



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

NEONATAL CALF DIARRHEA INDUCED BY ROTAVIRUS

G. CASTRUCCI,^{1*} F. FRIGERI,¹ M. FERRARI,² V. CILLI,¹
G. L. GUALANDI² and V. ALDROVANDI³

¹Istituto di Malattie Infettive, Profilassi e Polizia Veterinaria dell'Università di Perugia, Perugia,
²Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia, Brescia and ³Centro
Provinciale Svezamento Vitelli, Tripoli S. Giorgio, Mantova, Italy

(Received 11 February 1988)

Abstract—This presentation summarizes the results of a comprehensive study on rotaviruses isolated in Italy from calves and rabbits affected by neonatal diarrhea.

The results clearly indicated that rotavirus infection is widespread and supported the evidence for an etiologic role of these viruses in neonatal diarrhea. The evidence of differences in virulence among bovine rotaviruses appeared also to be confirmed.

Conventionally reared calves were fully susceptible to the experimental infection induced by three rotaviruses originating from heterologous hosts, i.e. monkeys, pigs and rabbits, respectively.

When rotavirus strains of bovine, simian, porcine and rabbit origin were compared by cross neutralization tests, it was found the simian and porcine strains were indistinguishable and both appeared to relate antigenically to the bovine strain. On the other hand, a reciprocal antigenic correlation was found between bovine and rabbit isolates.

Finally, it was proven that feeding newborn calves with colostrum of their dams, previously vaccinated with an inactivated rotavirus vaccine, could prevent the neonatal diarrhea from occurring.

Key words: Neonatal diarrhea, calves, rotavirus

DIARRHÉE NEONATALE DU VEAU PROVOQUÉE PAR ROTAVIRUS

Résumé—Cette présentation résume les résultats d'une étude d'ensemble sur des rotavirus isolés en Italie sur des veaux et lapins frappés de diarrhée néonatale.

Les résultats indiquaient clairement que l'infection par rotavirus est répandue, et corroboraient l'évidence d'un rôle étiologique de ces virus dans la diarrhée néonatale. L'existence des différences de virulence entre les divers rotavirus bovins apparaissait également confirmée.

Les veaux élevés de manière conventionnelle étaient pleinement réceptifs à l'infection expérimentale provoquée par trois rotavirus provenant d'hôtes hétérogènes—respectivement singe, porc et lapin.

Quand les souches de rotavirus provenant de bovin, singe, porc et lapin ont été comparées au moyen de tests de neutralisation croisés, il a été découvert que les souches simienne et porcine étaient indistinguables et qu'elles semblaient toutes deux s'apparenter antigéniquement à la souche bovine. D'autre part, une corrélation antigénique réciproque a été découverte entre les isolats de bovin et de lapin.

Enfin, il a été prouvé qu'en nourrissant les veaux nouveaux-nés avec le colostrum de leurs mères, préalablement vaccinées au moyen d'un vaccin de rotavirus inactivé, l'on pouvait éviter l'apparition de la diarrhée néonatale.

Mots-clefs: Diarrhée néonatale, veaux, rotavirus

*Address correspondence to: Dr G. Castrucci, Facoltà di Medicina Veterinaria, Via S. Costanzo 4, 06100 Perugia, Italy.

INTRODUCTION

Our study on this topic began with the publication in 1981 of a review on neonatal diarrhea in animals caused by viral agents [1]. In that article the various aspects of the disease from etiologic, pathogenetic and immunological points of view, were considered as well as for each viral infection the respective clinical-induced condition was also reported. Rotavirus infection was chosen for a detailed study because of its high frequency in the newborn and the consequent need to obtain useful data which eventually would lead to the preparation of a vaccine to be used for the prevention of the disease.

The study had several objectives, namely: (1) isolation and identification of bovine and rabbit rotavirus strains; (2) serological typing of rotavirus strains of calves; (3) experimental infection and cross immunity tests conducted with various strains of bovine rotavirus; (4) serological study on the distribution of bovine rotavirus in Italy; (5) comparison of rotavirus strains isolated from cattle, monkeys, rabbits and swine; (6) passive immunity to rotavirus infection in newborn calves.

The present paper summarizes the findings made.

EXPERIMENTAL PROCEDURES—RESULTS

Isolation and identification of rotavirus from calves and rabbits

A total of 12 rotavirus strains were isolated, of which 9 (81/31F, 81/32F, 81/33F, 81/35F, 81/36F, 81/37F, 81/38F, 81/40F, 82/80F) were from newborn calves [2] and 3 (82/309F, 82/310F, 82/311F) from young rabbits [3], all suffering from diarrhea. The isolates were obtained in cultures of an established cell line of fetal *Rhesus* monkey kidney (MA-104) inoculated with fecal specimens collected from the diarrheic animals. The isolates produced cytopathic effects (CPE) consisting of foci of rounded cells which tended to aggregate linearly and containing cytoplasmic inclusions of different sizes and shapes. The replication of the virus and the production of the CPE appeared to be strictly dependent on the presence of the trypsin (5 $\mu\text{g/ml}$) in the culture medium. On the other hand the attempts to grow the isolates on bovine embryo kidney (BEK) cell cultures, with or without trypsin, were unsuccessful.

