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.

Preventive Veterinary Medicine, 17 (1993) 65–75
Elsevier Science Publishers B.V., Amsterdam

The role of pathogenic *Escherichia coli* in the etiology of veal calf hemorrhagic enteritis

A. Mahdi Saeed*[,a], T. Bowersock[a,b], L. Runnels[b], R.F. Teclaw[a]
[a]*Department of Veterinary Pathobiology, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907, USA*
[b]*Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN 47907, USA*

(Accepted 5 February 1993)

Abstract

Veal calf hemorrhagic enteritis, a condition that has no identified specific etiology, is a fatal syndrome of veal calves and has recently become a major concern of the veal calf industry in the midwestern United States. To determine the possible role of common enteric pathogens in this disease, 40 veal calves with hemorrhagic enteritis (cases) and 25 dairy calves diagnosed with enteric infection (control) were investigated. The veal calves were negative for several known enteric pathogens except for pathogenic *Escherichia coli* isolates that expressed multiple virulence attributes. To determine whether such isolates have a significant association with hemorrhagic enteritis in veal calves, we compared the prevalence of pathogenic *E. coli* in the 40 veal calves with the prevalence of similar *E. coli* in the dairy calves that were diagnosed with colibacillosis within the same season of the year. *Escherichia coli* isolates from the two groups of calves were tested for several properties of *E. coli* related to pathogenicity, i.e. production of verotoxins, heat-stable enterotoxin (ST_a), heat-labile enterotoxin, enterohemolysin, K99 fimbrial antigens, hemagglutination activity, and attachment to Hep-2 tissue culture cells. *Escherichia coli* that produce ST_a were more commonly isolated from veal calves with hemorrhagic enteritis (45%) than from dairy calves with enteritis (12%) ($P<0.05$). Various patterns of attachment of *E. coli* to Hep-2 tissue culture cells were studied. The *E. coli* that demonstrated aggregative patterns of attachment were more commonly represented in veal calves (32%) than in dairy calves (8%). We observed that there was no correlation between ST_a production and K99 pili expression among the enterotoxigenic *E. coli* (ETEC) isolates that were recovered from veal calves. This may indicate the emergence of K99-negative ETEC, probably as a result of the wide use of K99-based vaccines.

Introduction

A fatal syndrome of veal calves which often includes hemorrhagic diarrhea has recently become a major concern of the veal calf industry in the midwestern United States. This disease has many synonyms including 'hemorrhagic veal calf disease', 'bloody scours', 'bloody calf disease' etc. The disease is characterized in the live calf primarily by anorexia, fever, diarrhea with

*Corresponding author.

mucus-containing stools which become bloody in the later stages, and hemorrhagic diathesis on the conjunctival surface of the eyes and the mucus surfaces of the mouth and nose (Espinasse et al., 1973; Harrison, 1978). Although no epidemiological surveys have been completed, this syndrome potentially is a source of considerable economic loss to veal calf producers. Until 1989, the Animal Disease Diagnostic Laboratory (ADDL), Purdue University, West Lafayette, IN, had examined veal calves with the signs and symptoms of generalized hemorrhagic disease. No specific etiologic agents which may relate to the condition were identified except the isolation of Bovine Virus Diarrhea (BVD) virus from three cases (18%). According to the reports of the virology section of the ADDL (Dr. C. Kanitz, personal communication, 1992), in over 70% of tissue specimens and fecal samples submitted from cattle and calves, BVD virus had been demonstrated by fluorescent microscopic examination of tissue culture inoculated with these specimens. Moreover, a large number of serum samples can be found positive for BVD virus. However, such demonstration of BVD virus clues could only be significant when suggestive lesions are recorded or a significant rise in serum titer is measured from paired sera. Calf scours is a disease complex and a number of different organisms have been associated with its etiology. The most common organisms are enterotoxigenic *Escherichia coli* (ETEC), *Salmonella* sp., *Campylobacter* sp., rota, corona, and BVD viruses, as well as some parasitic agents such as *Cryptosporidium* (Acres et al., 1977; Moon et al., 1978; Smith and Linggood, 1972).

