The Use of Non-Immune Plasma Powder in the Prophylaxis of Neonatal Escherichia coli Diarrhoea in Calves

H. Nollet1,4, H. Laevens2, P. Deprez1, R. Sanchez3, E. Van Driessche3, and E. Muylle1

Addresses of authors: 1Department of Internal Medicine and Clinical Biology of Large Animals, University of Gent, Faculty of Veterinary Medicine, Salisburylaan 133, B-9820 Merelbeke, Belgium; 2Department of Reproduction, Obstetrics and Herd Health, University of Gent, Faculty of Veterinary Medicine, Salisburylaan 133, B-9820 Merelbeke, Belgium; 3Institute for Molecular Biology and Biotechnology; Laboratory of Protein Chemistry, Vrije Universiteit Brussel, Paardenstraat 65, B-1640 Sint-Genesius-Rode, Belgium; 4Corresponding author

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Summary

The protective use of plasma powder from cattle and swine against experimentally induced neonatal E. coli diarrhoea in colostrum-deprived calves was examined. Diarrhoea was induced with a strain expressing F5+ fimbriae and a strain expressing F17+ fimbriae. In all groups supplemented with bovine plasma powder, diarrhoea and fever were less severe than in the control groups. For the groups infected with the F5+ E. coli strain, a reduction in excretion of the challenge strain by 2–4 orders of magnitude and by 1–2 orders of magnitude was seen when supplemented with bovine plasma powder at a dose of 25 g/l milk and 10 g/l milk, respectively.

The bovine plasma powder showed also beneficial effects in the F17+ infected groups. No mortality, no septicaemia and no severe clinical signs were observed. Concerning the excretion of the E. coli F17+ strain in the faeces, no significant difference with the control group was found.

Swine plasma powder showed little beneficial effect on E. coli diarrhoea in calves in this study.

Introduction

Neonatal diarrhoea in calves has generally been ascribed to E. coli strains producing F5, formerly named K99, and/or F41 fimbriae (Gaastra and De Graaf, 1982; De Graaf and Roorda, 1982). More recently, E. coli expressing F17 fimbriae, previously called Att25 in Belgium (Pohl et al., 1982, 1984) and F(Y) in France (Girardeau et al., 1980; Contrepois and Girardeau, 1985), have been shown to be involved in neonatal diarrhoea in calves and some strains are suspected to cause septicaemia (Morris et al., 1985; Shimizu et al., 1987; Van Driessche and Beeckmans, 1993A; Pohl and Mainil, 1995).

Interfering with mucosal colonization is obviously an essential step in the prevention of the disease. The use of antibiotics is limited by the emergence of antibiotic resistant variants (Chopra, 1986; Hinton et al., 1986). Vaccination of the dams has been conducted with success (Rutter et al., 1976; Contrepois et al., 1985) and is nowadays widely used in the prevention of neonatal E.T.E.C. diarrhoea. However, adequate colostrum intake by the calves and the presence of all types of adhesins in the vaccine are essential for clinical protection.

Blocking the receptor-binding site of bacterial lectins by receptor analogues such as glycoproteins constitutes an alternative and/or complementary way to prevent colonization.
vitro studies, carried out by MOURICOUT et al. (1990), showed that glycoprotein glycans of bovine plasma inhibit F5+ mediated E. coli adhesion to erythrocytes. Investigations by SANCHEZ et al. (1993a,b,c) revealed that cow plasma glycoproteins strongly inhibit the attachment of E. coli F17+ cells to mucus and brush border membranes all along the small intestine. Consequently, cow plasma preparations could be an excellent source of adhesion blockers.

These preparations must fulfil several requirements for their applicability in practice. First, they should bind with high affinity to bacterial adhesins, so that they can be used at reasonable concentrations. Secondly, their inhibitory power should not be lost upon partial or complete digestion of their polypeptide part in the stomach or intestine. Thirdly, they should be abundantly available at low cost. Fourthly, the inhibitors should be easy to deliver to the animals, preferentially as a feed additive.

