



**HAL**  
open science

**THE EXPERIMENTAL PRODUCTION OF  
DIARRHOEA IN COLOSTRUM DEPRIVED AXENIC  
\* AND GNOTOXENIC CALVES WITH  
ENTEROPATHOGENIC ESCHERICHIA COLI,  
ROTAVIRUS, CORONAVIRUS AND IN A  
COMBINED INFECTION OF ROTAVIRUS AND E.  
COLI**

Ph. Gouet, M. Contrepolis, H.C. Dubourguier, Y. Riou, R. Scherrer, J.  
Laporte, J.F. Vautherot, J. Cohen, R. L'Haridon

► **To cite this version:**

Ph. Gouet, M. Contrepolis, H.C. Dubourguier, Y. Riou, R. Scherrer, et al.. THE EXPERIMENTAL PRODUCTION OF DIARRHOEA IN COLOSTRUM DEPRIVED AXENIC \* AND GNOTOXENIC CALVES WITH ENTEROPATHOGENIC ESCHERICHIA COLI, ROTAVIRUS, CORONAVIRUS AND IN A COMBINED INFECTION OF ROTAVIRUS AND E. COLI. *Annales de Recherches Vétérinaires*, 1978, 9 (3), pp.433-440. hal-00901024

**HAL Id: hal-00901024**

**<https://hal.science/hal-00901024>**

Submitted on 11 May 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**THE EXPERIMENTAL PRODUCTION OF DIARRHOEA  
IN COLOSTRUM DEPRIVED AXENIC \* AND GNOTOXENIC CALVES  
WITH ENTEROPATHOGENIC *ESCHERICHIA COLI*, ROTAVIRUS,  
CORONAVIRUS AND IN A COMBINED INFECTION  
OF ROTAVIRUS AND *E. COLI***

Ph. GOUET \*\*, M. CONTREPOIS \*\*, H.C. DUBOURGUIER \*\*, Y. RIOU \*\*,  
R. SCHERRER \*\*\*, J. LAPORTE \*\*\*, J.F. VAUTHEROT \*\*\*, J. COHEN \*\*\*,  
et R. L'HARIDON \*\*\*

\*\* Laboratoire de Microbiologie - I.N.R.A. - C.R.Z.V. de Theix - 63100 Beaumont - France

\*\*\* Station de Virologie-Immunologie - I.N.R.A. - Route de Thiverval - 78850 Thiverval Grignon - France

**Résumé**

REPRODUCTION EXPERIMENTALE DE LA DIARRHÉE CHEZ DES VEAUX AXENIQUES OU GNOTOXENIQUES PRIVÉS DE COLOSTRUM, PAR UN *ESCHERICHIA COLI* ENTEROPATHOGENE, UN ROTAVIRUS, UN CORONAVIRUS, ET L'ASSOCIATION DU ROTAVIRUS-*E. COLI*. — La reproduction expérimentale du syndrome diarrhéique du veau nouveau-né a été tentée par infection orale de 17 veaux gnotoxéniques privés de colostrum avec deux sérotypes d'*E. coli* Ent<sup>+</sup> K99<sup>+</sup>, un rotavirus et un coronavirus.

Avec *E. coli* seul, une dose de  $2 \times 10^8$  bactéries à l'âge de 24 heures provoque une légère diarrhée seulement, alors que  $1 \times 10^{10}$  bactéries, conduisent à la mort avec déshydratation. Avec le rotavirus l'inoculation est suivie d'un syndrome diarrhéique plus ou moins prononcé, le déclenchement de la maladie correspond toujours à l'apparition de grandes quantités de virus dans les matières fécales. Ces animaux ont présenté une phase d'anorexie mais aucun n'est mort ni n'a été déshydraté.

Le coronavirus a provoqué une diarrhée aqueuse très abondante suivie de la mort de l'animal déshydraté.

L'inoculation du rotavirus, non létale en elle-même, suivie d'une inoculation d'*E. coli* aux doses non létales de  $3 \times 10^8$  à  $2 \times 10^9$  a entraîné la mort avec déshydratation.

