

## STUDIES ON NEONATAL CALF DIARRHOEA CAUSED BY ROTAVIRUS: TRANSMISSION OF THE DISEASE AND ATTEMPTED VACCINATION OF COLOSTRUM-DEPRIVED CALVES

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### ABSTRACT

THEODORIDIS, A., PROZESKY, L. & ELS, H. J., 1980. Studies on neonatal calf diarrhoea caused by rotavirus: Transmission of the disease and attempted vaccination of colostrum-deprived calves. *Onderstepoort Journal of Veterinary Research*, 47, 31-34 (1980).

Mild to severe scouring could be produced in colostrum-deprived calves with tissue culture-adapted rotavirus and faecal material from field cases of calf diarrhoea. The faeces of experimentally infected calves contained rotavirus for at least 3 days. Pathogenic bacteria were present in one calf only and this calf also showed the most severe gastroenteritis.

Eight calves were vaccinated with a live rotaviral calf diarrhoea vaccine and subsequently challenged with infective rotavirus. Mild scouring was observed after vaccination, but the calves remained normal after challenge. Rotavirus particles were detectable in the faeces for a few days after vaccination and challenge.

### Résumé

ÉTUDE SUR LA DIARRHÉE POST-NATALE DU VEAU AU VIRUS ROTA: TRANSMISSION DE LA MALADIE ET TENTATIVE DE VACCINATION DES VEAUX PRIVÉS DE COLOSTRUM

Une diarrhée faible à sévère peut être produite chez les veaux privés de colostrum avec un virus, rota en culture adapté aux tissus et du matériel fécal provenant de veaux diarrhéiques en pâturage extensif. Les fèces de veau infectés expérimentalement contenaient le virus rota pendant au moins trois jours. Des bactéries pathogènes étaient présentes chez un seul veau atteint d'une gastroentérite la plus sévère.

Huit veaux furent vaccinés avec un vaccin rota vivant, et ces veaux furent ensuite soumis à l'épreuve du virus rota infectieux. Une légère diarrhée fut observée sur les sujets après vaccination, mais les animaux demeurèrent normaux après cette épreuve.

Des particules de virus rota furent détectables dans les matières fécales pendant quelque jours après la vaccination et l'épreuve.

### INTRODUCTION

Rotavirus has repeatedly been identified as one of the aetiological agents of neonatal calf diarrhoea (Mebus, Underdahl, Rhodes & Twiehaus, 1969; Turner, Caple & Craven, 1973; Mayling, 1974; Woode, Bridger, Hall & Dennis, 1974). In the search for an effective vaccine against this virus, it was found that oral vaccination of new-born calves with a tissue culture-attenuated virus gave satisfactory results (Mebus, White, Bass & Twiehaus, 1973). The mode of action in this vaccine is uncertain, but presumably it stimulates cellular resistance or acts as an interference factor. The vaccination of pregnant cows reduced this scouring rate in those herds (Mebus *et al.*, 1973), but its protection is restricted to the colostrum period and depends on the concentration of IgG in the colostrum.

The results of the experimental work on rotavirus infection and the vaccination and challenge of colostrum-free calves are presented in this report.

### MATERIALS AND METHODS

#### Colostrum-free calves

Cross-bred Africander-Hereford cows were brought into the isolation stable from the Institute's farm in an advanced stage of pregnancy. The udders of the cows were covered with canvas bags to prevent the new-born calves from taking colostrum. Immediately after birth the calves were removed to a separate compartment, caged individually, and bucket-fed with milk which had been boiled and treated with 1 g/l of Furazolidone. Twenty-one calves were reared in this manner, being fed 2 l of milk twice daily. Faecal samples for virological and bacteriological investigations were collected before the calves were infected and as soon as a change in the consistency of the faeces was noted. When suitable samples were not available, calves were induced to defecate by applying light pressure on the

abdomen. The pre- and post-infection sera of the calves were examined for complement fixing antibodies.

#### Source of infectious material

1. *Tissue culture-adapted rotavirus.* A rotavirus strain designated R & C15/1976 isolated from calves with diarrhoea at the Municipal Farm Johannesburg (Theodoridis, Prozesky & Els, 1979) was used. Virus of the 4th tissue-culture passage in foetal calf kidney cells (FCK) and 1st passage in LLC-MK<sub>2</sub>\* cells were given to some of the calves (Table 2), each animal receiving 4 ml orally.