During this study, cattle and swine plasma preparations were tested as inhibitors of the adhesion of E. coli strains producing F5+ or F17+ fimbriae to the intestine of neonatal colostrum-deprived calves.

Materials and Methods

Animals

Twenty-four newborn Holstein Friesian male calves from different farms in the neighbourhood of Gent were used in this study. They arrived at the university between 1 and 6 hours after birth. All calves were colostrum deprived. The animals were randomly divided into eight groups of three calves and were kept in individual pens with straw bedding.

Feeding

The calves were fed 2 l of whole, sterilized milk three times daily until 2 weeks after infection. Starting from the second week, hay and commercial flakes for calves (Kweekkalverkorrels, N.V. Voeders Debaillie, Roeselare, Belgium) were available ad libitum. From the third week on, the sterilized milk was replaced by commercial milk powder for breeding calves.

Categorization of groups

Four groups of three calves were infected with the E. coli F5+ strain. One group was used as a control group and received no plasma powder. A second group received bovine plasma powder at a dose of 25 g/l milk 3 times a day before and until 14 days after the infection. A third group was supplemented with bovine plasma powder at a lower dose (10 g/l milk) during the same study period. Swine plasma powder (dose 25 g/l milk) was supplied to the fourth group infected with the F5+ strain in the same manner.

An identical treatment was applied to the four groups of calves infected with the E. coli strain expressing F17+ fimbriae: a control group receiving no plasma powder supplementation and three treatment groups supplemented as described previously for the groups of calves infected with the F5+ E. coli strain.

Plasma powder

Preparation. The plasma powder (bovine, VEPRO 75 BC; swine, VEPRO 85 PDC) was provided by N.V. VEOS (Zwevezele, Belgium). The plasma powder was prepared from blood, collected from healthy animals in EEC approved slaughterhouses. Trisodium citrate was added to the blood on collection to prevent coagulation. On arrival at the factory, the blood was centrifuged into two fractions: plasma and cells. In all steps, the blood was handled carefully to prevent haemolysis of the red blood cells. The plasma fraction was pasteurized at maximum 50°C for 15 min. After pasteurization the plasma was concentrated either in an evaporator at maximum 37°C or in an ultra filtration unit. The concentrated plasma powder was then spray-dried at a maximum product temperature of 40°C.
**Determination of antibodies against F17+ and/or F5+ fimbriae in the used plasma powder.** Titres of antibodies against F5 and/or F17 fimbriae were determined by ELISA: wells of microtitre plates (NUNC-immuno plate, maxisorp) were incubated overnight at 4°C with either 150 μl of F17+ fimbriae or F5+ fimbriae (2 μg/ml). Non-adsorbed material was removed by washing the plates twice with 20 mM PBS pH 6.8 containing 0.05 % (v/v) Tween 20 and twice with 20 mM PBS. Non-occupied sites were blocked by incubating the wells with blocking reagent (Boehringer Mannheim). All subsequent steps were performed at 37°C, i.e. wells were incubated consecutively with 150 μl of plasma proteins for 1 h, anti-cow IgG-alkaline phosphatase (SIGMA) for 1 h (or protein a-alkaline phosphatase). After each step, the plates were twice thoroughly washed with PBS containing 0.05 % (v/v) Tween 20 and twice with 20 mM PBS pH 6.8. Phosphatase activity was determined using p-nitro-phenylphosphate disodium salt as substrate (2 mg/ml) in N-ethylamine ethanol buffer (20 ml N-ethylamine ethanol and 200 ml 1 M HCl for 1 litre, adjusted to pH 10.2 with NaOH). the absorbance was measured at 405 nm in Biorad 3550 microplate reader.