Les auteurs concluent que la mort de l'animal avec déshydratation peut être provoquée par des doses importantes d'*E. coli* ou de coronavirus ou par l'absorption successive de deux doses non létales de rotavirus et *E. coli*.

**Introduction**

Research carried out in recent years has

done much to increase our knowledge into the etiology of diarrhoea in the newborn calf. The presence of rotavirus and coro-

\* Terminology used is from Raibaud *et al.* (1966): axenic = germfree, gnotoxenic = axenic animals inoculated with known bacteria, holoxenic = conventional animals, oligoxenic = animals with few bacterial species.

navirus which has been confirmed in a high proportion of faeces from diarrhoeic animals (Mebus *et al.*, 1969, Stair *et al.*, 1972, Woode and Bridger, 1975, Scherrer *et al.*, 1976), and the experimental production from these same viruses of diarrhoea, in colostrum-deprived gnotoxenic animals (Mebus *et al.*, 1969, 1971, 1973, Woode *et al.*, 1974), leave no doubt as to their importance in etiology.

The presence of *E. coli* in the blood and the organs of sick newborn calves and the high number in the jejunum are factors which have long been recognised (Smith-Orcutt 1925, Gay 1965). Much more recently it has been discovered that most of these strains possess a common antigen (K99) which enables them to attach themselves to the intestinal mucosa (Orskov, 1975) and to settle in the small intestine. These strains secrete an exclusively heat stable enteropathogenic toxin (enterotoxin) [Kaeckenbeck *et al.*, 1977] which causes diarrhoea (Dubourguier, 1977). The syntheses of the K99 antigen and the enterotoxin are coded by plasmids (Gyles, 1974). These facts leave no room for doubt as to the importance of these *E. coli* strains in the etiology of neonatal diarrhoea (Kaeckenbeck, 1977).

Attempts at producing diarrhoea experimentally, have been carried out using different serotypes of *E. coli*, however both the methods, on the one hand, and the results obtained on the other are often questionable, on account of the possibility of complications caused by other bacterial species of the intestinal microflora. Furthermore, the frequent co-occurrence of the virus and *E. coli* might suggest that a combined infection is responsible for the diarrhoea. Thus Stair *et al.* (1972, 1973) make a disputable claim, that *E. coli* may aggravate the outcome of the sickness brought on by the rotavirus.

The object of our present work was to produce diarrhoea by experimentally infecting colostrum-deprived gnotoxenic and normal calves. Using animals of different ages, we have administered orally or intra-duodenally several doses of the following pathogenic agents, either separately or in combination: 2 major serotypes of *E. coli* which have been established as enteropathogenic (Ent<sup>+</sup> K99<sup>+</sup>), rotavirus and coronavirus. We have recorded the clinical observations, the length of survival and the establishment of pathogenic *E. coli* in the digestive tract.

## Material and methods

### 1. ANIMALS

Most of the calves used in these experiments were delivered and reared according to gnotobiotic techniques (see terminology, foot note at the beginning of the article).

— Eight axenic calves (group A) were delivered by aseptic caesarian section into an isolator according to a technique which has already been described (Riou *et al.*, 1977) and three axenic calves (group B) delivered by normal caesarian section, were transferred immediately into a sterile isolation unit.

— Five oligoxenic calves (group C) were delivered under normal conditions and then transferred into the isolators through a germicidal liquid lock according to the technique used for lambs, described by Ducluzeau *et al.* (1976). These calves, having been thoroughly washed and rubbed down in the isolator with a one fifth concentration of iodined antiseptic (Bétadine gynecologic solution, Sarget Laboratories, Mérignac, France), received the following mixtures of antibiotics during the three days following birth: 4 g of bacitracin + 2 g of neomycin in the morning and 3 g of ampicillin + 4 g of streptomycin in the evening, diluted each time with one liter of milk. The first dose of antibiotics was administered one hour after birth and the second divided into 3 or 4 parts and given after four hours.

— Four calves (group D) were delivered normally and, having been bathed immediately in the iodined antiseptic, were transferred to a cleaned and disinfected room where they received 0.5 g of each of the antibiotics throughout the entire duration of the experiments.

