10

REPRODUCTION OF LESIONS AND CLINICAL SIGNS WITH A CNF2-PRODUCING *ESCHERICHIA COLI* IN NEONATAL CALVES

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1. SUMMARY

CNF2-producing necrotoxigenic *E. coli* (NTEC2) are associated with diarrhoea and septicaemia in calves. We orally inoculated neonatal calves with a NTEC2 strain in order to reproduce clinical signs and lesions. We observed diarrhoea in each inoculated calf, bacteraemia (80%), the presence of CNF2+ bacteria in the lungs (80%) and in the liver (20%). The observed lesions were inflammation of the entire gut, hypertrophy of the mesenteric lymph nodes and hepatisation of the lungs. We were unable to detect characteristic lesions that are classical signs of septicaemia.

2. INTRODUCTION

Necrotoxigenic *Escherichia coli* (NTEC) produce cytotoxic necrotizing factor 1 (CNF1) or 2 (CNF2). NTEC1 have been isolated from cases of extraintestinal infections in humans (Caprioli et al., 1987), of diarrhoea in calves and pigs (Holland, 1990), of extraintestinal infections in cattle, pigs, goats, cats and dogs (Pohl et al., 1993) and of diarrhoea in rabbits and haemorrhagic colitis in horses (Ansuini et al., 1994). NTEC2 are generally restricted to ruminants with diarrhoea and/or septicaemia (DeRycke et al., 1990; Pohl et al., 1993; Derycke et al., 1987; Oswald et al., 1991). CNF are 110–115 kDa toxins either encoded by the chromosome (CNF1) (Falbo et al., 1992) or by a

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transferable F-like plasmid called Vir (Oswald et al., 1989; Smith, 1974). In vitro, CNF toxins induce on various eukaryotic cells lines a drastic reorganization of the microfilamental network into thick stress fibres. This phenomenon is accompanied by a block of cytokinesis, leading to the formation of giant multinucleated cells. This phenotype is associated with the ability of CNF toxin to induce a postranlational modification of the 21 kDa Rho GTP-binding protein which is involved in the regulation of microfilament network (Oswald et al., 1994; Schmidt et al., 1997). In vivo, CNF toxins induce necrosis in rabbit skin. Experimental infection of neonatal pigs with NTEC1 O88 induced an early enteritis, progressing to enterocolitis and bacteraemic spread to the lungs. Infection with NTEC1 O32 produced a milder but similar enterocolitis, also with bacterial colonisation of the lungs. The histopathological changes in both cases were characteristic of a toxemia (Wray et al., 1993). Intravenous injection of partially purified CNF1 toxin to lambs induced the development of severe clinical signs starting six hours after the inoculation, and consisted mainly in neurological signs and mucoid diarrhoea. The most striking lesions were oedema and haemorrhage in the central nervous system and foci of coagulation necrosis in the myocardium (De Rycke et al., 1990). In natural infection in rabbits, Asuini et al. (1994), observed post mortem enteritis mainly of the ileum and the caecum. In horses with dysentery, post mortem examination revealed a severe haemorrhagic colitis and caecitis (Ansuini et al., 1994). If we consider the adhesins associated with NTEC strains: most NTEC1 isolates hybridized with Pap probe and either with Sfa or Afa probes. In contrast, most NTEC2 strains hybridize with F17 and/or Afa probes (Mainil et al., 1997). Here, we describe the clinical signs and lesions obtained after an oral challenge in neonatal calves with a NTEC2 bovine isolate.

3. EXPERIMENTAL PROCEDURES AND RESULTS

NTEC2 strain B20A was isolated from the faeces of a calf with diarrhoea and belongs to serotype O15:K14. It was positive for: serum resistance, aerobactin production, ability to adhere to calf intestinal villi, and hybridization with F17A and CNF2 probes (Oswald et al., 1991). Six newborn Friesan calves received 300 ml of colostrum just after the birth. This colostrum was tested by agglutination for the absence of specific antibodies against the B20A strain. At six hours, 5 calves received 250 ml of saline containing 10^9 to 10^{12} bacteria and one calf received 250 ml of saline.