**Calf inoculation procedure**

The calves were infected with 10^10 CFU of the *E. coli* strains expressing F5+ (strain B85; F5+, ST+) or F17+ (strain 32KH85; F17+). Both strains were selected for resistance to rifampicin. This property allowed to determine and count the excretion of the bacteria before and after infection. The strains were grown overnight on solid LB medium containing rifampicin (100 mg/l) at 37°C. LB medium consists of 10 g bactotryptone, 5 g yeast extract, 10 g NaCl and 100 mg rifampicin for 1 litre of medium, adjusted to pH 7.3 by the addition of NaOH. Bacteria were resuspended and harvested by centrifugation, washed with 20 mM sodium phosphate-buffered saline pH 7.0 and resuspended in milk to get 1.10^10 bacteria/ml.

The presence of F5+ or F17+ pili was tested by an agglutination test using specific antisera raised in rabbits against highly purified F17+ or F5+ fimbriae (VAN DRIESSCHE et al., 1993b).

The inoculum was administered into the back of the mouth with a syringe when the calves were 12–24-h-old and 6–20 h after the first administration of milk (supplemented or not with plasma powder depending on the group). Immediately after infection the calves were fed 21 of milk (and plasma powder as indicated for the groups).

**Parameters**

**Zinc sulphate turbidity (ZST) test.** Immunoglobulin status of the sera of the calves was checked at arrival before the administration of milk, at 5 days and at 38 days after the infection by the method described by MCEWAN et al. (1970). The data of the ZST test are received in units (IU/l). After converting these results, an idea of the corresponding IgG content (mg/ml) is showed in the results.

**Autopsy.** Autopsy was performed immediately after death. Segments of duodenum, midjejunum, ileum and colon and samples of the liver, kidney and spleen were collected for microbiological analysis.

**Body temperature.** Rectal body temperature was measured every morning before feeding.

**Clinical findings.** Appetite, degree of dehydration, general behaviour and appearance were observed during the feeding periods.

**Consistency of faeces.** Faecal consistency was graded every morning as: 0: normal faeces, 1: thick liquid faeces and 2: thin watery faeces.

**Excretion of total coliforms in the faeces.** One gram of rectally collected faeces was suspended in 10 ml of NaCl 0.15 M. Serial dilutions were made in saline (until x10^6) and numbers of CFU per gram of faeces were determined by conventional plating techniques on LB media.

**Excretion of the challenge strains.** The same procedure as described for total coliform excretion
was used for the quantification of the challenge strain excretions, except that a LB medium was used, supplemented with 100 mg of rifampicin per litre, for the F17+ strain and Minca medium supplemented with the same dose of rifampicin for the F5+ strain. Colonies were counted and identified by agglutination with specific antisera against F17+ or F5+ fimbriae.

Parasitological, virological and bacteriological analyses on faeces. Faecal samples were examined for the presence of Cryptosporidium spp. (at day 5), Rota and Corona viruses (at day 7) and Salmonella spp. (at day 7).

Statistical analysis

Differences in the immunoglobulin status (ZST test results) between groups, at days 0 and 5 and per experiment were analysed using one-way analysis of variance (ANOVA) (Statistix 4.0).

For each experimental infection, the influence of the different treatments on the survival time was assessed with Kaplan-Meier Survival Analysis (SPSS, SPSS Inc, IL 60611, USA). The survival times were defined as the period from the day of experimental infection to the day of death or euthanasia. Calves that survived the observation period were censored in the analysis on day 38. The survivor functions per treatment group were compared pairwise with the survivor function of the control group using the Breslow test (or generalized Wilcoxon test).

Fever was defined as a temperature of \(\geq 39.5^\circ\text{C}\). This critical temperature was equal to the one-sided 99\% confidence interval limit of the temperatures monitored on day 0 of the calves in both experimental infections. Febrile periods were described per calf and started when two successive temperatures were both \(\geq 39.5^\circ\text{C}\) and ended with the first of two successive temperatures both \(< 39.5^\circ\text{C}\).

Similarly, periods with abnormal stool were described per calf. Abnormal stool was present when the score was 1 or more (2). Periods with abnormal stool started when two successive scores were both \(\geq 1\) and ended with the first of two successive scores both \(< 1\).

