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Prevalence and characteristics of *Escherichia coli* serotype O157:H7 and other verotoxin-producing *E. coli* in healthy cattle

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SUMMARY

From February to July of 1994, 328 faecal samples from 32 herds were collected and verotoxin-producing *Escherichia coli* (VTEC) found on 84% of the farms. The proportion of animals infected varied from 0–63%. VTEC were recovered from 52 (20%) of 257 cows and from 16 (23%) of 71 calves. Although the VTEC belonged to 25 different serogroups, 7 (O8, O20, O22, O77, O113, O126 and O162) accounted for 46% of strains. Nearly 45% of the 83 bovine VTEC strains belonged to serogroups associated with haemorrhagic colitis and haemolytic uraemic syndrome in humans. However, only 2 (2%) of 83 VTEC strains isolated from cattle belonged to enterohaemorrhagic *E. coli* (EHEC) serotypes (O26:H11 and O157:H7), and only 8 (10%) were positive for the attaching and effacing *E. coli* (*eae*) gene sequence. Polymerase chain reaction (PCR) showed that 17 (20%) of VTEC strains carried VT1 genes, 43 (52%) possessed VT2 genes, and 23 (28%) carried both VT1 and VT2 genes. Characterization of VTEC isolates revealed a heterogeneous population in terms of serogroup and toxin type in the positive herds. This study confirms that healthy cattle are a reservoir of VTEC, but, the absence of *eae* genes in most bovine VTEC strains suggests that they may be less virulent for humans than *eae*-positive EHEC.

INTRODUCTION

Verotoxin-producing *Escherichia coli* (VTEC) are recognized as a cause of haemorrhagic colitis (HC) and the haemolytic-uraemic syndrome (HUS), in humans who contract infection following the consumption of contaminated foodstuffs like meat and unpasteurized milk [1–4]. Epidemiological investigations revealed that cattle frequently excrete VTEC in their faeces and thus may represent a source of infection [5–10]. However, the epidemiology of the organism remains unclear. Identification of the source of contamination of foods with VTEC is important to the understanding of the epidemiology of human VTEC infection and devising strategies for its control. To date most surveys of bovine VTEC infection have expressed the prevalence of infection as the proportion of infected animals in the population. Equally important, from a public health standpoint, is the determination of farm-level prevalence of infection, an aspect of the epidemiology of VTEC which has so far received little attention [11–13].

VTEC from different sources and geographical areas belong to many different O serogroups. However, most of the documented outbreaks of HC and HUS were attributed to only a few serotypes (e.g. O26:H11, O111:H- and O157:H7 or H-) which have been designated enterohemorrhagic *E. coli* (EHEC)

[1–4, 14]. It is not known whether all variants of VTEC are equally pathogenic for humans. However, the relatively low incidence of non-EHEC VTEC disease in humans suggest that VT production alone may not be sufficient for VTEC to cause disease [15, 16]. A factor that may affect virulence of VTEC is the ability to cause attaching and effacing lesions in the intestinal mucosa [15–17].

In this study the prevalence of VTEC infection in cattle in the province of Lugo (Galicia, Northwest Spain) was investigated at the herd and individual animal levels.

MATERIALS AND METHODS

Specimens collection and *E. coli* strains

Thirty-two farms in the province of Lugo (Galicia, Northwest Spain) were visited once between February and July of 1994. Both beef and dairy cattle were sampled. A single faecal swab was obtained from selected cows and from all calves aged 4 months or less. The swabs were placed in transport medium and taken to laboratory for immediate processing. They were used to inoculate lactose-MacConkey agar and cefixime tellurite sorbitol MacConkey (CT-SMAC) medium [7]. From each sample five suspect *E. coli* colonies (lactose positive, lactose negative or sorbitol negative) were chosen, identified and examined for verotoxin (VT) production. Identification was based on biochemical tests, including hydrogen sulphide, citrate, urease and indole. All VTEC strains were confirmed as *E. coli* using the API-20E system (bioMérieux, France). Reference *E. coli* strains, used as positive and negative controls, included: 933 (O157:H7 VT1$^+$, VT2$^+$ and *eae*$^+$), H19 (O26:H11, VT1$^+$ and *eae*$^+$) and K12-185 (non-toxigenic). Strains were stored at room temperature in nutrient broth with 0·75% of agar.

