted for five to twelve days. On the first two to three days body temperatures were slightly raised. During this period the calves were depressed and refused to drink or consumed only part of their milk. Thereafter no. 28 recovered quickly, but calves 58 and 33 remained ill and depressed. Sometimes they were too weak to stand. Recovery did not start before another week had passed.

Calves 30, 32 and 61 of exp. B 1 arrived four days after the challenge of the calves of exp. A. They were vaccinated and housed in a room adjacent to that of the exp. A calves (see Material and Methods). Within 48 hours all three developed diarrhoea. At the same time they started to excrete rotavirus. One calf, no. 32, also shed coronavirus. The faeces of the other two calves contained coronavirus the next morning. Diarrhoea lasted a few days or occurred intermittently. Oral inoculation with virulent rotavirus and coronavirus on day 6 had no apparent effect on this pattern (Fig. 1 B).

Calves 62 and 35 of experiment B 2 were housed completely isolated. After vaccination, both excreted rotavirus for a few days, and no. 35 also excreted coronavirus, but diarrhoea was not observed. After challenge both calves passed yellow faeces for one to two days. However, diarrhoea did not develop and virus shedding could not be detected by ELISA or by EM (Fig. 1 C).

The clinical observations in the various experiments were reflected by the mean weight curves of the groups (Fig. 1 D). Whereas the curve of calves in exp. B 2 showed a steady increase from day 6, the other two curves showed marked dips. In the case of the B 1 curve this already started on day 3. On average the maximum loss of weight in experiments A and B 1 was six to seven per cent. Individual calves, however, all lost at least this much, since the day when the maximum loss was observed was not always the same. For instance, calves 28, 58 and 33 in exp. A lost 10.5, 9.5 and 15 per cent of their body weight, respectively, before recovery commenced.

Calves receiving "positive" colostrum

The colostrum pool used had an ELISA titre (log_{10}) against rotavirus of 2.7 and against bovine coronavirus of 2.4.

Of the non-vaccinated control calves in exp. C, only one (237) developed severe diarrhoea. The other two calves passed somewhat loose and yellow faeces for a few days (Fig. 2 A). Coronavirus shedding was detected in calf 235 and rotavirus shedding in 236. Calf 237 excreted both viruses, but detection was limited to one day.

In experiment D three vaccinated calves were challenged on day 6 and three others on day 8, since it was considered possible, based on the results of experiments A and C, that

day 6 was too early, at least with the challenge inoculum employed, to induce severe disease in calves that had received positive colostrum. The results of exp. D are shown in Figs. 2 B and 2 C. Virus shedding was not detected by ELISA or by EM in any of the vaccinated calves until two days after challenge. All calves passed firm faeces during this period. The calves challenged on day 6 (exp. D 1) had detectable quantities of rotavirus in