The study of the growth curve was performed in MA-104 cell cultures with two representative strains of the virus (81/36F, 81/40F) (Fig. 1). After an initial period of about 2 h, during which time the titer underwent a reduction of 1.5 (81/40F)–2.0 (81/36F) log units, the curve increased logarithmically, reaching a maximum of $10^{5.50}$ tissue culture infectious doses (TCID)₅₀/0.2 ml, after 24 h in the case of 81/40F and of $10^{6.00}$ TCID₅₀/0.2 ml, after 48 h in the case of 81/36F. After 48 h of incubation the titer began to decrease slightly.

The isolates produced plaques on MA-104 after 5 days of incubation. The plaques were rounded, with irregular edges and varied from 1 to 5 mm in dia.

The isolates were completely resistant to a pH of 3.0 and to ether and partially sensitive to chloroform. They were inactivated by a temperature of 50°C and also, they were not stabilized by 2 M MgCl₂. The viruses contained ribonucleic acid (RNA), since their multiplication was not inhibited by 5-bromo-2'-deoxyuridine. The size of the viral particle was about 85 nm in dia. The electron microscopy study of negatively stained preparations of the virus, revealed a typical rotavirus morphology (Fig. 2).

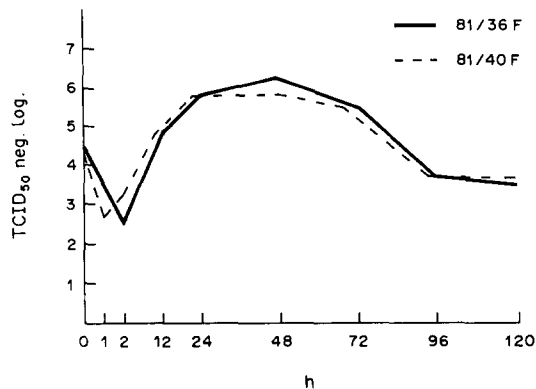


Fig. 1. Growth curve of two strains of bovine rotavirus in MA-104 cell cultures.

All 12 isolates were devoid of haemagglutinating activity for red cells of guinea-pig, rabbit, sheep and cattle.

Serological identification of the isolates was carried out by neutralization tests in presence of immune serums to the Nebraska Calf Diarrhea Virus (NCDV) and to the British Compton strain of bovine rotavirus.

Serological cross-neutralization reactions were exhibited by one representative of the rabbit isolates (strain 82/311F), and one strain of bovine rotavirus (81/36F) [3].

Serological typing of calf rotavirus isolates

It has been demonstrated that rotaviruses from various animal species can be differentiated by sero-neutralization tests [4]. However, it is not precisely known whether this procedure can be applied to rotaviruses isolated from the same species. It has been hypothesized that pathogenic strains of calf rotavirus exist which do not possess protective antigens [5].

A study on this subject has been conducted in this Institute on the 9 rotavirus strains isolated from cattle, using the plaque reduction neutralization tests [2]. These strains were compared with the reference NCDV and Compton rotavirus strains using immune serums obtained from rabbits. In these tests a fixed dilution of antiserum (20 median neutralizing doses) were tested against a fixed concentration (100 TCID₅₀) of either homologous or heterologous virus and the results were evaluated according to criteria used for enteroviral classification [6].

Of the 9 viral strains examined, 7 (81/31F, 81/33F, 81/35F, 81/36F, 81/38F, 81/40F, 82/80F) were identical as a reciprocal relationship was found among them. By contrast, there was a one way antigenic relationship between the remaining 2 strains (81/32F, 81/37F) and the other isolates [2]. A similar situation was found on comparing the 2 reference bovine strains of rotavirus with the isolates. In this case there was also a one way antigenic relationship in that neither NCDV nor Compton strains were able to neutralize any of the heterologous viruses tested.

Based on these results, the existence of an antigenic variability among the bovine rotavirus seems to be a possibility. On the other hand, this opinion appears to be supported by a similar study where haemagglutination-inhibition tests were used [7].

Experimental infection and cross immunity tests in calves with strains of bovine rotavirus

After the first isolation of a bovine rotavirus [8], the rotavirus infection was experimentally reproduced in gnotobiotic [8] and colostrum-deprived calves [9, 10], as well as in conventionally reared animals [5].

Following the isolation in Italy of 9 bovine rotaviruses [2] 4 of the strains (81/32F, 81/36F, 81/40F, 82/80F) were selected for experimental infection and 3 (81/36F, 81/40F, 82/80F) for cross protection tests in conventionally-reared calves [11]. Forty calves, 2–3 days of age, were used.

For the experimental infection study, 16 calves were selected. For each of the 4 viral strains under study, 3 calves were infected, each receiving 30 ml (9×10^7 TCID₅₀) of the virus culture orally. Each of the remaining 4 calves was given 30 ml of culture medium and served as control. Infected and control calves were kept in separate pens.

During the 1-month observation period all animals were clinically examined and their rectal temperatures were recorded daily. In addition fecal and pharyngeal swabs were taken at predetermined intervals after infection. Blood samples for serological testing were also taken just before virus inoculation (day 0) and at post infection day (PID) 30.