The animals of groups A, B and C, were fed with sterilised condensed milk diluted with two parts distilled water and those of group D received whole commercial U.H.T. milk.

The animals of groups C and D receiving antibiotics were used solely for the viral infections.

Bacteriological examinations were performed regularly on the faeces from animals of groups A, B and C, according to the techniques used by Riou *et al.* (1977).

## 2. PREPARATION OF THE INOCULA

### *E. coli*

The strains of *E. coli* used have the serotypes a = 0101:K99:H<sup>-</sup> and b = 09:K(A),K99:H<sup>-</sup>. The inocula were obtained after 18 hours growth on a Minca medium (Guinee 1976). A direct microscopic count with a Petroff-Hausser cell counter gives an idea of the number of bacterial cells present in the preparation of the bacterial inoculum, whereas the precise number of living bacteria actually administered is calculated « a posteriori » on a DCA (Difco) medium in Petri dishes.

### *Rotavirus*

The virus used in this work was obtained from the « Fumades » experimental farm (Puy-de-Dôme), where it was isolated from a six days old diarrhoeic calf which was excreting large quantities of rotavirus. The first inoculum was prepared by the following technique: the faeces were clarified first by dilution with three parts distilled water and centrifugation at 5.000 g for 30 min. (Scherrer *et al.*, 1976), and then the supernatant filtered through successive Millipore membranes (8  $\mu$ , 2.2  $\mu$ , 0.8  $\mu$ , 0.45  $\mu$ , 0.22  $\mu$ ): 20 ml of the filtrate was used to inoculate calf C2, which, in its turn, excreted large quantities of rotavirus. All the other inoculations were subsequently performed from the faeces of calf C2, by the same method.

All the inocula of rotavirus used were shown to be free of bacteria and they were kept at -70 °C up to the time of use.

### *Coronavirus*

The virus used was isolated from a calf which died from natural diarrhoea at the « Fumades » experimental farm. The calf's faeces were clarified by dilution and centrifugation as described above for rotavirus. Calf B1 was inoculated with 10 milliliters of the non-filtered crude supernatant and calf D2 was inoculated with 10 ml of filtered supernatant (Millipore membranes 8  $\mu$  to 0.22  $\mu$ ). The intestine of calf B1, which died from experimentally induced diarrhoea, was rinsed with distilled water, and this solution was used to infect two other calves

(D3, D4). These inocula were likewise filtered and kept at -70 °C.

Coronavirus was characterized by electron microscopy after negative staining and polypeptide composition analysis (Laporte, 1978).

## 3. ADMINISTRATION OF THE INOCULA

With the exception of calf B1, all the animals were inoculated orally from a feeding-bottle containing the bacterial or viral inoculum. Calf B1 was anaesthetised inside the isolator and the preparation of coronavirus was directly injected into the duodenum. The ages of the calves and the order in which the inocula were administered are shown in figure 1.

## 4. PARAMETERS MEASURED

The amount of milk consumed by each infected animal was noted; for the first three days the faeces were collected once or twice a day, and then again on the 5th, 7th, 14th and 30th days, in order to assess the quantity of virus in the excreta.

The quantity of virus excreted was estimated by examining the faeces by electron microscopy according to a technique previously described (Scherrer *et al.*, 1976).

The consistency of the faeces from all the animals was classified as normal, mucoid or liquid.

Finally, on the death of the animal, which had been under constant observation, the viscera and the samples were examined for a bacterial flora and viral content.

## Results

The outcome of the infections and the stages in the development of the sickness in each animal are shown in figure 1.

### 1. INFECTIONS BY *E. COLI*

Four calves (A) and one calf (B) received orally a dose of  $1 \times 10^8$  to  $2.1 \times 10^{10}$  *E. coli*, 24 hours after birth.