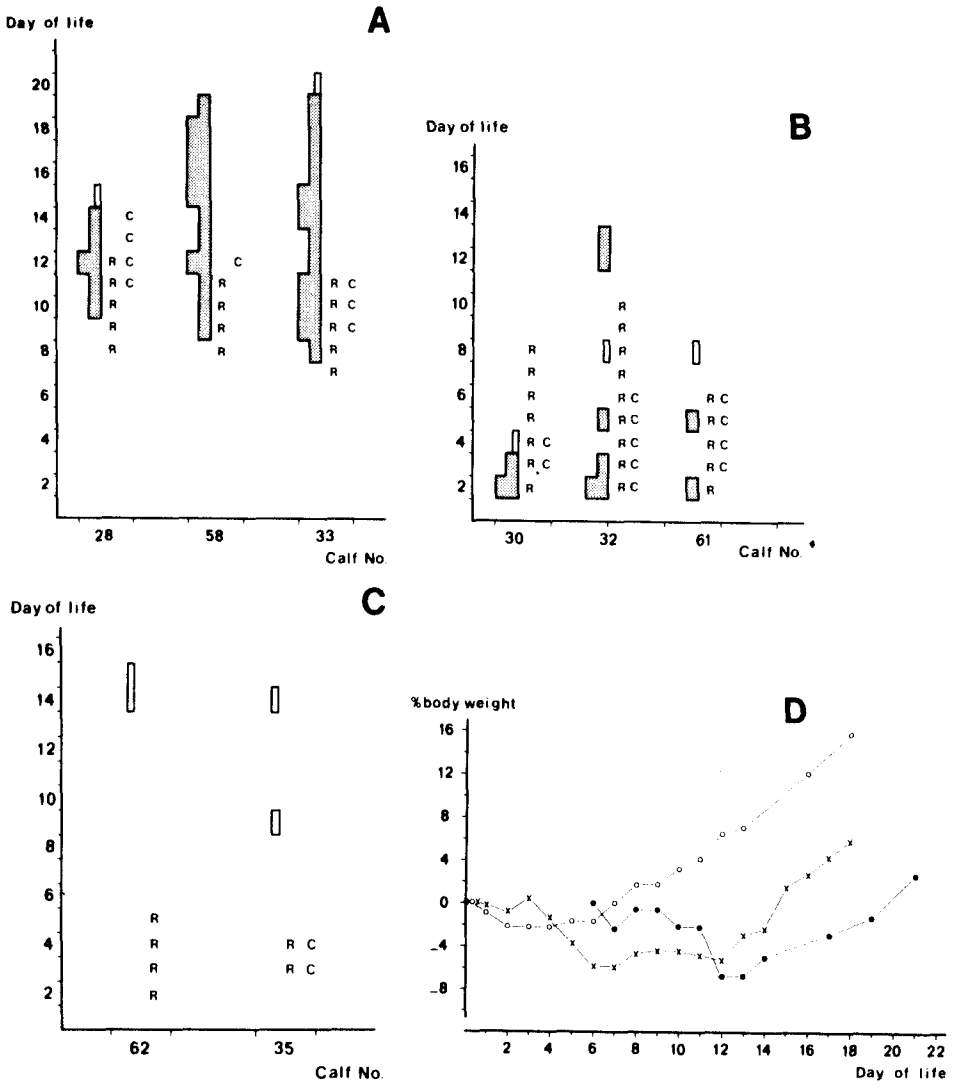

Fig. 1. Diarrhoea and virus excretion in calves fed colostrum without antibodies against rotavirus or bovine coronavirus

Faeces consistency liquid ■, semi-liquid ■, or (bright) yellow, but with normal consistency □. R = rotavirus detected in the faeces. C = bovine coronavirus detected in the faeces.

1 A: Non-vaccinated control calves challenged with a mixture of virulent rotavirus and coronavirus on day 6

1 B: Calves vaccinated orally with Scourvax®-2 one hour after the first feed and challenged on day 6; contrary to all other experiments calves were not housed completely isolated

1 C: Completely isolated calves vaccinated and challenged as above

1 D: Mean body weight curves of calves in exp. A (●), B 1 (x) and B 2 (○)

their faeces for up to seven days after challenge. Coronavirus excretion was only found in calf 243 on the 12th and 13th day post-challenge when diarrhoea had ceased (Fig. 2 B). The faeces consistency of all three calves was loose and yellow or semi-liquid 48 hours after challenge. During the next eight to ten days abnormal faeces was passed intermittently; all three calves were depressed and they consumed less milk for a few days. The calves challenged on day 8 (exp. D 2) were found to excrete rotaviruses two to three days later. In two calves coronavirus shedding was also detected, starting five to six days after the

