Verotoxin production among porcine strains of *Escherichia coli* and its association with oedema disease

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Summary. Strains of *Escherichia coli* isolated from documented cases of disease in pigs and belonging to a wide range of pathogenic serotypes were tested for their ability to produce a heat labile verotoxin (VT). The strains isolated from oedema disease all produced VT, indicating that the cytotoxin detected by the vero-cell assay was identical to “oedema disease principle”. Strains belonging to the serotypes associated with enterotoxigenic diarrhoea were VT⁻. Not all the strains belonging to the recognised oedema disease serotypes (O141:K85, O139:K82 and O138:K81) produced VT, but the VT⁻ strains were not associated with outbreaks of clinical disease.

Introduction

Oedema disease attacks thriving pigs about one week after weaning, the major feature being progressive incoordination leading to partial or complete paralysis and death. Post-mortem examination shows oedema in various sites, particularly the stomach wall and the mesentery of the colon (Sojka, 1965). The *Escherichia coli* strains associated with the disease are confined to the intestinal tract and it has been assumed, therefore, that they produce a neurotoxin, “oedema disease principle”, which is absorbed and gives rise to the pathological signs. The presence of this toxin has been demonstrated in “freeze-thaw” extracts of oedema disease strains; intravenous injection of these extracts into pigs can give rise to a condition that is very similar to natural oedema disease (Erskine *et al.*, 1957; Clugston and Nielsen, 1974).

Because study of the pathogenesis of oedema disease has been seriously hampered by the lack of a convenient in-vitro assay system for toxin, reports that some oedema disease strains produce a factor cytopathic for vero cells assume an added significance (Dobrescu, 1983; Smith *et al.*, 1983). Two *E. coli* “verotoxins” have already been identified, namely the “Shiga-like” toxin produced by many human enteropathogenic *E. coli* strains and the verotoxin produced by strains associated with diseases such as haemorrhagic enteritis and haemolytic uraemic syndrome (Konowalchuk *et al.*, 1977; Scotland *et al.*, 1980; O’Brien *et al.*, 1983 *a, b*;

Karmali *et al.*, 1985), but the porcine verotoxin (VT) appears to be different from these.

Smith *et al.* (1983) reported that about 63% of porcine strains belonging to the four serotypes associated with post-weaning disease (diarrhoea or oedema disease) in pigs were VT⁺. They did not, however, distinguish between the two types of post-weaning disease. We considered that further work was needed to clarify the role of VT in virulence. Accordingly we examined a large number of isolates from documented cases of *E. coli* disease in pigs to determine whether VT was specifically associated with either post-weaning diarrhoea or oedema disease.

Materials and methods

Source of E. coli strains

Most of the strains were obtained during the period 1978–1985 from farms in the UK. The diagnosis of *E. coli* disease was made by veterinary practitioners, and faecal swabs, or, occasionally post-mortem samples of intestinal contents, were sent to us for bacteriological examination. The samples came from both neonatal and weaned pigs, but all the O141:K85 and O139:K82 isolates analysed in detail in tables II and III came from weaned pigs between 5 and 8 weeks old. The samples were plated out on blood agar and a representative number of haemolytic and non-haemolytic *E. coli* colonies were then picked, subcultured, and serotyped.

Toxins prepared from *Shigella dysenteriae* type 1 and from two human *E. coli* strains were included for comparison. The human *E. coli* strains belonged to serotypes O26:K60:H11 and O157:H7 and had been

isolated from cases of diarrhoea and haemolytic uraemic syndrome respectively.

Antitoxic sera

Rabbits were immunised subcutaneously with cell-free VT preparations (culture supernates, see below) with Freund's complete adjuvant (Difco) for the first injection, and with Freund's incomplete adjuvant (Difco) for the second injection given 4 weeks later. The animals were bled 2 weeks after the second injection.

Toxin assays

Supernates of cultures grown in Mundell's medium (Ristaino *et al.*, 1983) were tested for VT, and also for the heat stable (STa) and heat labile (LT) toxins. VT was assayed by the vero-cell method of Konowalchuk *et al.* (1977), before and after heat treatment at 70°C for 30 min. LT was assayed by ELISA (Svennerholm and Holmgren, 1978) and STa by the suckling-mouse test (Dean *et al.*, 1972). In addition, LT assays were performed on cell lysates.