On PID 30, when the calves were completely recovered, they were used to produce immune serum to each virus strain. Accordingly, each calf was challenged with the same virus given originally, in a dose of 10 ml (3×10^7 TCID₅₀) by the oral route. In addition, 2 ml (6×10^6 TCID₅₀) of virus culture, mixed with an equal volume of Freund's incomplete adjuvant was given by subcutaneous inoculation to each calf. Two weeks after this treatment, calves were bled, the serum was separated and pools were made according to the groups of calves. The serum pools were then stored at -30°C in volumes of 100 ml until administered to calves in the cross protection tests.

Twenty-four calves were used for cross-protection tests. The animals were divided into 4 groups of 6. Each calf in group 1 received 100 ml orally of antiserum to 81/36F isolate; calves of groups 2 and 3 were orally given antiserum to isolates 81/40F and 82/80F, respectively. Each of the animals of group 4 was given culture medium and served as virus control. One hour later the calves were challenge exposed. Accordingly, each of the 3 challenge virus (81/36F, 81/40F, 82/80F), was given orally in a volume of 30 ml (9×10^7 TCID₅₀) to 2 calves of each of the 4 groups. Fecal swabbings for virus recovery and blood samples for serology were obtained from each calf at predetermined intervals after the challenge infection. The results of the experimental infection and cross immunity tests are presented in Tables 1 and 2 respectively.

Table 1. Clinical response of calves to inoculation with bovine rotavirus strains

Calves No.	Viral strain	Diarrhea			
		Calves affected No.	Onset ^a PID	Duration ^a days	Outcome of infection
3	81/36F	3	3.0	15.6	1D; 1K; 1S
3	82/80F	3	3.3	7.0	3S
3	81/32F	3	4.3	10.0	3S
3	81/40F	3	2.3	9.0	1K; 2S
Controls 4	MEM	0	NA	NA	NA

^aAverage value; PID: post infection day; D: dead; K: killed; S: survived; MEM: culture medium; NA: not applicable.

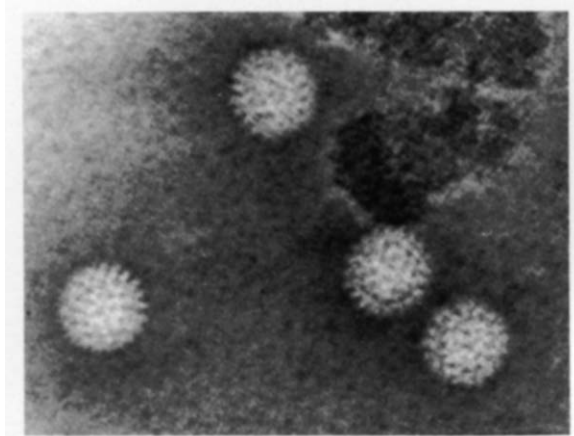


Fig. 2. 81/36F strain of bovine rotavirus. Negative staining $\times 98,000$

Table 2. Cross protection tests in calves with strains of bovine rotavirus

Calves No.	Immune serum to strain	Challenge viral strain	Diarrhea		
			Calves affected No.	Onset ^a PID	Duration ^a days
2	81/36F		0	NA	NA
2	81/40F		2	3.5	6.0
2 ^b	82/80F	81/36F	2	4.5	10.0
Controls					
2	MEM		2	3.5	12.5
2	81/40F		0	NA	NA
2	81/36F		1	2.0	1.0
2	82/80F	81/40F	1	5.0	1.0
Controls					
2	MEM		2	5.5	8.5
2	82/80F		0	NA	NA
2	81/36F		0	NA	NA
2	81/40F	82/80F	0	NA	NA
Controls					
2	MEM		2	3.0	9.5

^aAverage value.

^bOne calf died on post infection day (PID) 30; MEM: culture medium; NA: not applicable.

The findings from experimental infection can be summarized as follows: after 2–4 days of incubation, all animals became anorexic and depressed and most had a febrile reaction. All had diarrhea, 2–4 days after infection and voided yellowish, fluid feces sometimes containing mucous and /or blood. Most of the calves had sunken eyes and were markedly dehydrated. Symptoms were most severe in calves infected with 81/36F isolate. One calf in this group died on PID 28; this calf had profuse diarrhea for 25 days and it was particularly emaciated when it died. At necropsy its small intestines were thin-walled and the mucosa was generally congested. A second calf from the same group was killed on PID 9, having experienced extremely severe diarrhea and high fever. The lesions in this calf were limited to the ileum and were characterized by thickening of the mucosa. An additional calf, inoculated with 81/40F isolate, was killed on PID 3. When killed it was extremely depressed, febrile and had profuse diarrhea. At necropsy the mucosa of the small intestines appeared markedly congested. The surviving calves had diarrhea for a maximum of 7–10 days, then their general condition improved progressively, reaching complete recovery in approx. 2 weeks.

Virus was repeatedly recovered from the feces of the calves from PID 1 to PID 13. The calves infected with strain 81/40F had neutralizing antibody at a titer of 1:4 on day 0, while on PID 30 the average titer of the group was 1:180. The calves of the other 3 groups were negative for antibody on the day they were infected. On PID 30 the titer was 1:24 in the calves inoculated with 81/80F strain and 1:32 in the calves infected with either 81/32F or the 81/36F rotavirus strain.