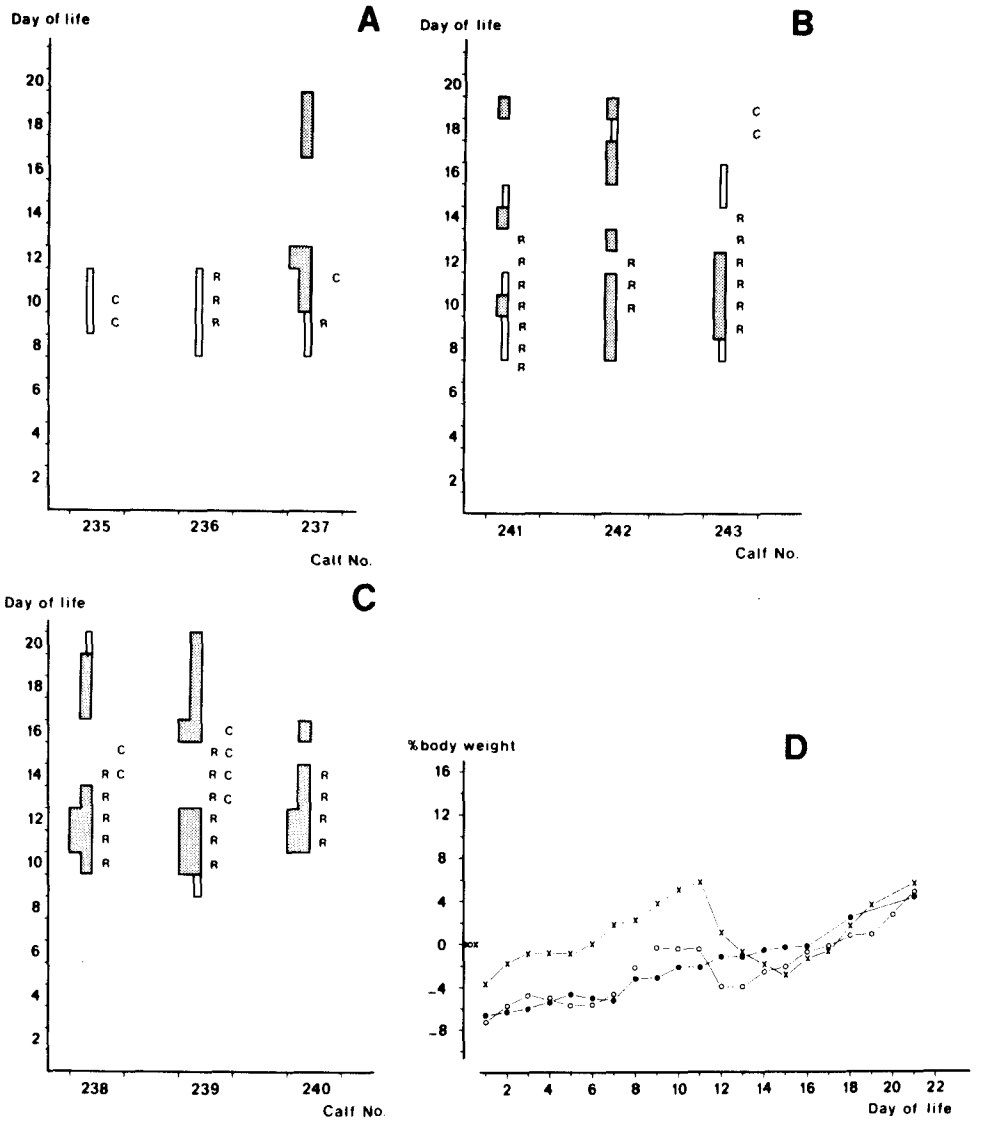

Fig. 2. Diarrhoea and virus excretion in calves fed "positive" colostrum
Symbols are the same as in Fig. 1; vaccine and challenge inoculum were identical.
2 A: Non-vaccinated control calves challenged on day 6
2 B: Calves vaccinated one hour after the first feed and challenged on day 6
2 C: Calves vaccinated as above, but challenged on day 8
2 D: Mean body weight curves of calves in exp. C (●), D 1 (○) and D 2 (x)

challenge. Severe diarrhoea, the beginning of which coincided with rotavirus excretion, was observed in all three calves (Fig. 2 C). Clinically the vaccinated calves challenged on day 8 were more severely affected than those challenged on day 6.