The role of pathogenic *E. coli* has been well investigated in dairy and beef herds in the past (Moon et al., 1976; Morin et al., 1976; Orskov and Orskov, 1984; Morris and Sojka, 1985). Only limited information, however, is available on the role of pathogenic *E. coli* in veal calf scours (Webster et al., 1985; Visser et al., 1987). Therefore, the epidemiology of enteric infections in veal calves may differ from those seen in dairy calves (Klaus et al., 1969; Thornton et al., 1972). The purpose of this study was to determine whether certain strains of pathogenic *E. coli* that express multiple virulence factors play an important role in the pathogenesis of veal calf hemorrhagic enteritis. The method used was a comparison of the prevalence of these strains of *E. coli* in veal calves suffering from this disease with their prevalence in a similar age group of dairy calves that were identified as colibacillosis cases by clinical diagnosis and laboratory testing.

Materials and methods

Description of the disease outbreak

A veal production farm with a herd size of 750 veal calves in central Indiana experienced an outbreak of hemorrhagic enteritis in August 1989. The

disease strikes healthy calves 7–10 days after entry to the farm. It was described as an initial febrile condition with a temperature of 39–41 °C, yellow loose stools which become bloody in the later stages, variable appetite during the course of illness, dehydration and cachexia at the terminal stage of the disease. The morbidity rate of this syndrome was estimated to be 30% with a case fatality rate approaching 80%. All the calves on this farm were male Holsteins purchased at an average weight of 45–52 kg from different markets and were kept in individual stalls with replacement on an all-in all-out basis. Feeding and the feeding schedule was supplied by the Real Veal Co., (Ixonia, WI) an important supplier of veal calf formula in the area. Bovitec (Lasolicid) was given as a routine feed medication. The following antibiotics and antibacterial agents were used in the treatment of affected calves as well as prophylactically on healthy calves during the 2 week period of the disease outbreak: Neomycin, Penicillin, Oxytetracycline, Nitrofurazone, Furazolidone, Sulfamethazin, and Sulfamethaxazole. Several drugs on this list were used on several occasions by the farmer without veterinary advice. All treatments failed to alter the course of the disease.

Selection of cases

Forty veal calves that manifested signs of diarrhea (including veterinary identified bloody diarrhea) within 1–2 weeks after the disease outbreak was reported on the farm, were included as cases. These cases were inspected by the investigators and were reported by the owner and/or the veterinarian to have had one or more of the following clinical signs: temperature of 39–42 °C, anorexia, depression and weakness, and petechial hemorrhages on the mucus surfaces of the mouth or the nose. The 40 diarrheic and dehydrated Holstein calves comprised two groups. Each group was chosen from a barn to include the 20 calves that were most recently identified as having the disease. The estimated age of these animals was 2 weeks (only estimation of the ages of auction-purchased calves was possible). Calves were selected at this age because more than 80% of the calves on the farm were approximately this age. However, 1- and 3-week-old calves reported to have been affected with the disease were also present on the farm.

Selection of control calves

All of the 25 Holstein dairy calves that were of a median age of 2 weeks and were presented, within the same season (August 1989 to February 1990), to Purdue University Animal Hospital and Animal Disease Diagnostic Laboratory (ADDL) with clinical diagnosis of colibacillosis were included as controls. The diagnosis of colibacillosis in these cases was confirmed by microbiologic testing for the absence of other major bacterial, viral, and parasitic

etiologies that are often associated with calf scours. Only 25 such animals were identified throughout the period of the study. These criteria for selection of control calves were chosen after the conclusion that the strains of *E. coli* that were isolated from the 40 veal calves (cases) expressed multiple virulence factors. There were no other major bacterial, viral, or parasitic etiologies identified in association with the cases of veal calf hemorrhagic enteritis.