Animals in the control group never had diarrhea and at the end of the observation period they appeared to be in good condition.

As previously mentioned, cross immunity tests were conducted with 81/36F, 81/40F and 82/80F isolates. Selection was based on the criterium of using viruses representing three distinct outbreaks of neonatal diarrhea. From Table 2 it can be seen that immune serums to the three isolates mentioned above protected, with two exceptions, against challenge

Table 3. Distribution of rotavirus neutralizing antibody among calves, heifers and adult cows in Italy

Animals	No. of serums	Year of collection of serum samples								
		1964			1972-1973			1981-1982		
		No.	%	AMT	Positive serums 1:4 or higher					
Calves	433	184	92.46	1:16	23	100	1:16	210	99.53	1:32
Heifers	547	167	95.97	1:16	34	100	1:8	338	99.71	1:64
Adults	1990	166	97.07	1:32	1046	99.71	1:32	764	99.22	1:32
Totals	2970	517	95.03		1103	99.72		1312	99.39	

AMT: Average maximum titer.

infection with either 81/40F and 82/80F isolates. This protection was complete, in that the animals did not show any clinical signs of the disease during the entire observation period. The two exceptions refer to calves from the group treated with immune serum to 81/36F and 82/80F isolates respectively, and challenged with 81/40F virus. These calves experienced diarrhea for 1 day on PID 2 and PID 5 respectively. A different situation was offered by calves infected with 81/36F isolate, where only the animals pretreated with homologous serum were protected, whereas those calves which were immunized with the heterologous serums were not. These animals were diarrheic, depressed and sometimes febrile. One of them died on PID 30. At necropsy the mucosa of the small intestines of this calf was thin-walled and congested.

Virus was recovered repeatedly from feces of calves that were challenged with 81/36F isolate, while in the case of calves challenged with other viruses, the isolation of virus was rather sporadic. Virus was not isolated from any tissue sample obtained from the calf infected with 81/36F isolate, which died.

Virus was recovered from PID 2 through 8 from all calves that served as control of the challenge viruses. All of them reacted with clinical signs of disease, i.e. depression, fever and diarrhea.

At the time of infection all calves had antibodies to the strains of rotavirus under study. On PID 30 the titers of the calves that were challenged with 81/36F isolate did not vary; whereas those of the animals infected with either 81/40F or 82/80F strains tended to decrease. This also occurred in the case of control calves.

A serologic survey of bovine rotavirus infection in Italy

Serums were obtained from calves, heifers and adults in 1964, 1972-1973 and 1981-1982 from several Italian regions. The total number of samples was 2970. Just prior to testing the serums were inactivated at 56°C for 30 min.

The serum neutralization test [12] was used for all serologic studies reported herein. It was carried out in 96-well microtiter plates, using strain 81/36F of bovine rotavirus in MA-104 cell cultures.

The results, which are shown in Table 3, indicate that rotavirus infection is very common in Italy, with the incidence being well over 90% of the cattle tested. The high percentage of positive results in serum samples collected in 1964 would seem to confirm the hypothesis that the virus was introduced in Italy previous to that year. Surveys, somewhat similar to the present one, have been conducted in several countries, such as Germany [13], Great Britain [5], Sweden [14], France [15], the United States [16] and Japan [17].

The apparent worldwide distribution of rotaviruses, their enormous host range, their multiplicity of serotypes and the fact they attack the newborn over the period of greatest risk, identify them as one of the the most serious agent with which the veterinarian and the livestock owner have to contend.

Comparative study of rotavirus originated from different animal species [18, 19]

Two very interesting findings have arisen as a result of the most recent studies on rotaviruses. First it has been shown that a rotavirus from one species can infect members of certain other species. Second, a serotypic similarity between human and several animal rotaviruses has been reported [20]. These findings, i.e. the possible inter-species transmission of rotaviruses, as well as the fact that certain human and animal rotaviruses may share subgroup or serotypic antigens, raise the question as to whether the taxonomic segregation of these viruses into different groups, according to the animal species of origin, could be supported any longer.

In the present study 4 strains of rotavirus of bovine (81/36F strain), simian (SA-11 strain), porcine (ED strain) and rabbit (82/311F strain) origin, respectively, are compared. The comparison included the serologic correlation among the viral strains by serum neutralization tests and experimental infection in calves.

The results of serologic comparison among the strains under study, can be summarized as follows: the SA-11 simian rotavirus seemed to be identical to ED porcine rotavirus. On the other hand, a one way antigenic relationship was found to exist between 81/36F bovine rotavirus and the simian and porcine rotaviruses, with the former virus (bovine rotavirus) probably representing an antigenically predominant strain among the three viruses under study [18]. The rabbit rotavirus strain was not compared with either simian or porcine rotaviruses, however, as formerly stated it appeared reciprocally related to 81/36F bovine rotavirus [3].

For the experimental infection trials, 23 conventionally-reared Friesian calves, 1–2 days of age, were divided into 5 groups, of which 3 groups consisted of 5 animals each, whereas the other groups were composed of 6 and 2 animals, respectively. The calves of the first 3 groups were exposed orally to rotavirus strains isolated originally from cattle, pigs and monkeys, respectively, whereas the calves in the group of 6 received the rabbit rotavirus. Each calf received approx. 9×10^7 TCID₅₀ of the appropriate virus. The remaining 2 calves were given minimum essential medium (MEM) and served as a control.