Fig. 2 D depicts the clinical patterns described above. The mean weight curves of calves challenged in experiments D 1 and D 2 each showed a marked dip, whereas that of calves in exp. C continued to rise, though slowly. Of this last group only calf 237 lost some four per cent of its body weight. In exp. D 1 the mean body weight loss was nearly four per cent (3 to 5) and in exp. D 2 nine per cent (3 to 19).

None of the calves used in these experiments excreted the K 99 antigen of *E. coli* or developed a specific antibody response against this antigen. All calves were serologically negative for rotavirus and coronavirus antibodies before they received positive colostrum or before they were vaccinated and challenged.

Discussion

The results obtained in this study with oral vaccination of calves fed colostrum free of specific antibodies against rotavirus and coronavirus are in accord with those of earlier experiments in which a monovalent rotavirus vaccine and calves deprived of colostrum were used (DE LEEUW et al., 1980 b). Two out of three vaccinated calves were found to be protected against virulent challenge three days later. However, another calf challenged after one day developed severe diarrhoea. It was concluded that for effective oral vaccination of colostrum-deprived calves against rotavirus induced diarrhoea, a period of at least three to four days was required between vaccination and challenge. WOODE et al. (1978) came to a similar conclusion, although others suggested a more rapid protection based on interference phenomena (MEBUS et al., 1972, 1973; THURBER et al., 1977). In the present study the "natural" infection pressure in exp. B 1 was probably not high and yet rotavirus and coronavirus associated diarrhoea developed in all three vaccinated calves before the experimental challenge was performed. Obviously, if no interference phenomena can be shown to occur, this raises questions with respect to the effectiveness of the vaccine in the field, as nearly all calves in endemically infected herds will be exposed to rotavirus and probably also to coronavirus immediately after birth (DE LEEUW et al., 1980 a). Protection against challenge on day 6 (exp. B 2) was to be expected, although at present there appears to be very little published information on the effect of a "bivalent" challenge. Whether our bivalent challenge was more severe than a challenge with the rotavirus or the coronavirus strain alone would have been, is difficult to say as no straight comparisons were made. However, as calves in the field may become infected with rotavirus and coronavirus at the same time we considered a "bivalent" challenge justified. It is noteworthy that in our control calves the start of diarrhoea coincided better with the beginning of rotavirus than with the beginning of coronavirus detection in the faeces. This indicates that at least the start of diarrhoea was usually due to the former virus.

The antibody titres against rotavirus and coronavirus of the "positive" colostrum pool were comparable to the mean values calculated for first-day colostrum in a number of Dutch dairy herds in the past (DE LEEUW et al., 1980 a, b) as well as in more recent studies (unpublished results). Feeding "positive" colostrum for two days had a favourable effect on the clinical course of the challenge on day 6, as suggested by the results of experiments A and C. Since with time the passive antibody content in the alimentary tract of calves fed colostrum for two days would decrease, whereas the vaccine would be given a better chance, three of the six vaccinated calves in the main experiment (D) were challenged on day 8. All six calves developed diarrhoea shortly after the challenge, coinciding mainly with rotavirus excretion (Figs. 2 B and 2 C). The vaccinated calves challenged on day 8 were more severely affected than those challenged on day 6, while both groups showed more diarrhoea than the controls (Fig. 2). Contrary to the situation in calves deprived of colostrum (DE LEEUW et al., 1980 b) or fed colostrum without specific antibody (Fig. 1 C), excretion of virus was not detected in the period between vaccination and challenge.

The above results in our opinion provide strong evidence for the "colostrum barrier" hypothesis expressed before (De Leeuw et al., 1980 b): effective oral vaccination of calves in the field against diarrhoea caused by rotavirus (or coronavirus) is hindered by the presence of specific antibodies in colostrum. Thus other schemes of vaccination and colostrum feeding would have to be tested to find an optimum application of the oral vaccine, but for obvious reasons one should be very careful with, for instance, changing the practice of feeding colostrum as soon as possible after birth. Experimental results in this field have been disappointing so far (Bürki et al., 1983). It appears, therefore, that a positive use of colostrum, that is through vaccination of the dam, deserves priority.