2. *Vaccine virus.* A rotavirus-modified live virus vaccine strain\*\* was further subcultured twice in FCK cells, lyophilized, and kept at -20 °C, at the virus concentration of  $2 \times 10^{5.5}$  TCID<sub>50</sub>/ml. Each animal received 4 ml of this virus suspension *per os*, either soon after birth or, in most instances, after the first feed (Table 2).

3. *Gut content.* Gut content from calves suffering from severe diarrhoea and proved by means of electron microscopy to contain rotavirus was used to infect and challenge the colostrum-deprived calves. The material was diluted 1:3 in phosphate buffer, homogenized and centrifuged at 1 500 rpm for 10 min. The supernatant was removed, treated with penicillin, colistim 500 IU/ml and streptomycin 500 mg/ml, and kept overnight at 4 °C. The material was further centrifuged at 4 000 rpm for 1 h and 5 ml of the supernatant administered to the calves *per os*. The details of the inoculation are shown in Table 1.

#### Electron microscopy

With some modifications (Theodoridis *et al.*, 1979), the crude virus preparation technique of Petric, Szymanski & Middleton (1975) was followed. Faecal

\* Lilly Laboratory Corporation, 307-E McCARTY Ind., U.S.A.

\*\* Obtained from Norden Laboratories, Lincoln, Nebraska 68501, U.S.A.

samples were taken as frequently as possible before and after infection, vaccination and challenge, and were kept at  $-20^{\circ}\text{C}$  before processing, the specimens being examined in a Siemens 102 EM.

#### Complement fixation test

The antigen for the complement fixation test (CFT) was prepared from the above-mentioned local rotavirus strain isolate. The LLC-MK<sub>2</sub> cell cultures in tubes were pre-treated with Eagle's media containing trypsin 25  $\mu\text{g}/\text{ml}$  and the maintenance medium 5  $\mu\text{g}/\text{ml}$  (Babiuk, Mohamed, Spence, Fauvel & Petro, 1977). A 1:1000 dilution of the FCK-adapted virus in media containing 25  $\mu\text{g}/\text{ml}$  trypsin was seeded into the tissue culture (TC) tubes. After 1 h adsorption at  $37^{\circ}\text{C}$  the tubes were washed and maintenance media without serum were added. The cultures were harvested at 48 h by scraping the cells off the glass when the cytopathic effect had involved more than 50% of the cells. The medium containing the infected cells constituted the final CF antigen. The CFT was performed according to the method of Cunningham (1973).

#### Fluorescent microscopy

Pieces of duodenum, jejunum and ileum were taken at autopsy and stored at  $-20^{\circ}\text{C}$ . Small pieces of these tissues were sectioned in a microtome to a thickness of 6–8  $\mu\text{m}$ , mounted on slides and fixed in cold acetone at  $-20^{\circ}\text{C}$  for 10 min, using the direct fluorescent antibody (FA) technique of Mebus, Stair, Underdahl & Twiehaus (1971). The conjugate, prepared against the calf rotavirus strain Nebraska\*, was kindly supplied by Mme A. M. Delvaux\*\*. It has been reported (Theodoridis *et al.*, 1979) that the Nebraska strain is antigenically related to the local rotavirus strain. The indirect FA technique was used to test the sera of infected and vaccinated calves. Rotavirus-infected LLC-MK<sub>2</sub> cells were treated with the calf sera at  $37^{\circ}\text{C}$ , washed in phosphate buffer and stained with conjugated antiovine globulin\*\*\* at 1:20 dilution.

## RESULTS

#### Transmission of rotavirus

Colostrum-deprived calves showed scouring when infected with either rotavirus-infected gut content or tissue culture-adapted rotavirus (Table 1). Rotavirus particles were invariably observed in the faeces of all infected calves (Table 1) and in the pre-infection faecal sample of Calf 10. No other calf had previously been kept in the cage of this animal. Except for Calves 15 and 16, the body temperatures of the others remained normal. The onset of scouring varied from 24–96 h (Table 1), the faeces being light-yellow in colour, and having a strong odour.

Calves 1, 2, 20 and 21, which received tissue culture-adapted virus, developed severe diarrhoea within 24 h and became listless. Calves 20 and 21 were sacrificed 36 h after infection. Calves 1 and 2 recovered within 4 days.