Production and detection of verotoxins in Vero and HeLa cells

For production of verotoxins, bacteria were grown for 20 h in Tryptone Soya Broth with mitomicin C at 37 °C (shaken at 200 rpm) and then centrifuged (6000 g) for 30 min at 4 °C. The Vero and HeLa cell culture assays were performed using nearly confluent cell monolayers grown in plates with 24 wells. At the time of assay, the growth medium (RPMI with polymixin sulphate) was changed (0·5 ml per well) and 75 μl undiluted culture supernatant added. Cells were incubated at 37 °C in a 5% CO_2 atmosphere and the morphological changes in cells observed after 24 and 48 h of incubation using a phase contrast inverted microscope [18].

Detection of VT1, VT2 and *eae* sequences by PCR

Bacteria were harvested from CFA agar, suspended in 200 μl of sterile water, incubated at 100 °C for 10 min to release the DNA, and centrifuged. The supernatant was used in the PCR reaction as described below. Base sequences, locations and predicted sizes of amplified products for the specific oligonucleotide primers used in this study are shown in Table 1 [19, 20]. Oligonucleotide primers were synthesized using a Gene Assembler Special (Pharmacia, LKB Biotechnology Inc.) according to the manufacturers instructions. Amplification of bacterial DNA was performed using 50 μl volumes containing 10 μl of the prepared sample supernatant; the oligonucleotide primers (90 ng for *eae* and VT2 primers; 150 ng for VT1 primers); 0·2 mM (each) dATP, dGTP, dCTP, and dTTP; 10 mM Tris HCl (pH 8·8); 1,5 mM $MgCl_2$; 50 mM KCl; and 1 U of DynaZyme DNA polymerase (Finnzymes OY, Finland). The reaction mixtures were overlaid with an equal volume of mineral oil. The conditions for the PCR were 94 °C for 2 min for initial denaturation of DNA within the sample followed by 30 cycles of 94 °C for 1 min (denaturation), 55 °C for 1 min (primer annealing) and 72 °C for 1 min (DNA synthesis) performed with a thermal cycler (model Gene ATAQ Controller; Pharmacia, LKB Biotechnology Inc, Sweden). The amplified product was visualized by standard submarine gel electrophoresis using 10 μl of the final reaction mixture on a 2% agarose (agarose MP, Boehringer Mannheim, Germany) gels in TBE buffer (89 mM Tris, 89 mM boric acid, 2·5 mM EDTA). The samples were electrophoresed for 40 min at 140 V. Amplified DNA fragments of specific sizes were located by UV fluorescence after staining with ethidium bromide (0·5 μg/ml). Molecular size markers (*Hae* III digest of ϕx174DNA) were included in each gel.

Serotyping

The isolates were serotyped using a modification [21] of the method of Guinée and colleagues [22]. O (O1–O171) and H (H1–H56) antisera were absorbed

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Table 1. *Primers used in PCR to amplify specific fragments from genes for VT1, VT2 and* eae

Primer*	Oligonucleotide sequence (5′-3′)	Location within gene†	Size of amplified product (bp)
VT1a	CAGTTAATGTGGTGGCGAAG	215–234	894
VT1b	CTGCTAATAGTTCTGCGCATC	1089–1109	
VT2a	CTTCGGTATCCTATTCCCGG	288–307	478
VT2b	GGATGCATCTCTGGTCATTG	747–766	
eae-1	ACGTTGCAGCATGGGTAACTC	1054–1074	815
eae-2	GATCGGCAACAGTTTCACCTG	1869–1849	

* References: VT1 and VT2 (20) and *eae* (19).
† In nucleotides.