Verotoxin neutralisation tests

Toxin preparations were serially diluted and an end-point titre was determined, i.e., the highest dilution giving a positive result in the assay. Doubling dilutions of the antisera were made in microtitration plates and an equal volume of diluted verotoxin was added to give a toxin concentration equivalent to the end-point titre. The plates were incubated at 37°C for 60 min and then 50 µl from each well assayed for verotoxic activity.

Results

Identification of three verotoxins

Neutralisation tests indicated two immunologically different verotoxins in the human *E. coli* cultures, the O26 verotoxin being similar to Shiga toxin. The porcine verotoxin was not neutralised by antisera to either of the human strain verotoxins.

The human verotoxins were inactivated by heat treatment at 80°C but were stable at 70°C. The verotoxins produced by all the porcine strains mentioned in this report were inactivated by heat treatment at 70°C.

Occurrence of porcine verotoxin

The association of the porcine (heat labile) verotoxin with serotype is shown in table I. Strains were divided into two groups. Group A comprised those serotypes encountered in colibacillary diarrhoea particularly in neonatal piglets. Group B

Table I. Verotoxin production among porcine strains of *E. coli*

Serotype	Number of strains tested	Number of VT ⁺ strains
Group A*		
O149:K91, K88ac	10	0
O141:K85, K88ab	2	2
O8:K87, K88ab/ac	4	0
O8:K85, K99	1	0
O157:K'V17', K88ac	6	0
O157:K'V17'	3	0
O45:K'E65', K88ac	2	0
O138:K81, K88ac	2	0
O147:K89, K88ac	1	0
Group B*		
O141:K85	30	23
O139:K82	8	4
O138:K81	11	6
'Untypables'	17	3

* The group A serotypes are usually associated with diarrhoea, particularly in neonatal pigs, and the group B serotypes with oedema disease or diarrhoea, particularly in weaned pigs.

contained serotypes more often found in weaned pigs and either associated only with oedema disease (O139:K82) or with both oedema disease and diarrhoea (O141:K85 and O138:K81) (Sojka, 1973). With the exception of the two O141:K85, K88ab strains, the group A strains were VT⁻, but two-thirds of the group B strains were VT⁺ (table I). Among the group of "untypables" were six strains isolated by J. E. T. Jones (Royal Veterinary College) from post-weaning pigs with "haemorrhagic enteritis", one of which produced VT. The remaining 11 untypable strains were part of the mixed faecal flora of healthy pigs; two of them produced VT.

The association of porcine verotoxin with disease is shown in tables II and III. It will be seen that VT⁺ *E. coli* was present in all pigs suffering from oedema disease, but when the same serotypes were isolated from healthy pigs, or pigs with post-weaning diarrhoea ("scours"), they were usually VT⁻. Two of three VT⁻ O141:K85 strains isolated from post-weaning diarrhoea were STa producers.

Discussion

Approximately 67% of *E. coli* strains belonging to serotypes associated with two serious post-weaning syndromes in pigs, diarrhoea and oedema disease, produced verotoxin. This "porcine verotoxin" could be clearly differentiated from two

Table II. Details of O141:K85 isolates

Farm	Year of isolation	Number of isolates tested	Syndrome	Toxins detected		
				VT	LT	STa
Ba	1979	1	OD	+	—	—
St	1979	2	OD	+	—	—
N	1979	1	OD	+	—	—
T	1980	2	OD	+	—	—
M	1982	4	OD	+	—	—
G	1983–1984	9	OD	+	—	—
G	1983–1984	1	OD	—	+	+
Nut	1985	3	OD	+	—	—
F	1979	1	Scours	—	—	+
D	1979	2	Scours	—	—	—
Sp	1980	1	Scours	—	—	+
Tw	1978	1	Healthy	+	—	—
Be	1979	1	Healthy	—	—	—
E	1979	1	Healthy	—	—	—

OD=clinical oedema disease.

Scours=diarrhoea with O141:K85 present in almost pure culture.