Calf 17 showed scouring for 11 days and on the 9th day the faeces had a brownish colour. Bacteriological examination showed that its faeces on the 10th day

were positive for pathogenic *Escherichia coli*, and on the 11th day this calf died. It must be mentioned here that faecal samples of the 6th–10th days were not examined bacteriologically.

Thin sections of intestine of the experimentally infected calves were negative when stained with the FA technique, using the Nebraska rotavirus conjugate.

#### Vaccination of calves

Of the 8 calves vaccinated with the tissue culture-attenuated rotavirus vaccine, 5 were challenged with faecal material positive for rotavirus (Table 2). Five calves showed scouring at least once after vaccination, but no scouring was observed after challenge (Table 2). Rotavirus particles were observed in the faeces after vaccination in 7 out of 8 animals and in some cases a corona-like virus was detectable (Table 2). Calf 5, which was weak at birth and had to be killed after vaccination, showed mucoid nasal discharge and excessive salivation. On several occasions its faeces contained blood, and its joints (carpal and metacarpal) were warm and swollen. A pure culture of *Klebsiella* 54 was cultivated from the joint fluids.

Calf 7 showed scouring on the 2nd day after birth and before vaccination. Its condition deteriorated suddenly on the day it was vaccinated and it had to be killed later that day. Bacteriological examination of the faeces before and after vaccination showed that only normal gut inhabitants were cultivated. Rotavirus was detected in the faeces before and after vaccination. Calf 12 was a poor drinker, hence the delay in the vaccination. Faeces were obtained only on the 4th day after vaccination and were negative for rotavirus, while post-challenge samples were positive (Table 2). No rotavirus particles were detected in the faecal samples of the control calf, which was kept in the same room, but in a separate cage.

#### Electron microscopy

Particles resembling rotavirus were observed in the majority of the samples examined after infection, vaccination and challenge (Tables 1 and 2), and coronavirus-like particles were detected in 3 of the calves (Table 2). In most instances single capsid particles were predominant. The concentration of particles was higher in the early samples (after infection, vaccination and challenge) than in the later samples.

#### Complement fixation test

The CFT titres of the sera of some experimental calves are presented in Table 3. Calves 2, 3 and 10 received an oral dose of virulent rotavirus, calves 8 and 12 received the rotavirus vaccine and were subsequently challenged with virulent virus, and calf 9 received only the vaccine. The test revealed that all 6 calves were negative for CF antibodies at birth but that they became positive after infection or vaccination.

#### Fluorescent antibody staining

All thin sections of intestinal tissues of the experimentally infected calves were negative when stained with the conjugate of the Nebraska rotavirus agent. All the post-infection sera gave a positive reaction at 1:4 dilution, while the pre-infection sera were negative. The fluorescence observed was confined to the cytoplasm and was diffuse in character.

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TABLE 1 Results of oral inoculation of rotavirus in colostrum-deprived calves

| Calf No. | Type and origin of inoculum                     | Age at inoculation (hours) | Onset of diarrhoea after infection (hours) | Electron microscopy of gut content |
|----------|---|----------------------------|--|------------------------------------|
| 1        | Tissue culture antigen R & C 15/76** FCK 4..... | 5                          | 48   | Positive rotavirus                 |
| 2        | Tissue culture antigen R & C 15/76** FCK 4..... | 3                          | 36   | Positive rotavirus                 |
| 3        | Gut content of Calf 16.....                     | 8                          | 36   | Positive rotavirus                 |
| 10       | Gut content of Calf 21.....                     | 54                         | 72   | Positive rotavirus                 |
| 15       | Gut content of natural case R & C 12/76-A.....  | 10                         | 96   | Positive rotavirus                 |
| 16       | Gut content of natural case R & C 12/76-A.....  | 10                         | 36   | Positive rotavirus                 |
| 17       | Gut content of natural case R & C 12/76-A.....  | 52                         | 48   | Positive rotavirus                 |
| 18       | Control.....                                    | —                          | —  | Negative                           |
| 19       | Gut content of Calf 17.....                     | 72                         | —*   | Positive rotavirus                 |
| 20       | Tissue culture antigen R & C 15/76 FCK 4.....   | 2                          | 31   | Positive rotavirus                 |
| 21       | Tissue culture antigen R & C 15/76 FCK 4.....   | 4                          | 24   | Positive rotavirus                 |

\*\* R & C 15/76 FCK 4=Reo and Corona, 15th sample, year of isolation passages in foetal calf kidney cell cultures