All strains of rotavirus used in this experiment appeared to be pathogenic for newborn calves. After an incubation period of 2–3 days all inoculated calves became depressed and anorexic; all were afebrile but had severe diarrhea for 3–10 days, and the virus was reisolated from their feces. Seven calves died, of which 2 were from the group inoculated with bovine rotavirus, 4 among those that were given porcine rotavirus and 1 of those exposed to the monkey isolate. The control calves remained healthy and virus was not isolated from their feces (Table 4).

Unfortunately, there is insufficient information to evaluate the possibility of inter-species infection under natural conditions. However, according to this study, and in view of many other successful experiments, it seems reasonable that inter-species spread of rotaviruses would be possible under field conditions also. If this could be proven, it might be interesting to investigate the possible existence of just one rotavirus which is potentially able to infect regardless of animal species.

Table 4. Response of calves to oral inoculation of rotavirus of bovine, simian, porcine and rabbit origin

Inoculation	Diarrhea					
	No. of calves	Affected calves No.	Onset ^a PID	Duration ^a days ^b	Survival rate ratio	Viral recovery from fecal swabs PID/No. of calves
Bovine 81/36F	5	5	3.0	7.7	3/5*	2/5, 4/4, 6/5, 8/3, 10/3
Simian SA-11	5	5	4.0	7.0	4/5**	2/5, 4/5, 6/3, 8/4, 10/2
Porcine ED	5	5	4.0	5.0	1/5***	2/5, 4/5, 6/4, 8/2, 10/2
Rabbit 82/311F	6	6	5.6	3.1	6/6	2/2, 4/5, 6/4, 8/6, 10/5
Control MEM	2	0	NA	NA	2/2	Negative

^a Average values.

^b As determined in calves that survived the infection; PID Post infection day; calves died on PID 7 and 10 (*), 12 (**) or 5, 11, 12 and 13 (***); MEM: culture medium; NA: not applicable.

Studies on passive immunity in calf rotaviral infections

Attempts to prevent rotavirus-associated diarrhea have been made since 1973. The first approach consisted of vaccinating newborn calves with an oral modified live vaccine [21]. The use of the vaccine apparently decreased calf morbidity and mortality [22, 23]. However, more recent field studies failed to substantiate the efficacy of the vaccine [24, 25].

The second attempt was to passively immunize the calf by stimulating the dam, through vaccination, to secrete antibody in the colostrum and milk [26, 27, 28]. There was evidence that feeding immune colostrum from cows vaccinated with inactivated calf rotavirus vaccine, delayed the onset of diarrhea and reduced its incidence, duration and severity in a naturally occurring outbreak of the disease [27].

In this section are reported the results of our studies, in which the efficacy of colostrum from vaccinated cows in protecting calves against experimentally induced [29] or naturally occurring rotavirus infection [30], was tested.

Protection of calves fed immune colostrum against experimentally-induced rotavirus infection. Strain 81/36F of bovine rotavirus [2] grown in MA-104 cell cultures was used as antigen. The virus was at its 18th passage and had a titer of $10^{6.74}$ TCID₅₀/0.2 ml.

The infectivity of the virus was inactivated by overnight (14 h) incubation at 4°C with 0.5% formaldehyde. One portion was then emulsified in an equal volume of Freund's incomplete adjuvant and drawn in volumes of 2.0 ml in disposable plastic syringes. The remainder of the suspension was distributed in vials in volumes of 10.0 ml. The two vaccine preparations were stored at 4°C until used.

Of 23 pregnant Friesian cows, 14 were vaccinated with the rotavirus for the production of immune colostrum, whereas the other 9 served as unvaccinated controls for the normal colostrum. Vaccination was started 6 weeks before calving when each cow received 2 ml of the emulsified vaccine subcutaneously in the dewlap. The second preparation of vaccine (10 ml) was given by the same procedure 2 weeks before calving. The cows were bled prior to vaccination and at calving time.

Colostrum from the first and second milkings after calving was obtained from vaccinated and not vaccinated cows. Pools of either the immune or normal colostrum were made and stored at -30°C until fed to calves. In addition, samples of milk for serology were taken from the vaccinated cows 10 days after calving [29].

Twenty-six Friesian calves, from 8 to 12 h old, born from rotavirus unvaccinated cows, were subdivided into 3 treatment groups: namely immune colostrum or group A with 10 calves; normal colostrum or group B with 8 calves and control group C with 8 calves. The colostrum supplement was fed to the calves by substituting 200 ml of milk with 200 ml of

appropriate colostrum pool, twice daily. Colostrum treatment of calves (group A and B) was started 24 h after their arrival at the laboratory, and was continued daily for the next 9 days.

Soon after the second colostrum-supplemented meal, all calves, i.e. the colostrum treated calves and the controls, were inoculated with 81/36F bovine rotavirus, each calf receiving 30 ml of the culture orally. The calves remained on experiment for 30 days. During this time they were observed twice daily, with particular attention to the character of the feces. Rectal swabbings for the recovery of virus [2] were taken from all calves at the time of infection (time 0), and again on PID 2, 4, 8, 10 and 12. In addition, blood samples for serology were obtained from each calf on PID 30.