Processing of samples

Blood samples and paired sera were collected from five calves selected at random (using the table of random numbers) from the 40 veal calves with clinical signs of the disease or which had manifested the signs at an earlier time. These samples were submitted to the clinical pathology laboratory for hematologic analysis (total protein, fibrinogen, hematocrit, hemoglobin, red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), total count of white blood cells (CWBC), differential white blood cells count, platelets count, and morphologic examination of the blood cells) and for determination of selenium and vitamin E levels. Based on previous cases of veal calf hemorrhagic enteritis that were investigated by the ADDL, no specific viral etiology was identified. However, the paired sera collected from the five calves were tested for serum neutralization titers to BVD, corona and rota viruses. Fecal and/or rectal swab samples collected from the 40 veal calves and from the 25 dairy calves (*E. coli* cultures from cases of colibacillosis were stored and maintained by the ADDL according to a collaborative project between the ADDL and the Department of Veterinary Pathobiology at Purdue University), were processed for common enteric bacterial pathogens following standard procedures (Nagy et al., 1976; Moon et al., 1977; Carter and Cole., 1990). Ten well isolated smooth colonies with an appearance typical of *E. coli* were chosen from MacConkey plates. Selection was made by placing the plate on a paper grid; two colonies were picked from each quarter that showed discrete colonies of bacterial growth. With the size of the grid used, most of the areas of discrete bacterial growth were covered. The rectal swabs or fecal samples were also inoculated on Campy-Bap medium containing 5% sheep blood and antibiotic supplementation (BBL Microbiology Systems, Cockeysville, MD) and incubated in an anaerobic jar with a Campy-Pak (BBL Microbiology Systems). The jars were sealed and incubated at 42°C for 48 h. The plates were examined for *Campylobacter* organisms following standard procedures. For *Salmonella* isolation, each rectal swab or fecal sample was inoculated into a tube with 10 ml of Selenite enrichment broth and incubated at 42°C for 24 h. Samples from each tube were streaked onto Brilliant Green agar plates with and without Novobiocin. After 24 h incubation at 37°C, the plates were examined for suggestive *Salmonella* growth and if positive were confirmed by standard biochemical and serologic methods. All *E. coli* isolates from both veal calves

and dairy calves were subcultured on Minca Isovitalex agar plates and tested for K99 fimbrial antigen (Guinee et al., 1977). *Escherichia coli* growth on this medium was also tested for hemagglutination (HA) as described by Evans et al. (1980). Testing of *E. coli* for enterotoxin production was performed by growing the isolates on casamino acid–yeast extract (CAYE) medium for the production of heat-stable enterotoxin (ST_a) and heat-labile enterotoxin (Guerrant et al., 1974; Saeed et al., 1984), on Trypticase Soy Broth (BBL Microbiology Systems) for verotoxin (VT) production and testing as described by Karmali et al. (1985). *Escherichia coli* isolates were tested for enterohemolysin as described by Beutin et al. (1988). The test for attachment to Hep-2 cells was performed as described by Cravioto et al. (1979) and interpreted according to Nataro et al. (1987). Five pools of *E. coli* colonies (two colonies per pool) for each fecal swab or sample were tested from each veal and dairy calf in this study. Positive test results by any pool were included in the overall classification of *E. coli* isolates from the respective calf. However, the profile presented belonged to the single bacterial pool that expressed the largest number of virulence factors. The association of the virulence factors with *E. coli* isolates from veal calves and dairy calves was tested by univariate analysis using χ^2 statistics (Fleiss, 1981). The association of virulent *E. coli* with hemorrhagic enteritis in veal calves and enteritis in dairy calves was also tested by χ^2 analysis ($\alpha=0.05$). Three veal calves that were showing typical signs of hemorrhagic disease were necropsied before death by the veterinarian on the farm.

Results

Gross lesions

There were no obvious gross lesions at necropsy in any of the three veal calves, except the signs of dehydration and cachexia that are consistent with enteric disease.

Histopathologic examination

Alterations in multiple sections of small intestine consisted of edema of villous tips; moderate shortening and fusion of villi was also present. Multifocal sites of deep mucosal necrosis were present in sections taken from the terminal colon.

Laboratory findings

Hematological analysis showed remarkable hypoproteinemia in all five veal calves (less than 6 g dl^{-1}) and two of the five calves showed moderate neutrophilia (Table 1). One veal calf had slightly decreased platelets. Results of

Table 1
Hematologic values of five veal calves with hemorrhagic enteritis

Test	Veal calf number					Units
	1	2	3	4	5	
Total protein	5.6	5.9	5.0	5.1	5.3	g dl^{-1}
Fibrinogen	800	400	500	500	600	mg dl^{-1}
Hematocrit	24.9	38.4	30.2	27.0	11.0	%
Hemoglobin	8.6	13.2	10.1	9.5	3.6	g dl^{-1}
RBC	6.70	9.87	7.66	7.04	2.81	$\times 10^6$
MCV	37.0	39	39	38	37	fl
MCHC	35	34	33	35	33	g dl^{-1}
CWBC	8.80	14.50	4.60	18.90	6.50	$\times 10^3$
Band	0	0	0	0.38	0.2	$\times 10^3$
Neutrophil	2.29	9.28	1.47	15.5	2.34	$\times 10^3$
Lymphocyte	6.25	4.64	2.94	2.65	3.97	$\times 10^3$
Monocyte	0.26	0.58	0.14	0.38	0	$\times 10^3$
Eosinophil	0	0	0	0	0	$\times 10^3$
Basophil	0	0	0.05	0	0	$\times 10^3$
Morphology of platelets	adeq.	adeq.	adeq.	adeq.	dec.	
Poikilocytosis	2+	2+	2+		1+,anis. 1+	

adeq., adequate; dec., decreased; anis., anisocytosis.