\* Animal was sacrificed 24 h after infection

TABLE 2 Response of vaccinated calves to the challenge of virulent rotavirus

| Calf No. | Age vaccinated (hours) | Scouring post-vaccination (hours) | Challenge post-vaccination (hours) | Scouring post-challenge | Virus demonstration in electron microscope |                         |
|----------|------------------------|-----------------------------------|------------------------------------|-------------------------|--|-------------------------|
|          |                        |                                   |                                    |                         | Post-vaccination                           | Post-challenge          |
| 4        | 24                     | 24                                | 120                                | Neg.                    | Rotavirus                                  | Corona-like             |
| 5        | 24                     | 48                                | N.C.*                              | —                       | Rotavirus                                  | N.C.                    |
| 6        | 4                      | 48                                | 98                                 | Neg.                    | Rotavirus                                  | Rotavirus               |
| 7        | 72                     | **                                | **                                 | **                      | Rotavirus                                  | N.C.                    |
| 8        | 16                     | 32                                | 168                                | Neg.                    | Rotavirus                                  | Corona-like             |
| 9        | 5                      | 48                                | N.C.                               | —                       | Rotavirus                                  | —                       |
| 11       | 72                     | Neg.                              | 48                                 | Neg.                    | Rotavirus + corona-like                    | Rotavirus + corona-like |
| 12       | 96                     | Neg.                              | 96                                 | Neg.                    | Neg.***                                    | Rotavirus               |

\* N.C. = Not challenged, died soon after vaccination

\*\* = Killed 6 h after vaccination

\*\*\* = Faeces were taken only on 4th day post-vaccination

TABLE 3 CF antibody demonstration in the sera of experimental calves

| Calf No. | CF titres             |                              |                    |                |
|----------|-----------------------|------------------------------|--------------------|----------------|
|          | USA rotavirus vaccine |                              | Virulent rotavirus |                |
|          | Pre-vaccination       | Post-vaccination & challenge | Pre-infection      | Post-infection |
| 2        | —                     | —                            | < 1:4              | 1:16           |
| 3        | —                     | —                            | < 1:4              | ≥ 1:48         |
| 10       | —                     | —                            | < 1:4              | ≥ 1:48         |
| 8        | < 1:4                 | 1:48                         | —                  | —              |
| 9*       | < 1:4                 | 1:48                         | —                  | —              |
| 12       | < 1:4                 | 1:27                         | —                  | —              |

\* Received only USA vaccine

< Less than

≥ More than or equal to

#### DISCUSSION

The detection for several days of rotavirus in the faeces of infected calves was in line with the observations of Mebus *et al.* (1973) and Woode, Bridger, Jones, Flewett, Bryden, Davies & White (1976). Similarly, the calves in this experiment recovered after a short period of the scouring and exhibited the listlessness described by Bridger & Woode (1975), Mebus *et al.* (1973) and MacNulty, McFerran, Bryson, Logan

& Curran (1976). This observation supports the notion that this infection may frequently take place in a subclinical form.

Many of the calves involved in our experiment, before being infected or vaccinated, developed scouring for a brief period 48 h after birth, but neither rotavirus nor coronavirus-like particles were present in their faeces. Similarly, McNulty *et al.* (1976) observed that the scouring of many calves was not

associated with rotavirus infection. Calf 7 may have contracted the infection prior to treatment from the bars of the cage where previously another calf had been kept.

Mild attacks of diarrhoea similar to those of the infected calves were observed in the group of vaccinated animals. Rotavirus particles were detectable in the faeces of these animals until the 3rd day after vaccination. However, no scouring was observed after challenge of the vaccinated calves, though rotavirus and coronavirus-like particles were observed until the 4th day. The shedding of rotavirus for 3 days after vaccination and 4 days after challenge-inoculation indicates an active replication of these viruses in the epithelial cells of the gut, possibly accompanied by considerable cell destruction. It is also evidence that the attenuated strain does not interfere or inhibit the replication of the virulent strain in the intestine epithelium and, if pathogenic agents such as *E. coli* had been present, the clinical outcome might have been different. In view of these findings it is not surprising that, on several farms in South Africa, there has been no reduction in scouring among calves immunized with this oral vaccine.

#### ACKNOWLEDGEMENTS

The authors wish to thank Dr M. M. Henton for the bacteriological tests. Thanks are also due to Mrs E. J. du Plessis, Miss A. E. Bodenstein and Mr W. C. Holscher for their excellent assistance.

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