Calves A2 and A3 which were inoculated 24 hours after birth with  $2 \times 10^8$  *E. coli*, had an attack of diarrhoea, but it was not fatal ;

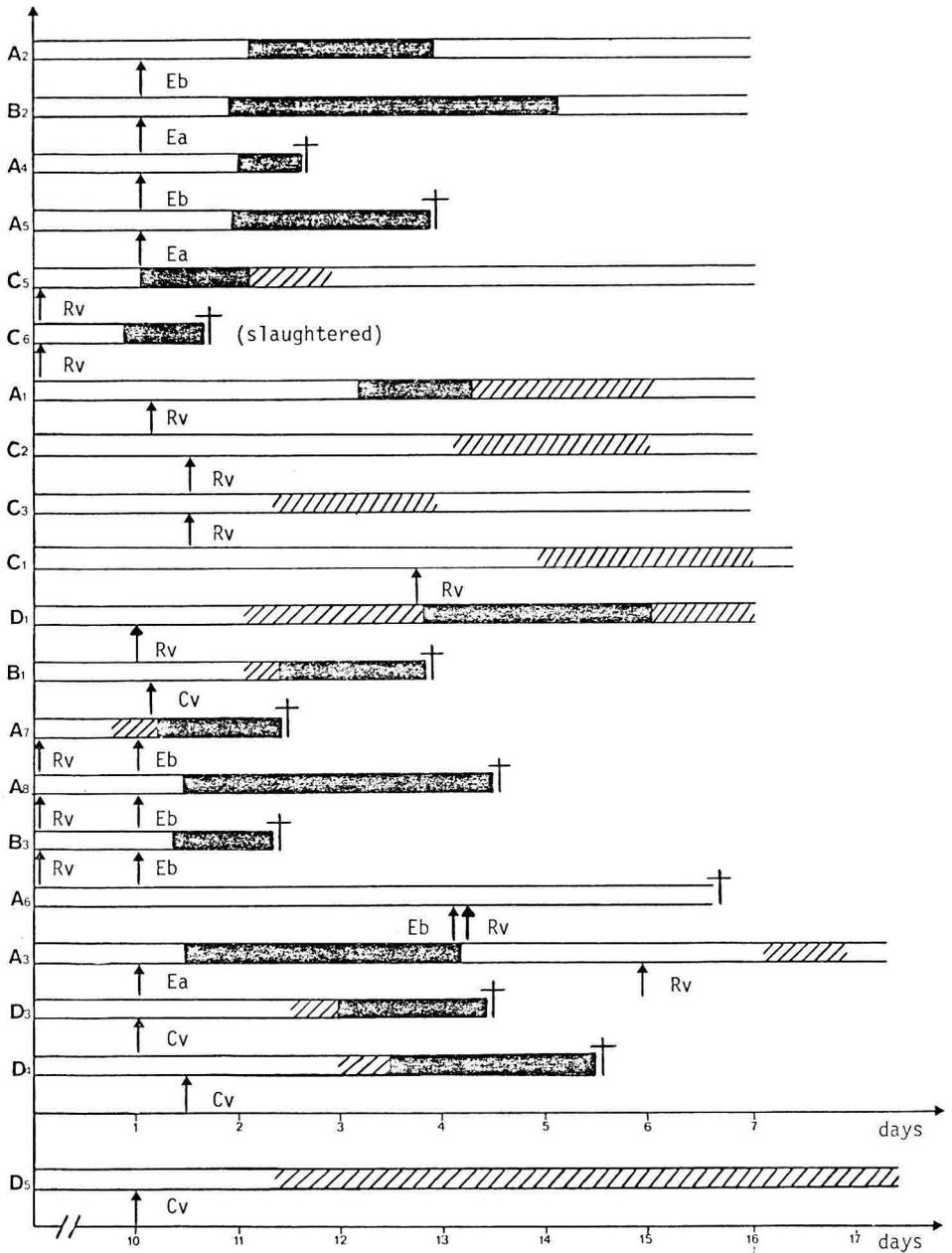


Figure 1.—Evolution and consistency of feces after inoculation by:  
*E. coli* (a) 0101:K99:H: ↑ Ea  
*E. coli* (b) 09:K(A),K99:H ↑ Eb  
 Rotavirus ↑ Rv  
 Coronavirus ↑ Cv  
 Feces normal ——— mucoïd  liquid   
 Death †

Calf B2 which received  $3.6 \times 10^9$  *E. coli* also survived, but suffered very severe diarrhoea. A dose of  $1 \times 10^{10}$  *E. coli* (calves A4 and A5) produced a particularly severe attack of diarrhoea and proved fatal.