hematologic testing were obtained for only seven of the 25 dairy calves. All of the seven dairy calves had blood parameter values within the normal range. Selenium and vitamin E levels were within normal ranges in the sera of the five veal calves. No data on these parameter were available on the 25 control dairy calves.

Microbiology results

Serum samples that were collected from the five veal calves did not have significant neutralization titers against BVD, rota, and corona viruses. Sera from only three of the 25 dairy calves had positive results by fluorescent antibody test for rota virus. Results from fecal cultures for *Salmonella* sp., *Campylobacter* sp. and *Cryptosporidium* were negative for the 40 veal calves and for the 25 dairy calves. However, *E. coli* of several colonial morphologies were isolated from the fecal sample taken from each calf of the two groups. The results of the virulence analysis of *E. coli* isolates from the 40 veal calves and isolates from the 25 dairy calves are presented in Tables 2 and 3. ST_a-positive ETEC isolates were more common in veal calves (40%) than in dairy calves (12%) ($\chi^2=7.6$, 1 d.f., $P<0.005$; Table 2). There was no significant difference between the total number of *E. coli* isolates from the two groups of calves

Table 2
Frequency distribution of virulence factors among *E. coli* isolates from veal calves (cases) and dairy calves (controls)

Virulence factor	No. of calves positive (%)		χ^2	d.f.	*P*-value
	Veal calves ($n=40$)	Dairy calves ($n=25$)			
VT	9 (22.5)	5 (25)	0.06	1	0.72
K99	11 (27.5)	9 (36)	0.52	1	0.38
ST_a	18 (45)	3 (12)	7.66	1	0.005
HA	25 (62.5)	18 (72)	0.6	1	0.62
Hemolysis	18 (45)	7 (28)	1.87	1	0.375
Attachment[1]	26 (65)	15 (60)	0.16	1	0.6

[1]Any pattern of attachment to Hep-2 cells.

Table 3
Patterns of attachment to Hep-2 cells by *E. coli* isolates from veal calves with hemorrhagic enteritis and dairy calves with non-hemorrhagic colibacillosis

Attachment patterns	No. of calves positive (%)		χ^2	d.f.	*P*-value
	Veal calves ($n=40$)	Dairy calves ($n=25$)			
MRAA[1]	13 (32)	2 (8)	5.2	1	0.02
MRLA	9 (22)	11 (44)	3.34	1	0.08
MRDA	4 (10)	2 (8)	0.07	1	0.82

[1]Mannose-resistant aggregative attachment (this attribute was tested for independence from production of ST_a enterotoxin in *E. coli* isolates using χ^2 and found to be a separate risk factor for veal calf hemorrhagic enteritis: $\chi^2=1.23$, 1 d.f., $P=0.62$).

in the demonstration of any form of attachment to Hep-2 cells (Table 2). However, *E. coli* that demonstrated explicitly mannose-resistant aggregative attachment (MRAA) were more prevalent among the veal calves (32%) than among the dairy calves (8%). In contrast, expression of mannose-resistant local attachment (MRLA) did not differ significantly among the *E. coli* isolates of the two groups of calves (Table 3). There were no significant differences in the numbers of *E. coli* isolates from veal and dairy calves that were positive for VT, K99, HA, and enterohemolysin production (Table 2).