The results of the bacteriological counts were transformed to logarithmic values. The data were analysed statistically using general ANOVA (Statistix 4.0). The least significant difference multiple comparison method was used to detect differences in every paired comparison. Significance was determined at the \(P < 0.05, 0.01\) and 0.001 levels.

Results

Determination of antibodies against F17+ and/or F5+ fimbriae in the plasma powder preparations

In the ELISA experiments no antibodies against either F5 or F17 could be detected in plasma powder preparations used in the experiments described in this paper.

Immunoglobulin status: Zinc sulphate turbidity (ZST) test

The Ig content of the newborn calves, measured on arrival at the University, ranged from 0.05 to 2.25 mg/ml (average 0.39 ± 0.5 mg/ml). The fifth day after the inoculation the Ig content ranged from 1.20 to 4.30 (average 2.54 ± 0.9 mg/ml. At the end of the experiment (38 days after onset) the values varied between 3.43 and 10.15 mg/ml (average 6.83 ± 2.1 mg/ml). For both experiments, no differences could be observed at days 0 and 5, respectively, between the control group and the groups supplemented with either bovine or swine plasma powder (\(P\)-values 0.46 and 0.99, respectively, for the F17+ infected groups at day 0 and 5 and \(P\)-values 0.61 and 0.45, respectively, for the F5+ infected groups at day 0 and 5).

Mortality and autopsy

All control calves inoculated with either the \(E.\ coli\) strain expressing F5+ or F17+ fimbriae died between day 1 and day 7 after the inoculation.

In the group infected with the F17+ bearing \(E.\ coli\) strain and supplemented with pig
plasma powder, two of the three calves were euthanized at day 6 after infection for ethical reasons. In these two calves and also in one control calf infected with the F17 strain, the challenge strain was isolated from the small and large intestine. The isolation of the F17+ strain from spleen and liver was an indication for septicaemia.

In the group infected with the E. coli F5+ strain and supplemented with bovine plasma powder at a dose of 10 g/l, one calf was euthanized at day 7 because of its terminal condition. One calf infected with the E. coli F5+ strain and supplemented with plasma powder of pigs died at day 6 after infection. Bacteriological examination of liver, kidney and spleen of these two F5 infected calves was negative for E. coli F5+.

For each of the experiments, the survivor function, describing the time from inoculation to death, of the control group differed from the survivor functions of the group supplemented with 25 g/l bovine plasma powder (P = 0.0399 for both comparisons in the F17+ and F5+ strain experiment) and the group supplemented with 10 g/l bovine plasma powder (P = 0.0399 for both comparisons in the F17+ and F5+ strain experiment). For the F5+ strain experiment, the comparison of the survivor functions between the control group and the group supplemented with 25 g swine plasma powder per litre was borderline significant (P = 0.0665). For the F17+ strain experiment, the survivor functions between these two groups did not differ significantly (P = 0.4913).

**Clinical signs**

All the calves of both control groups showed signs of lethargy and loss of appetite from day 1 after infection onwards until death.

In the two groups (F5+ infected and F17+ infected) the supplementation with swine plasma powder did not protect against the infection. The calves had poor appetite and showed a marked weight loss. As mentioned above, three of the six calves in these two groups died during the experimental period. A fourth calf died from emaciation two weeks after the end of the experiment. the surviving two calves suffered from poor growth.

The calves supplemented with bovine plasma powder at a dose of 10 g/l also had a loss of appetite during the first 5 days following infection. Those infected with F5+ E. coli strain seemed to suffer more than the F17+ infected group.

The calves supplemented with bovine plasma powder at a dose of 25 g/l remained completely healthy and showed no loss of appetite during the whole duration of the experiment regardless of the infective agent.

**Rectal body temperature (Figs 1, 2)**

Infection with E. coli F17+ induced no significant rise in mean temperature immediately after infection in the group supplemented with the highest dose of bovine plasma powder. One calf supplemented with bovine plasma powder at a dose of 10 g/l had fever (up to 39.7°C) during two days. A second calf of the same group also had a rise in temperature on day 6 and 7. The mean temperature in the latter group raised to maximal 39.6°C. All the calves of the control group and the group supplemented with swine plasma powder had fever (up to 40.4°C) during at least one to four days. On day 6, the mean temperature of the group supplemented with the swine plasma powder even decreased under 38°C, due to hypothermia of the two dying calves in the last hours before death.