Healthy=no *E. coli* disease; faecal swabs yielded very heterogeneous cultures.

“human verotoxins” on the basis of antigenic cross-reactivity and heat stability. Previous studies had revealed the general association of porcine VT with serotype, but we found that an analysis of the strains based on the clinical signs in the infected pigs, rather than simply on serotype, provided much stronger evidence for the role of verotoxin in virulence.

Verotoxin-producing *E. coli* strains were isolated from all cases of oedema disease examined in this laboratory over a 7-year period, but when the same

Table III. Details of O139:K82 isolates

Farm	Year of isolation	Syndrome	Toxins detected		
			VT	LT	STa
K	1962	OD	+	—	—
Sw	1978	OD	+	—	—
C	1979	OD	+	—	—
We	1979	Healthy A	—	—	—
J	1980	Healthy A	—	—	—
Th	1978	Healthy B	—	—	—
Spex	1979	Healthy B	+	—	—
R	1979	Healthy B	—	—	—

OD=clinical oedema disease.

Healthy A=strain present in almost pure culture in faeces but no OD.

Healthy B=no *E. coli* disease; faecal swabs yielded very heterogeneous cultures.

serotypes were isolated from healthy pigs, or pigs suffering from post-weaning diarrhoea, they were usually VT[—].

It was possible to divide the O141:K85 strains into three “types”, as follows. (i) Strains producing VT but not enterotoxin, that had the potential to produce oedema disease but not diarrhoea and were isolated from all cases of oedema disease. (ii) Strains producing enterotoxin but not VT, which were isolated from animals with diarrhoea but without oedema disease. (The diarrhoea observed on Farm D may have been due to another agent such as rotavirus, or the strain may have been producing STb, which is not detected in the infant mouse assay.) (iii) Strains that did not produce VT or enterotoxin and were probably non-pathogenic; they were found in the mixed faecal flora of healthy pigs.

Strains of the O139:K82 serotype are non-enterotoxigenic and are associated with oedema disease but not diarrhoea in weaned pigs (Sojka, 1973). They predominated in oedema disease in the UK in the 1950s and 1960s (Sojka *et al.*, 1960; Sojka, 1965), but in our survey all cases of clinical oedema disease in the UK between 1978 and 1985 were associated with O141:K85 strains. O139:K82 strains were usually found as minor constituents of the mixed faecal flora of healthy animals. Three strains of O139:K82 isolated from cases of oedema disease are referred to in table III—one isolated in the UK in 1962, one (Sw/78) in Switzerland, and the other (C/79) in Spain. The O139:K82 serotype may have declined in importance as an oedema disease pathogen in the UK, but it is still significant in other parts of Europe.

The three O139 strains isolated from diagnosed cases of oedema disease were VT producers. When only VT[—] O139 strains were isolated there were no signs of oedema disease on the farms concerned, even on Farms We and J where the strains were present in almost pure culture in faecal swabs. According to the veterinarian in attendance, Farm J had been affected by oedema disease 15 years earlier, but it had disappeared. Possibly the disappearance of the disease coincided with the loss of the ability of the strain to produce VT, even though it could still colonise the pig intestine.

Both the O141:K85, K88ab strains in the survey produced VT and were the only examples of strains that produced verotoxin and classical enterotoxins. Natural loss of the K88 and Hly plasmids by these strains did not affect their ability to produce VT. One of them was the O141:K85, K88ab strain used by Smith and Linggood (1971) to infect pigs. It was used in repeated experiments and found to produce

severe diarrhoea in both neonatal and weaned pigs. Signs of oedema disease were never observed. One possible explanation is that any VT produced *in vivo* was flushed out by the diarrhoea induced by the enterotoxins, and therefore was not absorbed.

Our major observation was that VT⁺ *E. coli* were isolated from all cases of oedema disease. This strongly suggests that the cytotoxin measured by the vero cell assay is identical to the oedema disease principle responsible for the clinical signs of oedema

disease. It should also be noted that although the serotype of a strain may give some indication of its potential to cause oedema disease, not all strains belonging to "oedema disease serotypes" produce VT ("oedema disease principle"). Such VT⁻ strains were not associated with clinical outbreaks of the disease.

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