Discussion

The microbial etiology of the recently described veal calf hemorrhagic enteritis has yet to be identified. However, calf scours is known as a disease

complex with multiple etiology. This multiple etiology may include infectious agents, stress factors, lack of a protective level of serum immunoglobulin, and the disturbances of the intestinal microflora. *Escherichia coli* infection is considered to be the most important disease of young calves (Smith and Halls, 1968; Moon et al., 1978; Morris and Sojka, 1985). Veal calves are reared on a complete liquid diet such as milk replacers and other feeds that are often supplemented with several antibiotics and other antimicrobial agents at subtherapeutic levels. Such practices were always suspected of selecting for some drug resistant enteric bacterial pathogens by disturbing the intestinal microflora of these young animals. These calves usually originate from a large number of farms and are marketed at approximately 1 week of age (Webster et al., 1985; Visser et al., 1987). Mixing large numbers of susceptible animals and subjecting them to stress prior to rehousing in intensive systems creates favorable conditions for the spread of infectious agents and the development of disease (Fisher et al., 1975). In this study we have investigated *E. coli* isolates from veal calves with clinical manifestation of hemorrhagic enteritis (cases) and from dairy calves diagnosed with *E. coli* enteritis (controls) for the expression of several virulence factors related to pathogenicity. The experimental induction of diarrheal disease in newborn calves using *E. coli* whole culture (Smith and Halls, 1968) and by pure ST_a has been reported (Saeed et al., 1986). We have noted that large doses of pure ST_a can cause hemorrhagic enteritis in challenged calves (Saeed et al., unpublished observations, 1984). We identified ST_a production among 45% of the *E. coli* isolates from veal calves manifesting clinical signs of hemorrhagic enteritis but among only 12% of the dairy calves with non-hemorrhagic diarrhea. Most *E. coli* strains produce fimbrial adhesive antigens (K99) which attach the pathogen to the intestinal mucosal epithelium (Orskov et al., 1975; Guinee et al., 1977; Smith et al., 1985). Adherence brings the bacteria into close association with the intestinal villous cells, allowing colonization and facilitating the effect of enterotoxin on the secretory cells. That more *E. coli* isolates from veal calves than from dairy calves had aggregative attachment suggests that expression of these virulence factors by some strains of *E. coli* could have a role in the pathogenicity of the disease. In most previously described K99-positive strains of ETEC, a positive correlation between ST_a production and the presence of K99 antigen has been established (Orskov et al., 1975; Isaacson et al., 1978; Moon et al., 1978). Our results indicate a lack of correlation between these two virulence factors in *E. coli* isolates from diseased veal calves. It is possible that some virulent ETEC failed to express K99 antigen or that K99-negative virulent ETEC may produce other types of pili which facilitate colonization. Adhesion and colonization by K99-positive ETEC can be blocked by K99 antibody produced by the K99 pilus vaccines which are extensively used in cattle. However, K99 antibodies could lead to the emergence of K99-negative

ETEC in vivo (Mainil et al., 1987). The enterotoxigenic aggregative adhering *E. coli* may be opportunistic organisms in some circumstances. Verotoxin was produced by 22.5% of total and 44% of ST_a-producing ETEC from veal calves. Verotoxins (which by definition are cytotoxic for vero cells in vitro) are produced by some *E. coli* strains such as the hemolytic uremic (O:157, H:7) and other strains that have been identified as playing important roles in human hemolytic uremic syndrome and hemorrhagic colitis. This toxin can be also produced by some *Salmonella* and *Shigella* strains in association with a variety of disease conditions (Gonzalez and Blanco, 1985; Karmali et al., 1985; Bopp et al., 1987). However, the role of VT in the pathogenesis of veal calf hemorrhagic enteritis is not clearly defined. In this study, VT production in association with ST_a could be an important factor in the causation of hemorrhagic enteritis. The importance and prevalence of the aggregative type of attachment of *E. coli* should be assessed further. Several combinations of virulence factors could be described among *E. coli* isolates from this study. This may further expand the nomenclature of *E. coli* that express multiple virulence attributes. Possible limitations of this study are:

(1) the selection bias of case and control calves (case calves were all males that were probably deprived of colostrum after birth, raised under different conditions such as having one or two animals in a crate often chained by the neck, which may be considered as more stressful than the conditions under which dairy calves are raised);
(2) the maintenance of veal calves on feeds with antibiotic supplements (the use of many of these drugs during the disease outbreak could have affected the rate of successful isolation in the laboratory of potential enteric pathogens which may have been sensitive to these drugs;
(3) the use of the antibiotics predisposing for or aggravating the enteric infection through the selection for drug resistant intestinal pathogens;
(4) the availability of only 25 dairy calves with clinical diagnosis of colibacillosis, confirmed by laboratory tests, as a comparison group.