The calves which received immune colostrum (group A) remained clinically healthy throughout the observation period, while all calves which were given normal colostrum (group B), and 7 of 8 of the group of the control calves (group C), had diarrhea. The main live weight gain was 201 g per day for calves which were fed the immune colostrum, whereas it was 45 and 71 gs in the case of calves which received the normal colostrum and in the control calves, respectively.

Virus was isolated only once, on PID 2, from rectal swabbings of 2 calves which were treated with immune colostrum. By contrast, virus was isolated repeatedly from PID 2 to 12 from the control calves and from those which received normal colostrum.

Antibody titers of the serums of vaccinated cows averaged 1:18 prior vaccination; the titer increased to 1:200 at calving. The whey prepared from the colostrum pool of these cows (immune colostrum) was 1:1024, whereas in milk samples taken from the cows 10 days after calving the antibody titer dropped to 1:23. The serums taken at calving from unvaccinated cows had a titer of 1:50, while the colostrum of these cows (normal colostrum) neutralized the virus at a titer 1:32.

When the calves were subdivided into three treatment groups (time 0), the average neutralizing antibody titer to 81/36F bovine rotavirus was 1:15, for group A, 1:12 for group B and 1:25 for group C. At PID 30 the antibody titer did not vary in group C, whereas there was a slight increase in the other two groups, the titer being 1:35 (group A) and 1:25 (group B).

Protection of calves fed immune colostrum against naturally-occurring rotavirus infection. The trials were conducted in 26 dairy herds with a history of neonatal diarrhea in the last 5 years. The selected cows in each herd were randomly subdivided into two groups. Cows in one group (248 head in total) were vaccinated according to the procedure mentioned above, whereas cows in the other group (210 head in total) served as unvaccinated controls.

A pool of colostrum from the first and second milkings after calving was obtained from each vaccinated or control cows. The newborn calves were raised according to the existing management practices of the herd, with the exception that, beginning the second day after birth, and for the next consecutive 7–10 days, each calf was fed a supplement of 400 ml of colostrum obtained from its dam. The calves were observed for 30 days for the appearance of diarrhea. When diarrhea occurred, fecal swabbings were taken and cultured for virus.

Tests for the presence of neutralizing antibody to 81/36F bovine rotavirus were performed on 80 colostrum samples obtained from cows of 6 randomly selected herds among the 26 considered for the vaccination trial.

Attempts to recover rotavirus were made from 34 fecal swabbings obtained from diarrheic calves in 12 herds. Of these, 7 were from calves born from vaccinated cows

Table 5. Neutralizing antibody to 81/36F strain of bovine rotavirus in the colostrum of vaccinated^a and unvaccinated cows in six randomly selected herds

Herd No.	Antibody in the colostrum of:			
	Vaccinated cows		Unvaccinated cows	
	Samples No.	Titer ^b	Samples No.	Titer ^b
2	5	496.00	5	48.00
3	10	492.00	10	44.00
11	5	224.00	5	20.00
17	5	224.00	5	40.00
19	7	840.00	7	63.00
21	8	475.00	8	77.00
Totals	40	458.50	40	48.75

^a81/36F bovine rotavirus, inactivated vaccine.

^bAverage reciprocal value.

whereas the remainder 27 samples were collected from calves delivered by unvaccinated dams.

The whey prepared from the colostrum of cows from 6 randomly selected herds, had an average neutralizing titer to 81/36F bovine rotavirus of 1:458.50 or 1:48.75, respectively, for vaccinated cows or for the untreated controls (Table 5).

The diarrhoea (Table 6) occurred in 86 of the 210 (40.9%) calves which were delivered by unvaccinated cows (control calves) in 24 herds out of the 26 selected for the trials. Diarrhoea was also observed in 7 calves of the 248 (2.8%) born from vaccinated cows (immune calves). The latter were found from 4 herds included in the 24 mentioned above. In the control calves, diarrhoea usually appeared within 24–72 h from birth and was consistently associated to a marked dehydration which in 52 calves terminated with death 3–15 days after onset of diarrhoea. In the calves which survived, the diarrhoea lasted from 10 to 15 days; however, the calves recovered very slowly and their general condition was still very poor several weeks after recovery.

The diarrhoea which was observed in the 7 calves which were born from vaccinated cows developed within 3 days from birth and lasted from 3 to 4 days. The diarrhoeic calves did not show any other sign of disease, and all recovered in about one week without any significant loss in their condition.

Virus was not isolated from any of the 7 diarrhoeic calves born from vaccinated cows. By contrast, rotavirus was isolated from 11 of the 27 fecal swabbings that were collected from the control calves.

Table 6. The incidence of diarrhoea and mortality associated with diarrhoea, in calves fed with colostrum from their vaccinated^a or unvaccinated dams

Herds ^f No.	Calves							
	Fed with immune colostrum ^b				Fed with normal colostrum ^c			
	Diarrhoea		Dead		Diarrhoea		Dead	
	No. ^d	%	No. ^e	%	No. ^d	%	No. ^e	%
26/24	7/248	2.8	0/248	0	86/210	40.9	52/210	24.7

^a81/36F bovine rotavirus, inactivated vaccine.

^bFrom vaccinated cows.

^cFrom unvaccinated cows.