In almost all the calves infected with the F5+ bearing E. coli strain, a rise in temperature could be observed. The mean temperature rise was minimal in the group supplemented with the bovine plasma powder at the highest dose. One calf had fever for 2 days. In the other groups (supplemented with the bovine plasma powder at the dose of 10 g/l, the swine plasma powder and the control group) temperature rise lasted longer (from 2 to 33 days) and was more severe.
Fig. 1. Clinical findings concerning temperature and faecal consistency of the calves infected with the *E. coli* strain 32KH85 (F17'). Group 25B: supplemented with bovine plasma powder (25 g/l); Group 10B: supplemented with bovine plasma powder (10 g/l); Group 25S: supplemented with swine plasma powder (25 g/l); Control: unsupplemented control group.
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Fig. 2. Clinical findings concerning temperature and faecal consistency of the calves infected with the *E. coli* strain B85 (F5*'). Group 25B: supplemented with bovine plasma powder (25 g/l); Group 10B: supplemented with bovine plasma powder (10 g/l); Group 25S: supplemented with swine plasma powder (25 g/l); Control: unsupplemented control group.

- : Mean rectal temperature; : Mean faecal consistency score; : Individual febrile period; : Individual period of abnormal faecal consistency; †: Death of the calf at a given point in time; C1, C2, C3: calf 1, calf 2 and calf 3 of the group.
Consistency of faeces (Figs 1, 2)

The infected, non-supplemented calves had watery diarrhoea from the first day after infection until death.

The faeces of the infected (F5 or F17) calves treated with the highest dose of bovine plasma powder remained normal (score = 0) during the first 5 days after the infection. Afterwards some calves developed mild diarrhoea (score = 1).

The calves that received bovine plasma at a lower dose and infected with the \textit{E. coli} strain expressing F17\(^+\) fimbriae developed mild diarrhoea (score = 1) at an earlier stage, starting from day 3, 8 and 10, respectively. The diarrhoea stopped after a few days and the calves remained hydrated. Calves to which the same dose of bovine plasma powder was administered but that were infected with the F5\(^+\) carrying \textit{E. coli} strain showed more severe diarrhoea (score = 2) starting from day 1–2 after infection. The diarrhoea became milder (score = 1) but lasted up to the end of the study period.

The groups supplemented with the swine plasma powder of swine had diarrhoea beginning from day 1–3 after infection.

Excretion of total coliforms in the faeces

In all the calves, the excretion of total coliforms in the faeces showed a similar pattern. \(10^4–10^5\) CFU per gram of faeces were found immediately on arrival. The number increased to \(10^7–10^9\) and remained at that level during the subsequent study period.

Excretion of the challenge strain

Before infection, none of the calves excreted the pathogenic \textit{E. coli} strains. One day after infection with the \textit{E. coli} strain expressing F17\(^+\) fimbriae (Fig. 3), \(10^3–10^5\) CFU per gram of faeces were isolated. The excretion of this strain decreased during the study period to \(10^2–10^3\) bacteria per gram of faeces but never decreased under the detection limit.

![Fig. 3. Number of CFU (log10) of the \textit{E. coli} strain 32KH85 (F17\(^+\))/g faeces during the first 5 days after infection (= day 0). G\(_{25B}\): group supplemented with bovine plasma powder (25 g/l); G\(_{10B}\): group supplemented with bovine plasma powder (10 g/l); G\(_{25S}\): group supplemented with swine plasma powder (25 g/l); Control: unsupplemented control group.](image-url)
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Fig. 4. Number of CFU (log$_{10}$) of the E. coli strain B85 (F5+)/g faeces during the first 5 days after infection (= day 0). G$_{25B}$: group supplemented with bovine plasma powder (25 g/l); G$_{10B}$: group supplemented with bovine plasma powder (10 g/l); G$_{25S}$: group supplemented with swine plasma powder (25 g/l); Control: unsupplemented control group.