Summary

The efficacy of oral vaccination of calves with a modified live virus vaccine (Scourvax®-2, Smith Kline) against rotavirus and/or coronavirus induced diarrhoea was examined under controlled conditions. Calves were vaccinated one hour after the first feed. Challenge was done orally with a mixture of virulent bovine rotavirus and bovine coronavirus.

In the first experiments calves received colostrum free of antibodies against these viruses. Three control calves, challenged on the sixth day of life, developed severe diarrhoea after two to three days. Diarrhoea lasted for five to twelve days and on average the calves lost 12.5 per cent body weight. Rotavirus and coronavirus shedding was found in all three. In contrast, no diarrhoea was observed in two vaccinated calves challenged on day 6. After vaccination one shed rotavirus and one rotavirus plus coronavirus, but no virus excretion was found after the challenge. Three other vaccinated calves, that were not held completely isolated, all developed diarrhoea within three days, i.e. before the experimental challenge was performed. In each calf the beginning of diarrhoea coincided with rotavirus and coronavirus excretion. These calves lost six to seven per cent body weight.

In further experiments calves were fed "positive" colostrum (from a pool collected on a Dutch dairy farm) for two days. Antibody titres of this pool against rotavirus and bovine coronavirus were as expected for mixed first-day colostrum of dairy cows under the Dutch conditions. One out of three control calves fed colostrum from this pool developed severe diarrhoea after challenge on day 6; the others only passed somewhat loose or yellow faeces for a few days. The first calf shed both viruses, the last two either rotavirus or coronavirus. Weight losses were limited or did not occur. More severe diarrhoea was observed in six vaccinated calves, three of which were challenged on day 6 and three on day 8. None of these calves excreted rotavirus or coronavirus before the challenge. Two to four days after the challenge all six developed diarrhoea, the beginning of which coincided with the start of rotavirus excretion. In three calves coronavirus excretion was also found, but this started a few days later. The calves challenged on day 6 were less severely affected than those challenged on day 8; body weight losses averaged four and eight per cent, respectively.

These results show that effective oral vaccination of calves against diarrhoea induced by rotavirus or bovine coronavirus may be blocked by specific antibodies present in colostrum and thereby confirm an earlier hypothesis based on observations in the field.

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Zusammenfassung

Laboruntersuchungen zur oralen Impfung von Kälbern gegen Rotavirus- und Coronavirus-bedingten Durchfall

Die Wirksamkeit einer oralen Impfung von Kälbern mit einer modifizierten Lebendvakzine (Scourvax®-2, Smith Kline) gegen Rotavirus und/oder Coronavirus-bedingten

Durchfall wurde unter kontrollierten Bedingungen getestet. Die Kälber wurden eine Stunde nach der ersten Fütterung geimpft. Die Testinfektion erfolgte mit einer Mischung aus virulentem bovinem Rota- und Coronavirus.

Ein Teil der Kälber erhielt Kolostrum, welches keine Antikörper gegen beide Viren enthielt. Drei Kontrollkälber aus dieser Gruppe entwickelten zwei bis drei Tage nach Testinfektion am 6. Lebenstag schwere Durchfallsymptome. Die Durchfalldauer betrug 5—12 Tage. Die Kälber verloren im Durchschnitt 12,5 % an Körpergewicht. Alle drei Tiere schieden Rota- und Coronavirus aus. Im Gegensatz dazu wurde bei zwei geimpften Kälbern nach Testinfektion am 6. Lebenstag kein Durchfall beobachtet. Nach der Impfung schied ein Kalb Rotavirus aus, das andere Rota- und Coronavirus. Nach der Testinfektion wurde jedoch keine Virusausscheidung mehr beobachtet. Drei andere Kälber, die nicht streng isoliert gehalten wurden, bekamen innerhalb von 3 Tagen nach der Impfung Durchfall und schieden gleichzeitig Rota- und Coronavirus aus. Diese Kälber verloren 6—7 % an Körpergewicht.