Reductions in appetite of varying duration occurred simultaneously with the establishment of these strains in the digestive tract. Thus, calf A3 was anorectic for ten hours prior to the onset of diarrhoea. Calf B2 showed a reduction in appetite while the diarrhoea lasted (2 liters of milk a day instead of 4), but recovered at the end of diarrhoea. Calf A5 consumed approximately 3 liters of milk during the 3 days of very severe diarrhoea and it died in a state of dehydration. An analysis of the bacteria in the intestine revealed very few *E. coli* in the abomasum (3/gram), whereas  $2.5 \times 10^5$ /gram were counted in the jejunum,  $1.2 \times 10^8$ /gram in the ileum and  $8.10^9$ /gram in the caecum. An examination of the blood showed that in this case there was a septicaemia in the final stage (more than  $1 \times 10^4$  *E. coli*/ml of blood).

## 2. VIRAL INFECTIONS

### 2.1. Rotavirus

A total of seven calves were infected with rotavirus:

C5 and C6: 2 hours after birth

A1, C2, C3, D1: between 24 and 36 hours after birth

CA: 4 days after birth

Diarrhoea occurred after each of these inoculations and was of varying degrees of severity and duration (2 to 4 days). There was an excellent correlation between the onset of the sickness and the appearance of large quantities of virus in the faeces. Rotavirus was excreted throughout the entire duration of runny or mucoid diarrhoea and in some cases (A1, C1, C3) beyond this period (1 to 4 days).

Calves C5 and C6 which had been inoculated immediately after birth, produced very runny diarrhoea between 20 and 24 hours after inoculation. In all the other calves which had been inoculated more than 24 hours after birth the diarrhoea occurred later, between approximately 48 and 72 hours after infection. Out of these calves, some produced runny diarrhoea (A1, D2, C3). None of these animals showed any signs of dehy-

dration and they all survived. All the animals were anorectic for the first 24 hours.

With the exception of calves A1 and D1, all were free from bacteria when they were infected with the virus. Calves C2 and C3 were infected with a *Streptococcus* 24 and 36 hours respectively after the onset of diarrhoea. Calves C1, C5 and C6 remained sterile beyond the time of diarrhoea.

### 2.2. Coronavirus

All the animals which had been inoculated with coronavirus in the first 48 hours after birth (B1 intraduodenally, D2 and D3 orally) produced large quantities of watery diarrhoea and died in a state of extreme dehydration. Calf D4, which had received the virus when it was 10 days old, was the only one to survive, after it had produced mucoid diarrhoea for 6 days.

The temperature of the animals rose steadily as the sickness progressed and 48 hours after infection it reached  $39.5^\circ\text{C}$ . It dropped suddenly just before death. The period of hyperthermia was accompanied by a loss of appetite.

Observation by electron microscopy showed that the virus first appeared in the faeces at the onset of diarrhoea. There was a large number present in the few hours before death.

Calf D4 produced the maximum number of virus particles on the 3rd and 4th day; on the seventh day there was no trace of virus in the faeces. Calf B1, which had been sterile, was carrying 4 types of bacteria including *E. coli*, after inoculation with crude coronavirus preparation.

## 3. COMBINED INFECTIONS WITH ROTAVIRUS AND *E. COLI*

A total of five calves (4 of group A, and 1 of group B) were infected with both a rotavirus and *E. coli*.

Calves A7, A8 and B3 which received the inoculum of rotavirus 2 to 3 hours after birth, and then respectively  $3 \times 10^8$ ,  $5 \times 10^8$  and  $2 \times 10^9$  *E. coli* at the age of 24 hours, died with very severe diarrhoea and in a state of dehydration, within 31 and 78 hours after the administration of *E. coli*. In the abomasum of calf A7, which was autopsied a few minutes after it died, we counted  $2 \times 10^7$  cells of *E. coli* per gram, with  $6 \times 10^8$  in the

jejunum and more than  $1 \times 10^8$  per ml of blood. The death from diarrhoea was thus accompanied by septicaemia.