A faeces sample was taken each 4 hours post inoculation, diluted and platted on Gassner Agar medium. The plates were incubated overnight at 37°C. The number of CFU was counted and the colonies were blotted onto a filter in order to perform a colony hybridization (Mainil et al., 1990) with a CNF2 probe (Oswald et al., 1994). This allowed us to calculate the excretion rate of $CNF2^+$ bacteria. We noticed that the fecal excretion started 24–32 hours post inoculation and persisted until death (Table 1). The rate of excretion ranged from 10^7 CFU/ml to 10^{10} CFU/ml. Diarrhoea occurred in each calf inoculated with the NTEC2 B20A strain, but was absent in the control calf. Diarrhoea started 28–40 hours post inoculation and persisted until death (Table1). Therefore, there was a good correlation between fecal excretion of $CNF2^+$ bacteria and diarrhoea. We confirmed identity between the inoculated strain and the $CNF2^+$ bacteria recovered from faeces by pulse field gel electrophoresis (data not shown). The faeces were tested by ELISA for the presence of Enterotoxigenic *E. coli*, Cryptosporidium, Rota and Corona viruses. All the samples were negative. The calves were euthanasied

Table 1. Fecal excretion of NTEC2 B20A strain and diarrhoea appearance

Calves	Death hour	Diarrhoea appearance	Diarrhoea end	NTEC2 strains appearance in the faeces	NTEC2 strains disappearance in the faeces
7	36 Pi	28 Pi	36 Pi	24 Pi	36 Pi
8	74 Pi	28 Pi	74 Pi	28 Pi	74 Pi
9	39 Pi	28 Pi	39 Pi	24 Pi	39 Pi
10	45 Pi	40 Pi	45 Pi	28 Pi	45 Pi
11	57 Pi	32 Pi	57 Pi	32 Pi	57 Pi
control	45 Pi	—	—	—	—

between 36 and 74 hours post inoculation. Samples were taken from: the intestinal content of all parts of the gut, the mesenteric lymph nodes, the liver, the kidneys, the spleen, the lungs and from the heart blood. These samples were analysed for the presence of CNF2+ bacteria as described above. No $CNF2^+$ bacteria were isolated from the kidneys, spleen or mesenteric lymph nodes. By contrast, $CNF2^+$ bacteria were isolated from the small intestine (5/5) and colon (5/5), lungs (4/5), heart blood (4/5), and liver (1/5). The necropsy analysis revealed in each inoculated calf: inflammation of the entire intestine, hypertrophy of the mesenteric lymph nodes and a hepatisation of the cranial lobes of the lungs.

4. CONCLUSIONS

NTEC2 strains were associated with diarrhoea and/or septiceamia in calves. By oral inoculation of neonatal calves with a NTEC2 strain, we were able to consistently reproduce diarrhoea. The fecal excretion of the inoculated strain and diarrhoea correlated, and no other classical cause of diarrhoea (Enterotoxigenic *E. coli*, Rota and Corona -viruses) was detected. These observations allow us to conclude that the inoculated strain was probably the cause of the diarrhoea. Moreover, the uninoculated calf did not exhibit diarrhoea. The presence of CNF2-positive bacteria in the blood indicates that the calves developed bacteraemia, but there were no lesions characteristic of septicaemia. The presence of CNF2 positive bacteria in the lungs confirmed the observation made by Wray et al. (1993) that NTEC possesses a tropism for lungs. There was no correlation between the severity of the lesions and the inoculation or the euthanasia time. The absence of bacteria in the mesenteric lymph nodes indicates that the NTEC2 reach the blood directly and from there to the organs without passage through the lymphatic system. We conclude from these experiments that we have developed an in vivo model allowing for reproduction of diarrhoea, bacteraemia and bacterial invasion. This model will be useful for the study of the virulence factors of NTEC2 strains.

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REFERENCES

Ansuini, A., Candotti, P., Vecchi, G., Falbo, V., Minelli, F., and Caprioli, A., 1994, Necrotoxigenic *Escherichia coli* in rabbit and horses, Vet. rec., 134:608.