Ein anderer Teil der Versuche wurde an Kälbern durchgeführt, die zwei Tage lang Antikörper-haltiges Kolostrum bekommen hatten. Dieser Kolostrum-„Pool" enthielt Antikörper gegen beide Viren in einer für die holländischen Betriebe typischen Titerhöhe. Eines von drei Kontrollkälbern, die mit diesem Kolostrum gefüttert wurden, bekam nach der Testinfektion am 6. Lebenstag schweren Durchfall und schied beide Viren aus. Die beiden anderen Kälber zeigten nur leichte Durchfallsymptome über einige Tage und schieden nur jeweils Rota- oder Coronavirus aus. Gewichtsverluste waren gering oder nicht vorhanden. Schwerere Durchfallsymptome wurden dagegen bei 6 geimpften Tieren beobachtet. Drei dieser Tiere wurden am 6. Lebenstag und drei am 8. Lebenstag testinfiziert. Keines dieser Tiere schied Rotavirus oder Coronavirus vor der Testinfektion aus. Zwei bis vier Tage nach der Testinfektion bekamen jedoch alle sechs Tiere Durchfall und schieden Rotavirus aus. Drei Tiere schieden einige Tage später zusätzlich Coronavirus aus. Kälber, die am 6. Lebenstag testinfiziert wurden, erkrankten weniger stark als am 8. Lebenstag testinfizierte Tiere. Die Gewichtsverluste betrugen 4 bzw. 8 %.

Die Ergebnisse zeigen, daß eine erfolgreiche aktive Impfung von Kälbern gegen Rota- oder Coronavirus induzierten Durchfall durch spezifische Kolostrumantikörper verhindert werden kann. Damit werden frühere Beobachtungen bestätigt, die auf Feldstudien basierten.

Résumé

Recherches de laboratoire sur la vaccination orale de veaux contre la diarrhée à Rotavirus et à Coronavirus

L'efficacité d'un vaccin oral pour des veaux avec un vaccin vivant modifié (Scourvax®-2, Smith Kline) contre la diarrhée à Rotavirus et/ou à Coronavirus a été testée dans des conditions contrôlées. Les veaux ont été vaccinés une heure après le premier repas. L'infection-test a été faite avec un mélange de Rota- et de Coronavirus virulents bovins.

Une partie des veaux ont reçu du colostrum qui ne contenait pas d'anticorps contre les deux virus. Trois veaux de contrôle de ce groupe ont développé de graves symptômes de diarrhée deux à trois jours après l'infection-test, au 6ème jour de vie. La durée de la diarrhée fut de 5—12 jours. Les veaux ont perdu en moyenne 12,5 % du poids. Les trois veaux excrétèrent Rota- et Coronavirus. On n'a pas observé par contre de diarrhée au 6ème jour de vie après une infection-test chez deux veaux vaccinés. Un veau a excrété du Rotavirus et un autre Rota- et Coronavirus après la vaccination. Aucune excrétion virale n'a été observée après l'infection-test. Trois autres veaux qui n'avaient pas été strictement isolés ont fait une diarrhée dans les trois jours suivant la vaccination et ont excrété Rota- et Coronavirus. Ces veaux ont perdu 6—7 % de leur poids.