^dNo. of calves with diarrhoea/No. of calves considered.

^eNo. of calves which died/No. of calves considered.

^fHerds considered/herds where calf diarrhoea occurred.

The results of this study seem to strengthen both the opinion that the rotaviruses have an important etiological role in the neonatal diarrhoea of calves, and the suggestion that passive immunity would represent a logical way to prevent the disease [27, 30]. As far as the latter aspect is concerned, the aim might be attained by the way indicated in these tests and also suggested by others [27, 28, 30], i.e. (a) vaccination of the dams with an inactivated vaccine and, (b) feeding newborn calves throughout the period of greatest risk (the first 7–10 days of their life) with colostrum of their dams as part of the diet.

CONCLUSIONS

Calf diarrhoeas have been recognized for decades as a serious disease problem in eventually all areas where calves are reared, especially under intensified production methods. However, little progress had been made in resolving their etiology or in developing control measures until the studies of Mebus *et al.* in the late 1960s [8]. Despite having isolated strains of rotavirus and demonstrating their capacity to cause disease, their work was largely ignored. However, its veracity was recognized years later when it was found [31] that by addition of a minute amount of trypsin to cell culture medium, the virus could be isolated with relative ease and recognized by its characteristic CPE.

The serological results clearly indicate that rotavirus infection is widespread and it poses a serious threat to the calf rearing industry [12].

It has been confirmed that rotaviruses are able to induce experimental infection in conventionally reared calves [11]. This finding supports the evidence for an etiologic role of bovine rotavirus in neonatal calf diarrhoea. The evidence of differences in virulence among the bovine rotaviruses seems also to be confirmed. This finding could explain the variability observed in the severity of the disease in natural occurring outbreaks.

It seems noteworthy that conventionally reared calves were fully susceptible to the experimental infection induced by three rotaviruses originating from heterologous hosts (simian, porcine and rabbits) [18, 19]. These findings lend support to the possibility that interspecies spread of rotaviruses may occur under natural conditions.

Moreover when these 4 isolates were compared by cross serum neutralisation tests it was found the simian SA-11 and porcine strain ED were indistinguishable and both appeared to relate identically to bovine strain 81/36F. On the other hand a reciprocal antigenic correlation was found also, between bovine and rabbit isolates.

Data obtained from these studies seem to confirm the existence of antigenically predominant rotavirus strains such as 81/36F bovine rotavirus, which could eventually be used for developing immunizing products with a wide range of uses.

Acknowledgements—The present researches were supported by Consiglio Nazionale delle Ricerche, Progetto Finalizzato per il Controllo delle Malattie da Infezione, grant Nos 82.02391.52, 83.00637.52, 84.01841.52, 85.00835.52, 86.01581.52 and by the Department of Education, funds for scientific researches, 40 and 60%.

REFERENCES

1. Cilli V. and Castrucci G. Viral diarrhoea of young animals: a review. *Comp. Immun. Microbiol. infect. Dis.* **4**, 229–242 (1981).
2. Castrucci G., Ferrari M., Frigeri F., Cilli V., Donelli G., Angelillo G. and Bruggi M. A study of cytopathic rotavirus strains isolated from calves with acute enteritis. *Comp. Immun. Microbiol. infect. Dis.* **6**, 253–264 (1983).
3. Castrucci G., Ferrari M., Frigeri F., Cilli V., Perucca L. and Donelli G. Isolation and characterization of cytopathic strains of rotavirus from rabbits. *Archs Virol.* **83**, 99–104 (1985).
4. Thouless M. E., Bryden A. S., Flewett T. H., Woode G. N., Bridger J. C., Snodgrass D. R. and Herring