Data from this observational study suggest that infection with *E. coli* strains that produce enterotoxins and cytotoxins and demonstrate an aggregative pattern of attachment to Hep-2 cells should be considered risk factors for hemorrhagic enteritis in veal calves.

Acknowledgments

The authors thank the faculty and staff of Purdue University Animal Disease Diagnostic Laboratory for their help and support.

References

Acres, S.D., Saunders, J.R. and Rodostit, O.M., 1977. Acute undifferentiated neonatal diarrhea of beef calves: The prevalence of ETEC, reo-like (rota) virus and other enteropathogens in cow-calf herds. Can. Vet. J., 18: 113–121.

Beutin, L., Prada, J., Zimmerman, S., Stephan, R., Orskov, I. and Orskov, F., 1988. Enterohemolysin, a new type of hemolysin produced by some strains of enteropathogenic *E. coli* (EPEC). Zentralbl. Bakteriol. Hyg., 267: 576–588.

Bopp, C.A., Greene, K.D., Downes, F.P., Sowers, E.G., Wells, J.G. and Wachsmuth, I.K., 1987. Unusual verotoxin-producing *Escherichia coli* associated with hemorrhagic colitis. J. Clin. Microbiol., 25: 1486–1489.

Carter, G.R. and Cole, J.R., 1990. Diagnostic Procedures in Veterinary Bacteriology and Mycology, 5th edn. Academic Press, San Diego, CA, pp. 107–127.

Cravioto, A., Gross, R.G. and Scotland, S.M., 1979. An adhesive factor found in strains of *Escherichia coli* belonging to the traditional enteropathogenic serotypes. Curr. Microbiol., 3: 96–99.

Espinasse, J., Bonea, B. and Cabanie, P., 1973. Observation and comments on a hemorrhagia syndrome of beef calves fed on dried milk. Rev. Med. Vet., 124: 1503–1514.

Evans, D.J., Jr., Evans, D.G., Young, L.S. and Pitt, J., 1980. Hemagglutination typing of *E. coli*: Definition of seven hemagglutination types. J. Clin. Microbiol., 12: 235–242.

Fisher, E.W., Martinez, A.A., Trainin, Z., et al., 1975. Studies of neonatal calf diarrhoea. II. Serum and faecal immune globulins in enteric colibacillosis. Br. Vet. J., 131: 402–415.

Fleiss, J.L., 1981. Statistical Methods for Rates and Proportions, 2nd edn. Wiley, New York.

Gonzalez, E.A. and Blanco, J., 1985. Production of cytotoxin VT in enteropathogenic and non-enteropathogenic *Escherichia coli* strains of porcine origin. FEMS Microbiol. Lett., 26: 127–130.

Guerrant, R.L., Brunton, L.L., Schnaitman, T.C., Ribhun, L.I. and Gilman, A.G., 1974. Cyclic adenosine monophosphate and alteration of Chines hamster ovary cell morphology: a rapid, sensitive in vitro assay for the enterotoxins of *Escherichia coli*. Infect. Immun., 10: 320–327.

Guinee, P.A.M., Veldkamp, J. and Jansen, W.H., 1977. Improved Minca medium for the detection of K99 antigen in calf enterotoxigenic strains of *Escherichia coli*. Infect. Immun., 15: 676–678.

Harrison, L.R., 1978. A hemerrhagic disease syndrome of the veal calf. In: Proc. 21st Annu. Meet. Am. Assoc. Vet. Lab. Diagnosticians. Harrisburg, PA. Department of Agriculture, pp. 117–125.

Isaacson, R.E., 1977. K99 surface antigen of *E. coli*: Purification and partial characterization. Infect. Immun., 15: 272–279.

Isaacson, R.E., Moon, H.W. and Schneider, B.S., 1978. Distribution and virulence of *E. coli* in the small intestine of calves with and without diarrhea. Am. J. Vet. Res., 39: 11, 1750–1755.

Karmali, M.A., Petric, M., Lim, C., Fleming, P.C., Arbus, G.S. and Lior, H., 1985. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. J. Infect. Dis., 151: 775–782.

Klaus, G.G.B., Bennett, A. and Jones, E.W., 1969. A quantitative study of the transfer of colostral immunoglobulins to the newborn calf. Immunology, 16: 293–299.

Mainil, J.G., Sadowski, P.L., Tarsio, M. and Moon, H.W., 1987. In vivo emergence of enterotoxigenic *Escherichia coli* variants lacking genes for K99 fimbriae and heat-stable enterotoxin. Infect. Immun., 55: 3111–3116.