Animal A6 was not infected until it was 4 days old, when it received the rotavirus and  $2 \times 10^9$  *E. coli* simultaneously; it died in a state of extreme dehydration 60 hours after the inoculation. We did not observe diarrhoea because of intestinal occlusion. The gut was swollen with fluid in front of the occlusion. We counted  $1 \times 10^9$  *E. coli* per gram in the jejunum; the sample taken from the abomasum was free from bacteria.

Calf A3 was inoculated first at 24 hours after birth with  $2 \times 10^8$  *E. coli* and then with the rotavirus on the sixth day. Diarrhoea followed the establishment of *E. coli*, then the faeces reverted to their normal consistency. 40 hours after the administration of the rotavirus there was a slight loosening of the faeces, but no other special observations were made.

Calf A7 consumed little milk in the 36 hours it was sick. Calf A8 was anorectic for a short time when the diarrhoea appeared and finally, calf B3 was feeding more or less normally. We did not observe any abnormalities in the feeding behaviour of calves A6 and A3 during the period of combined infection.

## Discussion

In experimentally producing diarrhoea it was necessary to use colostrum-deprived calves in order to avoid passive immunity against the pathogenic agents we were using. Additionally, we had to protect the animals against any risk of infection from outside contaminants. Hence we resorted either to the techniques for rearing gnotobiotic animals (calves in isolators), or to the isolation of the animals under continuous antibiotic treatment in a cleaned and disinfected room.

### 1. INFECTIONS WITH *E. COLI*

The discrepancies noted in the attempts at experimentally producing diarrhoea with *E. coli* (Sojka, 1965) are largely due to the use of animals with an unknown bacteriological status as well as to the use of strains of bacteria of whose pathogenic potentialities was little known.

It is becoming increasingly evident that susceptibility to the infection decreases rapidly during the first 24 hours after birth. The serotype of *E. coli* used, the culture medium (Guinee, 1976) and the number of bacterial cells administered are, like the microbiological status of the animal, predisposing factors, which, if not specified, can lead to totally contradictory results. Contrefois *et al.* (1977) had already shown with axenic lambs that the same inoculum would produce a more severe experimental infection the earlier it was administered, and that, depending on the dose, it was possible to induce a moderate attack of diarrhoea which was not fatal, or else the rapid death of the animals, sometimes, even without diarrhoea. In the present work it is evident that on its own, a small dose ( $1 \times 10^8$ ) of *E. coli* Ent<sup>+</sup> K99<sup>+</sup> will induce a passing attack of diarrhoea, and a large dose ( $1 \times 10^{10}$ ) will induce fatal diarrhoea and dehydration. These results emphasise the importance of selecting the strain of *E. coli* and might explain the failure of Stair *et al.* (1972, 1973) who were unable to bring on the characteristic diarrhoea with their strain of *E. coli*. Finally it has been shown that « non-septicaemic » *E. coli* K99<sup>+</sup> may be present in large numbers in the blood and the organs.

### 2. VIRAL INFECTIONS

Experiments done on gnotoxenic calves with a rotavirus and a coronavirus, which had been isolated in France, indicate clearly, as Mebus *et al.* have already shown (Mebus *et al.*, 1969, 1971, 1973) that these agents on their own can cause diarrhoea, accompanied by a loss of appetite in the newborn calf. Most of our observations on the development of the sickness in animals inoculated with one or the other virus are, moreover, very similar to those described by these authors. Like them, we have noted the comparatively mild nature of certain forms of diarrhoea caused by rotavirus. In our experiments all the animals, passed through a phase of diarrhoea spread over 2 to 3 days, which in all cases was concomitant with the excretion of a large number of viral particles, but all the animals survived and none showed any signs of real dehydration. Since a single isolate of rotavirus was used all the way through this work, we cannot conclude

that other wild strains of the virus all act in the same way.