- J. A. Serological relationships between rotaviruses from different species as studied by complement-fixation and neutralization. *Archs Virol.* **53**, 287–294 (1977).
5. Woode G. N. Epizootiology of bovine rotavirus infection. *Vet. Rec.* **103**, 44–46 (1978).
 6. Melnick J. L., Dalldorf G., Enders J. F., Gelfand H. M., Hammon W. McD., Huebner R. J., Rosen L., Sabin A. B., Syverton J. T. and Wenner H. A. Classification of human enteroviruses. *Virology* **16**, 501–504 (1962).
 7. Spence L., Fauvel M., Petro R., Gillani A. and Babiuk L. A preliminary study of antigenic relationships among isolates of bovine rotavirus. *Proc. 2nd Int. Symp. Neonatal Diarrhea, University of Saskatchewan*, pp. 263–272 (1978).
 8. Mebus C. A., Underdahl N. R., Rhodes M. B. and Twiehaus H. J. Calf diarrhea (scours) reproduced with a virus from a field outbreak. *Neb. Agric. exp. Stat. Res. Bull.* **233**, 1–16. (1969).
 9. Bridger J. C. and Woode G. N. Neonatal calf diarrhoea: identification of a reovirus-like (Rotavirus) agent in faeces by immunofluorescence and immuno electron microscopy. *Br. vet. J.* **131**, 528–535 (1975).
 10. De Leeuw P. W., Van Nieuwstadt A. P. K. M. I., Van Balken J. A. M. and Ellens D. J. Rotavirus infection in calves. II. Experimental infections with a dutch isolate. *Tijdschr. Diergeneesk.* **102**, 515–524 (1977).
 11. Castrucci G., Frigeri M., Frigeri F., Cilli V., Caleffi F., Aldrovandi V., Rampichini L. and Tangucci F. Experimental infection and cross protection tests in calves with cytopathic strains of bovine rotavirus. *Comp. Immun. Microbiol. infect. Dis.* **6**, 321–332 (1983).
 12. McKercher D. G., Castrucci G., Frigeri F., Ferrari M. and Cilli V. A serologic survey of bovine rotavirus infection in Italy. *Comp. Immun. Microbiol. infect. Dis.* **7**, 165–169 (1984).
 13. Reissbauer K., Romer H., Wagenseil F., Albrecht E. and Oesterle P. Rotavirus infection in cattle herds in the Tübingen area. *Tierärztl. Umsch.* **36**, 334–336 (1981).
 14. Moreno-Lopez J. A serosurvey of viruses outbreaks of acute respiratory and/or enteric disease in Swedish cattle. *Zentbl. Vet Med. B* **26**, 634–640 (1979).
 15. Schwers A., Barrat J., Blacou J., Maenhoudt M. and Pastoret P. Recherche d'anticorps anti-rotavirus dans des serums d'animaux sauvages en France. *A. Med. Vet.* **127**, 651–654 (1983).
 16. Schlafer D. H. and Scott F. W. Prevalence of neutralizing antibody to the calf rotavirus in New York cattle. *Cornell Vet.* **69**, 262–271 (1979).
 17. Sato K., Inaba Y., Shinozaki T., Matumoto M. Neutralizing antibody to bovine rotavirus in various animal species. *Vet. Microbiol.* **6**, 259–261 (1981).
 18. Castrucci G., Frigeri F., Ferrari M., Cilli V., Aldrovandi V., Gatti R. and Rampichini L. A comparison of rotavirus strains of bovine, simian and porcine origin. *Eur. J. Epid.* **1**, 274–280 (1985).
 19. Castrucci G., Frigeri F., Ferrari M., Cilli V., Aldrovandi V., Caleffi F. and Gatti R. Comparative study of rotavirus strains of bovine and rabbit origin. *Comp. Immun. Microbiol. infect. Dis.* **7**, 171–178 (1984).
 20. Hoshino Y., Wyatt R. G., Greenberg H. B., Flores J. and Kapikian A. Z. Serotypic similarity and diversity of rotavirus of mammalian and avian origin as studied by plaque-reduction neutralization. *J. infect. Dis.* **149**, 694–702 (1984).
 21. Mebus C. A., White R. G., Bass E. P. and Twiehaus M. J. Immunity to neonatal calf diarrhoea virus. *J. Am. vet. med. Ass.* **163**, 880–883 (1973).
 22. Thurber E. T., Bass E. P. and Beckenahuer W. H. Field trial evaluation of a reo-coronavirus calf diarrhea vaccine. *Can. J. comp. Med.* **41**, 131–136 (1977).
 23. Twiehaus M. J. and Mebus C. A. Licensing and use of the calf scour vaccine. *Proc. A. Meet. U.S. Anim. Hlth. Ass.* **77**, 55–58 (1974).
 24. Bürki F., Schusser G. and Szekely H. Clinical, virological and serological evaluation of the efficacy of peroral live rotavirus vaccination in calves kept under normal husbandry conditions. *Zentbl. Vet Med. B* **30**, 237–250 (1983).
 25. De Leeuw P. W., Ellens D. J., Talmon F. P. and Zimmer G. N. Rotavirus infections in calves: efficacy of oral vaccination in endemically infected herds. *Res. Vet. Sci.* **29**, 142–147 (1980).
 26. Snodgrass D. R., Fahey K. J., Wells P. W., Campbell I. and Whitelaw A. Passive immunity in calf rotavirus infections: maternal vaccination increases and prolongs immunoglobulin G1 antibody secretion in milk. *Infect. Immun.* **28**, 344–349, (1980).
 27. Snodgrass D. R., Stewart J., Taylor J., Krantil F. L. and Smith M. L. Diarrhoea in dairy calves reduced by feeding colostrum from cows vaccinated with rotavirus. *Res. vet. Sci.* **32**, 70–73 (1982).
 28. Van Opendenbosch E., Wellemans G., Strobbe R., De Brabander D. L. and Boucqué Ch.V. Evolution des anticorps anti rota dans le lait de vaches traitées en fin de gestation soit par le vaccin anti rota complet, soit par l'adjuvant seul. *Comp. Immun. Microbiol. infect. Dis.* **4**, 293–300 (1980).
 29. Castrucci G., Frigeri F., Ferrari M., Cilli V., Caleffi F., Aldrovandi V. and Nigrelli A. 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 (1984).
 30. Castrucci G., Frigeri F., Angelillo V., Ferrari M., Cilli V. and Aldrovandi V. Field trial evaluation of an inactivated rotavirus vaccine against neonatal diarrhoea of calves. *Eur. J. Epid.* **3**, 5–9 (1987).
 31. Babiuk L. A., Mohammed K., Spence L., Fauvel M. and Petro R. Rotavirus isolation and cultivation in the presence of trypsin. *J. clin. Microbiol.* **6**, 610–617 (1977).