Moon, H.W., Whipp, S.C. and Skartvedt, S.M., 1976. Etiologic diagnosis of diarrheal diseases of calves: Frequency and methods for detecting enterotoxin and K99 antigen production by *E. coli*. Am. J. Vet. Res., 37: 1025–1029.

Moon, H.W., Nagy, B., Isaacson, R.E. et al., 1977. Occurrence of K99 antigen of *E. coli* isolated

from pigs and colonization of pig ileum by K99+ ETEC from calves and pigs. Infect. Immun., 15: 614–620.

Moon, H.W., McClurkin, A.W., Isaacson, R.E., Pohlenz, J., Skartvedt, S.M., Gillette, K.G. and Baetz, A.L., 1978. Pathogenic relationship of rotavirus, *E. coli* and other agents in mixed infections of calves. J. Am. Vet. Med. Assoc., 173: 577–583.

Morin, M., Lariviere, S. and Lallier, R., 1976. Pathological and microbiological observations made on spontaneous cases of acute neonatal calf diarrhea. Can. J. Comp. Med., 40: 228–240.

Morris, J.A. and Sojka, W.J., 1985. *Escherichia coli* as a pathogen in animals. In: M. Sussman (Editor), The Virulence of *Escherichia coli*. Academic Press, London, pp. 47–77.

Nagy, B., Moon, H.W. and Isaacson, R.E., 1976. Colonization of porcine small intestine by *Escherichia coli*: Ileal colonization and adhesion by pig enteropathogens that lack K88 antigen and by some capsular mutants. Infect. Immun., 13: 1214–1220.

Nataro, J.P., Kaper, J.B., Robins-Browne, R., Prado, V., Vial, P. and Levine, M.M., 1987. Patterns of adherence of diarrheagenic *E. coli* to Hep-2 cells. Pediatr. Infect. Dis. J., 6: 829–831.

Orskov, F. and Orskov, I., 1984. Serotyping of *Escherichia coli*. Methods Microbiol., 14: 43–112.

Orskov, I., Orskov, F., Smith, H.W. et al., 1975. The establishment of K99, a thermolabile, transmissible *Escherichia coli* K antigen previously called 'Kco', possessed by calf and lamb enteropathogenic strains. Acta Pathol. Microbiol. Scand. Sect. B, 83: 31–36.

Saeed, A.M., Magnuson, N.S., Sriranganathan, N., Burger, D. and Cosand, W., 1984. Molecular characterization of heat-stable enterotoxins produced by bovine enterotoxigenic *Escherichia coli*. Infect. Immun., 45: 242–247.

Saeed, A.M., Magnuson, N.S., Guy, C. and Greenberg, R.N., 1986. Characterization of heat-stable enterotoxin from hypertoxigenic *Escherichia coli* pathogenic for cattle. Infect. Immun., 53: 445–447.

Smith, H.W. and Halls, S., 1968. The experimental infection of calves with bacteraemia-producing strains of *Escherichia coli*. The influence of colostrum. J. Med. Microbiol., 1: 61–78.

Smith, H.W. and Linggood, M.A., 1972. Further observations on *Escherichia coli* enterotoxins with particular regard to those produced by atypical piglet strains and by calf and lamb strains. The transmissible nature of these enterotoxins and of a K antigen possessed by calf and lamb strains. J. Med. Microbiol., 5: 243–250.

Smith, H.R., Scotland, S.M. and Rowe, B., 1985. Genetics of *Escherichia coli* virulence. In: M. Sussman (Editor), The Virulence of *Escherichia coli*. Academic Press, London, pp. 227–269.

Thornton, J.R., Willoughby, R.A. and McSherry, B.J., 1972. Studies on diarrhea in neonatal calves: The plasma proteins of normal and diarrheic calves during the first ten days of age. Can. J. Comp. Med., 36: 17–25.

Visser, N.A.P.C., de Breukink, H.J., Zejdervild, F.G., van de Leeuw, P.W., 1987. Enteric infections in veal calves: A longitudinal study on four veal calves units. Vet. Q., 9: 289–296.

Webster, A.J.F., Saville, C., Church, B.M., Gnanasakthy, A. and Moss, R., 1985. Some effect of different rearing systems on health, cleanliness, and injury in calves. Br. Vet. J., 141: 472.