Caprioli, A., Falbo, V., Ruggeri, F.M., Baldassari, L., Bisicchia, R., Ippolito, G., Romoli, E., and Donelli, G., 1987, Cytotoxic necrotizing factor production by hemolytic strains of *Escherichia coli* causing extraintestinal infections. J. Clin. Microbiol., 25:146–149.

De Rycke, J., Guillot, J.F., and Boivin, R., 1987, Cytotoxins in non-enterotoxigenic strains of *Escherichia coli* isolated from feces of diarrheic calves, Vet. Microbiol., 15:137–157.

De Rycke, J., Gonzalez, E.A., Blanco, J., Oswald, E., BLanco, M., and Boivin, R., 1990, Evidence for two types of cytotxic necrotizing factor in human and animal clinical isolates of *Escherichia coli*, J. Clin. Microbiol., 28:694–699.

Falbo, V., Famigelietti, M., and Caprioli, A., 1992, Gene block encoding production of cytotoxic necrotizing factor 1 and haemolysin in *Escherichia coli* isolates from extraintestinal infection, Infect. Immun., 60:2182–2187.

Holland, R.E., 1990, Some infectious causes of diarrhoea in young farms animals, Clin. Microbiol. Rev., 3:345–375.

Mainil, J.G., Jacquemin, E., Kaeckenbeeck, A., and Pohl, P., 1993, Association between the effacing (*eae*) gene and theShiga-like toxin-encoding genes in *Eschrichia coli* isloates from cattle, Am. J. Vet., Res., 54:1064–1068.

Mainil, J.G., Jacquemin, E., Herault, F., and Oswald, E., 1997, Presence of pap-, sfa-, and afa- related sequences in necrotoxigenic *Escherichia coli* isolates from cattle: evidence for new variants of the AFA family, Can. J. Vet. Res., 61:193–199.

Oswald, E., De Rycke, J., Guillot, J.F., and Boivin, R., 1989, Cytotoxic effect of multinucleation in HeLa cell cultures associated with the presence of Vir plasmid in *Escherichia coli* strains, FEMS Microbiol. Lett., 58:95–100.

Oswald, E., De Rycke, J., Lintermans, P., Van Muylen, K., Mainil, J., Daube, G., and Pohl, P., 1991, Virulence factors associated with the cytotoxic nevrotizing factor type 2 in bovine diarrheic and septicaemic strains of *Escherichia coli*, J. Clin. Microbiol., 29:2522–2527.

Oswald, E., Pohl, P., Jacquemin, E., Lintermans, P., Van Muylen, K., O'Brien, A.D., and Mainil, J., 1994, Specific DNA probes to detect *Escherichia coli* strains producing CNF1 or CNF2, J. Med. Microbiol., 40:428–434.

Pohl, P., Mainil, J., Devriese, L., Haesebrouck, F., Broes, A., Lintermans, P., and Oswald, E., 1992, Escherichia coli productrices de la toxine cytotoxique nécrosante de type 1 (CNF1) isolées à partir de processus pathologiques chez des chiens et des chats, Ann. Med. Vet. 137:21–25.

Pohl, P., Oswald, E., Van Muylen, K., Jacquemin, E., Lintermans, P., and Mainil, J., 1993, *Escherichia coli* producing CNF1 and CNF2 cytotoxins in animals with different disorders, Vet. res., 24:311–315.

Schmidt, G., Sehr, P., Wilm, M., Selzer, J., Mann, M., and Aktories, K., 1997, Gln 63 of Rho is deaminated by *Escherichia coli* cytotoxic necrotizing factor-1, Nature, 387:725–729.

Smith, H.W., 1974, A search for transmissible pathogenic characters in invasive strains of *Escherichia coli*: the discovery of a plasmid-controlled lethal character closely associated, or identified with colicin V., J. Gen. Microbiol., 83:95–111.

Wray, C., Piercy, D.W.T., Carroll, P.J., and Cooley, W.A., 1993, Experimental infection of neonatal pigs with CNF toxin-producing strains of *Escherichia coli*, Res. Vet. Sc., 54:290–298.