Table 2. *Prevalence of VTEC strains in healthy cattle*

Age group	Number of animals	Number of animals with VTEC strains				
		Total	$VT1^+$	$VT2^+$	$VT1^+VT2^+$	eae^+
Cows	257	52 (20%)	7 (3%)	33 (13%)	16 (6%)	2 (1%)
Calves	71	16 (23%)	7 (10%)	6 (8%)	4 (6%)	5 (7%)

with the corresponding cross-reacting antigens to remove the non-specific agglutinins. The antisera were obtained from the National Institute of Public Health and Environmental Protection (Bilthoven, The Netherlands). The O antigen was established in all VTEC detected, whereas the flagellar antigen was determined in VTEC belonging to serogroups O26, O111 and O157.

Statistical methods

Results were evaluated using the χ^2 test with Yates' correction for continuity.

RESULTS

Prevalence of VTEC in healthy cattle

A total of 1469 *E. coli* colonies from 328 animals from 32 different farms were investigated for verotoxin production in Vero and HeLa cells. VTEC were recovered from 52 (20%) of 257 cows and from 16 (23%) of 71 calves (Table 2) from 27 (84%) of 32 farms examined. The estimated proportion of animals infected on each farm ranged from 0–63% (Table 3).

Serogroups and EHEC serotypes

A total of 83 VTEC strains were identified in this study, 60 from cows and 23 from calves. VTEC strains belonged to 25 different serogroups; however, 46% (38 of 83) were among 7 serogroups (O8, O20, O22, O77, O113, O126, O162) (Table 4). In 16 (24%) animals with VTEC, 2 (14 animals) or 3 (2 animals) serogroups were identified. In many farms a variety of VTEC strains with different O serogroups were detected (Table 3). Only two VTEC strains, isolated from calves, belonged to EHEC serotypes (O26:H11 and O157:H7).

Verotoxins (VT1 and VT2) synthesized by VTEC strains and attaching and effacing *E. coli (eae)* sequences

PCR demonstrated that 17 (20%) of VTEC strains carried VT1 genes, 43 (52%) possessed VT2 genes, and 23 (28%) carried both VT1 and VT2 genes (Table 4). $VT1^+$ strains were more prevalent in VTEC obtained from calves than from cows (39% *vs.* 13%) ($P < 0{\cdot}05$), whereas $VT2^+$ strains were more commonly recovered from cows (58% *vs.* 35%). In contrast, similar proportions of $VT1^+VT2^+$ strains were observed in adult (28%) and young (26%) animals (Table 5). Only 8 (10%) of the 83 VTEC strains were positive for *eae* genes. Presence of *eae* gene was significantly more frequent among VTEC strains isolated from calves (26%) than from cows (3%) ($P < 0{\cdot}01$).

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Table 3. *Distribution of VTEC strains in the 32 farms investigated*