Some experiments done in the USA and in Great Britain (Mebus *et al.*, 1969, Woode *et al.*, 1974) indicate the possible existence of wild, distinctly more pathogenic strains.

Moreover, the severity of the infections might depend also on the amount of the input virus in the absence of any other determining factor. We must also bear in mind that, on account of the very fact that our isolate of rotavirus did not induce mortality, it was particularly well-suited to the experiments aimed to show that there was a synergy between rotavirus and *E. coli*.

The results obtained with a coronavirus isolated in France confirm that this type of virus might prove to be very pathogenic for the newborn calf. Diarrhoea developed 24 and 36 hours after infection and there was always a large quantity of virus in the faeces. Death occurred between the third and fourth day in animals which have been inoculated within 48 days after birth. The high mortality rate indicates that this virus might have a significant influence on neonatal calf diarrhoea. However it is still difficult to judge at present, for, as yet, we have only a vague idea as to its rate of incidence in the field, on account of the difficulty in detecting this type of virus. Preliminary results (Laporte, 1978) obtained by hemagglutination inhibition test show that our strain of coronavirus is antigenically distinct from the one isolated by Stair *et al.* (1972).

### 3. COMBINED INFECTIONS

The results of combined infections from

*E. coli* and rotavirus demonstrate unequivocally the synergistic effect of the infection from *E. coli* on the infection from rotavirus: the combination of rotavirus and *E. coli* K99<sup>+</sup> in doses not lethal in themselves, induce severe diarrhoea which terminates in dehydration and death. Furthermore, it would be useful to examine the order in which the pathogenic agents are established. Synergy is doubtless most important when there are natural infections from rotavirus, which are, as we know, very common in newborn calves.

### Conclusion

Diarrhoea was reproduced in colostrum-deprived newborn calves by rotavirus, coronavirus or enteropathogenic *E. coli* K99<sup>+</sup>. Dehydration and death can be caused either by a large dose of *E. coli* or coronavirus, or by the inoculation of rotavirus and *E. coli* in two separate doses, not lethal in themselves, administered with a gap of several hours between the two.

### Acknowledgements

We are grateful to Mr. Lefavre, Station de Recherches sur l'Élevage for his capable surgical help, to Mr. Chavarot, Vert, Andant (Laboratoire de Microbiologie, I.N.R.A.) and to Chantal Feynerol and Françoise Rossi (Station de Virologie et d'Immunologie, I.N.R.A.) for their excellent technical assistance.

*Accepted for publication, March 1st 1978.*

### Summary

We attempted to produce diarrhoea experimentally in the newborn calf by orally injecting 17 colostrum-deprived calves with two serotypes of *Escherichia coli* Ent<sup>+</sup> K99<sup>+</sup>, a rotavirus and a coronavirus.

With *E. coli* alone, a dose of  $2 \times 10^9$  bacteria administered 24 hours after birth causes a mild attack of diarrhoea, whereas  $1 \times 10^{10}$  bacteria leads to dehydration and death.

An inoculation of rotavirus is followed by diarrhoea which always contains large quantities of rotavirus. These animals were anorectic for a time, but none was dehydrated or died. With coronavirus, there were large quantities of watery diarrhoea, which led to dehydration and death.

The inoculation of rotavirus, not lethal in itself, followed by a similarly non lethal inoculation of *E. coli* in doses of  $3 \times 10^8$  to  $2 \times 10^9$  led to dehydration and death.

The authors conclude that dehydration and death of the animal can be caused by large doses of *E. coli* or coronavirus or by two non-lethal doses of rotavirus and *E. coli* administered one after the other.