No significant difference ($P=0.22$) between the excretion of the challenge strain by the control group and by the group that received bovine plasma powder at 25 g/l was found. The average excretion of the F17$^+$ expressing E. coli in the groups that had received bovine plasma powder at 10 g/l or swine plasma powder differed 10$^2$–10$^3$ CFU per gram of faeces from the average excretion of the control group during the first 2 days after infection.

The excretion of the F5$^+$ E. coli strain (Fig. 4) showed a different pattern. The first day after infection, an excretion of 10$^3$–10$^9$ CFU per gram of faeces was found. The excretion of this strain decreased faster and after 6–20 days, no bacteria could be detected in the faeces of several calves supplemented by the bovine plasma powder during the experiment. In the group supplemented by the swine plasma powder the pathogens were isolated during the whole observation period. When comparing the average excretion of the treated groups with the control group, a significant decreased excretion ($P < 0.05$) of 2–4 orders of magnitude in the group supplemented with bovine plasma powder at the highest dose was evident during the first days after infection. The average excretion was 1–2 orders of magnitude lower in the group supplemented with the lower dose than in the control group. In the group receiving the swine plasma powder no clear difference with the control group was detected.

Additional analysis on faeces

Sporadically Rotavirus, Coronavirus or Cryptosporidium parvum was detected, but no correlation with the absence or presence of diarrhoea could be found.

Discussion

The results of this trial show that plasma powder from cattle which was shown to be devoid of specific antibodies against the fimbriae of the challenge strain can be used in the prevention of E. coli diarrhoea. This is especially important when we know that the calves were colostrum-deprived. The bovine calf is born virtually agammaglobulinaemic and maternal immunoglobulins (Ig) are acquired from colostrum to protect the calf from neonatal infections.
Calves who have not absorbed sufficiently colostral immunoglobulins ('failure of passive transfer') are more sensitive to infections than others who have absorbed sufficient quantities (BOYD, 1972; WITTUM and PERINO, 1995). Mortality reaches up to 50% in the calves who have a ZST score less than 10 IU/l (MCEWAN et al., 1970). When the value of the ZST test is between 10 and 20 IU/l, mortality varies from 7 to 25%. CALDOW et al. (1988) concluded that where the incidence of disease is low, the importance of passive immunity is reduced. Therefore improved management can at least partly overcome the effect of low circulating IgG concentrations in calves. In the present study, the calves were hypogammaglobulinemic and were infected at the first day of life with a lethal dose of pathogens ($10^{10}$ E. coli). The administration of bovine plasma powder induced a good protection against clinical symptoms and mortality in those hypogammaglobulinemic calves. In the experiment with the F17+ strain protection against septicemia was also obtained, although the amount of circulating antibodies, as determined by the zinc sulphate turbidity test, was not raised by the administration of plasma powder. The low circulating gammaglobulin concentrations, and the absence of specific antibodies in the plasma are indicative for the fact that the protection offered to the calves by the plasma powder is probably due to the non-immunoglobulin fractions of the plasma powder.

These results confirm the findings of earlier in vitro experiments (MOURICOUT and JULIEN, 1986, 1987; SANCHEZ et al., 1993C) on the adhesion inhibition effects of glycoproteins in plasma from cattle. They showed an inhibition of adhesion of E. coli cells expressing F5+ or F17+ fimbriae by several glycoproteins found in bovine plasma. VAN DRIESSCHE (1988) also proved that oxidation of the monosaccharides of the villar glycoconjugates with sodium metaperiodate, a reagent which cleaves the C-C bond between vicinal hydroxyl groups of sugars, completely prevented E. coli F17+ from binding to the villi. This indicates that the carbohydrate part of the glycoconjugates is involved in the interaction with the lectins. Glycan moieties of glycoproteins in cow plasma mimic the glycan moieties of natural receptors.