Une autre partie de l'essai a été faite chez des veaux qui avaient reçu durant deux jours du colostrum avec des anticorps. Ce »pool«-colostrum contenait des anticorps contre les deux virus avec un titre typique pour l'exploitation hollandaise. Un des trois veaux de

contrôle ayant reçu ce colostrum a fait une grave diarrhée au 6ème jour après l'infection-test et a excrété les deux virus. Les deux autres veaux ne développèrent que de légers symptômes de diarrhée durant quelques jours et excrétèrent seulement soit Rota- soit Coronavirus. Les pertes de poids furent minimes ou inexistantes. De graves symptômes de diarrhée ont été par contre observés chez 6 animaux vaccinés. Trois veaux ont subi une infection-test au 6ème et trois au 8ème jour de vie. Aucune de ces bêtes n'a excrété du Rotavirus ou du Coronavirus avant l'infection-test. Tous les six veaux ont fait une diarrhée 2 à 4 jours après l'infection-test et excrétèrent du Rotavirus. Trois animaux excrétèrent en plus du Coronavirus quelques jours plus tard. Les veaux infectés au 6ème jour de vie furent moins fortement atteints que les veaux infectés au 8ème jour de vie. Les pertes de poids furent respectivement de 4 et 8 %.

Les résultats montrent qu'une vaccination active valable des veaux contre la diarrhée à Rota- et Coronavirus peut être inhibée par des anticorps spécifiques du colostrum. Des observations antérieures, basées sur des études dans le terrain, sont ainsi confirmées.

Resumen

Experimentos de laboratorio sobre la vacunación oral de terneros contra la diarrea inducida por rotavirus o coronavirus

Con una vacuna viva modificada (Scourvax®-2, Smith Kline) se contrastó, bajo condiciones controladas, la eficacia de una vacunación oral en terneros contra la diarrea condicionada por rotavirus y/o coronavirus. Los terneros fueron vacunados una hora después de recibir la primera alimentación. La infección de prueba se llevó a cabo con una mezcla de rotavirus y coronavirus bovinos virulentos.

Una parte de los terneros recibió calostro que no contenía anticuerpos frente a ambos virus. Tres terneros testigos de este grupo desarrollaron síntomas graves de diarrea dos hasta tres días después de la infección de prueba en el día 6° de vida. La duración de la diarrea fue de 5—12 días. Los terneros perdieron como media 12.5 % de peso corporal. Todos los tres animales eliminaron rota y coronavirus. Por el contrario, en dos terneros vacunados no se observó diarrea después de la infección de prueba el día 6° de vida. Tras la vacunación eliminó un ternero rotavirus, el otro rotavirus y coronavirus. Sin embargo, después de la infección de prueba no se observó ya ninguna eliminación de virus. Otros tres terneros, los cuales no habían sido aislados de modo riguroso, tuvieron diarrea en el plazo de 3 días tras la vacunación y eliminaron al mismo tiempo rota y coronavirus. Estos terneros perdieron 6—7 % de peso corporal.

Otra parte de los ensayos se llevó a cabo con terneros que habían recibido durante dos días calostro que contenía anticuerpos. Este fondo común de calostro comprendía anticuerpos contra ambos virus con un nivel de título típico para explotaciones holandesas. Uno de los tres terneros testigos alimentados con este calostro sufrió una diarrea grave y eliminaba ambos virus tras la infección de prueba en el día 6° de vida. Los restantes dos terneros solo mostraban síntomas ligeros de diarrea a lo largo de algunos días y cada vez no eliminaban más que rotavirus o coronavirus. Las pérdidas de peso eran escasas o no las hubo. En cambio, se observaron síntomas graves de diarrea en 6 animales vacunados. Tres de estos animales se infectaron de prueba el día 6° y tres el día 8° de vida. Ninguno de estos animales había eliminado rota o coronavirus antes de iniciarse la infección de prueba. Sin embargo, dos hasta cuatro días después de la infección de prueba padecieron diarrea todos los seis animales, eliminando rotavirus. Tres animales difundieron aditivamente unos días más tarde coronavirus. Los terneros que fueron infectados el día 6° enfermaron con gravedad menor que los animales infectados el día 8°. Las pérdidas de peso fueron del orden de un 4 % y 8 %, resp.

Muestran los resultados obtenidos que una vacunación activa eficaz en terneros frente a la diarrea inducida por rotavirus o coronavirus se puede impedir por medio de anticuerpos calostrales específicos. Con eso se confirman observaciones hechas anteriormente, las cuales se basaban en estudios campales.

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