### References

- CONTREPOIS M., GOUET Ph., GOBY J.F., 1977. Production expérimentale de diarrhées colibacillaires chez le veau et l'agneau. Equilibres bactériens dans le tube digestif de veaux et agneaux gnotoxéniques. *S.T.A.L.* Vol. II, 2<sup>e</sup> trimestre, 118-119.
- DUBOURGUIER H.C., 1977. *Etudes des entérotoxines d'Escherichia coli. Importance dans la pathogénie des diarrhées du veau nouveau-né.* Thèse Docteur Ingénieur n° 80. Université Clermont II.
- DUCLUZEAU R., RAIBAUD P., LAUVERGEON B., GOUET Ph., RIOU Y., GRISCELLI C., GHNASSIA J.C., 1976. Immediate postnatal decontamination as a means of obtaining axenic animals and human infants. *Can. J. Microbiol.*, **22**, 563-566.
- GAY C.C., 1965. *E. coli* and neonatal disease of calves. *Bact. Rev.*, **29**, 75-101.
- GUINEE P.A.M., JANSEN W.H., AGTERBARG C.M., 1976. Detection of the K99 antigen by means of agglutination and immunoelectrophoresis in *E. coli* isolated from calves and its correlation with enterotoxigenicity. *Inf. Immun.*, **13**, 1369-1377.
- GYLES C.L., SO M., FALKOW S., 1974. The enterotoxin plasmids of *E. coli*. *J. Inf. Dis.*, **130**, 40-49.
- KAECKENBEECK A., 1977. A propos de l'étiopathogénie de la colibacillose du veau. *Ann. Med. Vet.*, **121**, 149-158.
- KAECKENBEECK A., SCHOENAERS F., PASTORET P.P., JOSSE M., 1977. Absence d'entérotoxine thermolabile chez les *E. coli* entéropathogènes isolées du veau. *Ann. Med. Vet.*, **121**, 55-57.
- LAPORTE J., 1978. Neonatal calf diarrhea coronavirus: purification and polypeptides structure (to be published).
- MEBUS C.A., UNDERDAHL N.R., RHODES M.B., TWIEHAUS M.J., 1969. Calf diarrhea (scours): reproduced with a virus from a field outbreak. *Research. Bull. Univ. Nebraska*, **233**, 1-16.
- MEBUS C.A., STAIR E.L., UNDERDAHL N.R., TWIEHAUS M.J., 1971. Pathology of neonatal calf diarrhea induced by a reo-like virus. *Vet. Pathol.*, **8**, 490-505.
- MEBUS C.A., STAIR E.L., RHODES M.B., TWIEHAUS M.J., 1973. Pathology of neonatal calf diarrhea induced by a coronavirus-like agent. *Vet. Pathol.*, **10**, 45-64.
- RIOU Y., GOUET Ph., DUBOURGUIER H.C., CONTREPOIS M., DARDILLAT C., LEFAIVRE J., 1977. techniques for obtaining, fistulization and rearing of axenic or gnotoxenic lambs, kids and calves. *Ann. Rech. Vet.*, **8**, 13-24.
- SCHERRER R., COHEN J., L'HARIDON R., FEYNEROL C., FAYET J.C., 1976. Reovirus like agent (rotavirus) associated with neonatal calf gastroenteritis in France. *Ann. Rech. Vet.*, **7**, 25-31.
- SMITH Th., ORCUTT M.L., 1925. The bacteriology of the intestinal tract of young calves with special reference to the early diarrhea (scours). *J. Exp. Med.*, **41**, 89-106.
- SOJKA W.J., 1965. *Escherichia coli* in animals. Commonwealth Agricultural Bureau. Farnham Royal, England.
- STAIR E.L., RHODES M.B., WHITE R.G., MEBUS C.A., 1972. Neonatal calf diarrhea: Purification and electron microscopy of a Coronavirus-like agent. *Am. J. Vet. Res.*, **33**, 1147-1156.
- STAIR E.L., MEBUS C.A., TWIEHAUS M.J., UNDERDAHL N.R., 1973. Neonatal calf diarrhea. Electron microscopy of intestines infected with a Reovirus like agent. *Vet. Pathol.*, **10**, 155-170.
- WOODE G.N., BRIDGER J.C., HALL G., DENNIS M.J., 1974. The isolation of a reovirus-like agent associated with diarrhea in colostrum deprived calves in Great Britain. *Res. Vet. Sci.*, **16**, 102-105.
- WOODE G.N., BRIDGER J.C., 1975. Viral enteritis of calves. *Vet. Rec.*, **96**, 85-88.