In vivo experiments performed by MOURICOUT et al. (1990) with glycoprotein glycans derived from bovine plasma, revealed a significant reduction in excretion of the pathogen strain in the faeces. When these authors measured the adhesion of the bacteria in the small intestine, they found a significant reduction in adhesion of two orders of magnitude in the treated group. However, these investigators used partly purified glycoprotein glycans derived from plasma instead of a dried whole plasma preparation and reported that whole plasma was without effect. In our trial we found in the F5+ infected groups a reduction in excretion of the challenge strain in the faeces by 2–4 orders of magnitude when supplemented with bovine plasma powder at a high dose (25 g/l) and by 1 or 2 orders of magnitude when supplemented with bovine plasma powder at a low dose (10 g/l). As far as the swine plasma powder is concerned SANCHEZ et al. (unpublished results) also found an inhibition of adhesion of E. coli F5+ or F17+ strains in vitro by using swine plasma powder. However, this inhibition by swine plasma powder was less pronounced when compared to bovine plasma powder. In our in vivo experiments, the administration of swine plasma powder to calves infected with E. coli F5+ strain had no protective effect. One possible explanation could be a difference in glycoprotein composition in the plasma between pigs and cattle, although we could not find any information in the literature comparing the composition of plasma glycoproteins in different species.

Although cow plasma powder protected newborn calves from developing diarrhoea upon infection with E. coli F17+, the excretion of the F17+ bacteria in the faeces of the infected calves was very variable and lasted for many weeks in the supplemented calves. This was in contrast with the excretion of the E. coli F5+ strain. This excretion was no longer detectable (< 10^2 bacteria/g faeces) at 6–20 days after infection in the groups treated with bovine plasma powder. The excretion of the E. coli F17+ strain in the faeces of older calves might be the result of a colonization of the colon which is not inhibited by the plasma components, whereas the E. coli F5+ strain is more consonant with a colonization of the small intestine. At this age, the F17+ strain probably behaves like a commensal and is no longer harmful for the carrier.

The fact that the isolation of total coliform bacteria per gram of faeces remains constant indicates that the plasma powder probably does not influence the intestinal flora adversely.

The clinical signs we recorded were also consonant with those reported by MOURICOUT et al. (1990) for the bovine plasma powder. There was indeed a great difference between the
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controls and the groups supplemented with plasma powder. Diarrhoea and fever were less severe in the latter group and the results were better in the bovine plasma powder groups than in those supplemented with the swine plasma powder. In the latter groups no significant better clinical results than in the control groups were obtained.

Although the analyses of the excretion were made on the faeces and not on the epithelial cells or the mucus layer itself, the obvious differences in clinical signs, mortality, septicaemia and general health status between the control groups and the groups supplemented with bovine plasma appeared to be related with an inhibition of adhesion of the bacteria to the intestinal wall.

In the present experiment, where the calves were hypogammaglobulinemic, the most effective protective dose was 25 g bovine plasma powder/l milk 3 times a day. The lower dose of 10 g/l was less effective in the group infected with the F5+ strain but had still reasonable effect in the F17+ infected group.

The administration of these receptor analogues as prophylaxis of *E. coli* diarrhoea in neonatal calves is promising for the future. These analogues are simple to use (oral as feed additive), their inhibitory power is not lost upon digestion (the calf intestine appears to have only weak oligosaccharidase activity) and they are derived from a cheap raw material (blood plasma). The efficiency could be explained by the fact that several glycoprotein glycans in bovine plasma are recognized by the F5+ and F17+ fimbriae. One of the major drawbacks of supplementing bovine plasma powder in calves is the possible transmission of pathogenic agents. Although a maximum of control procedures are being implemented in order to guarantee a relative safe product, the fear of transmitting BSE prions remains a major concern. At present there is no indication that an infection can be transmitted by plasma products, but on the other hand there are no quick and reliable procedures to detect the presence of BSE prions in the plasma products. Further research is necessary to find prion detection methods and/or to develop efficient procedures to inactivate the prion in plasma products.

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