Farm	Number VTEC+/ Number of animals examined		Serogroups of VTEC
	Cows	Calves	
1	1/7	0/2	NT* (1)†
2	2/5	0/5	O8 (1), NT (2)
3	1/8	1/2	O77 (1), O171 (1)
4	2/10	—	O113 (1), NT (1)
5	0/4	2/5	O22 (1), O88 (1)
6	0/8	0/2	—
7	5/8	—	O8 (1), O22 (1), O77 (1), NT (2)
8	2/7	0/2	O4 (1), O113 (1)
9	3/7	0/1	O2 (1), O20 (2)
10	2/8	0/1	O116 (1), O162 (1), NT (1)
11	1/8	1/2	O138 (1), NT (2)
12	1/8	0/2	O113 (1)
13	2/6	0/4	O77 (1), O113 (1), NT (1)
14	0/4	0/1	—
15	2/10	0/1	O8 (1), O96 (1)
16	3/10	—	O113 (3), NT (1)
17	3/9	0/1	O138 (1), O140 (1), O168 (1), NT (2)
18	2/8	0/2	O22 (1), NT (1)
19	1/9	1/3	O15 (1), O20 (1), O149 (1), NT (1)
20	2/6	1/4	O7 (1), O77 (1), NT (1)
21	1/7	1/3	O22 (1), O26 (1)
22	2/9	1/2	O8 (1), O81 (1), NT (3)
23	1/6	3/6	O20 (1), O77 (1), O116 (1), O126 (3), NT (1)
24	3/8	0/2	O77 (2), O162 (1)
25	3/10	—	O22 (2), O136 (1)
26	1/6	1/1	O77 (1), O113 (1)
27	0/8	0/2	—
28	0/8	—	—
29	1/15	0/5	O136 (1)
30	0/5	0/5	—
31	1/5	4/5	O9 (1), O15 (1), O157 (1), NT (2)
32	4/20	—	O20 (1), O110 (1), O162 (1), NT (1)

* Not typeable strains.

† Number of animals yielding VTEC strains belonging to indicated serogroups.

DISCUSSION

The results of this study indicate that VTEC infection is widespread among healthy cattle in the province of Lugo. VTEC strains were present in similar proportions in adult and young animals (20% *vs.* 23%). In contrast, to some previous studies, in which VTEC were more frequently recovered from young dairy cattle [9, 12, 13]. In a study, comparable in many respects to this one, Wilson and co-workers [12] estimated the prevalence of VTEC infection in cows and calves on Ontario (Canada) dairy farms at 9% and 25% respectively. While, Wells and colleagues [9] in the USA found VTEC in 8% of adult cows and 19% of heifers and calves, and Montenegro and co-workers [11] in Germany identified VTEC in 17% of cows and 9% of bulls.

PCR demonstrated that 20% of bovine VTEC strains isolated in this study carried VT1 genes, 52% possessed VT2 genes, and 28% carried both VT1 and VT2 genes. Several other authors have reported a similar distribution in VTEC from healthy cattle [6, 9, 10, 11]. In contrast, Pohl [23], Wieler [24], Dorn and colleagues [25] observed that the majority of VTEC isolated from calves with diarrhoea in Belgium, Germany and USA produced VT1 only while in previous studies [5, 26, 27] we found that 72% were $VT1^+$, 23% were $VT2^+$ and 5% were $VT1^+VT2^+$ (data

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Table 4. *Serogroups, type of VT and* eae *gene in VTEC strains recovered from healthy cattle*

	Number of VTEC strains					
Serogroup	Total		VT1$^+$	VT2$^+$	VT1$^+$VT2$^+$	*eae*$^+$
O2	1	(1/0/1)*	0	1	0	0
O4	1	(1/0/1)	0	1	0	0
O7	1	(0/1/1)	1	0	0	0
O8	4	(3/1/4)	0	4	0	0
O9	1	(0/1/1)	0	1	0	0
O15	2	(2/0/2)	0	1	1	0
O20	5	(4/1/4)	0	1	4	0
O22	6	(5/1/5)	0	4	2	0
O26	1	(0/1/1)	1	0	0	1
O77	8	(6/2/7)	1	2	5	1
O81	1	(1/0/1)	0	0	1	0
O88	1	(0/1/1)	0	1	0	0
O96	1	(1/0/1)	0	0	1	0
O110	1	(1/0/1)	0	1	0	1
O113	8	(8/0/6)	1	7	0	0
O116	2	(1/1/2)	0	2	0	0
O126	4	(1/2/1)	1	0	3	0
O136	2	(2/0/2)	2	0	0	0
O138	2	(1/1/2)	2	0	0	1
O140	1	(1/0/1)	0	1	0	0
O149	1	(0/1/1)	1	0	0	0
O157	1	(0/1/1)	0	1	0	1
O162	3	(3/0/3)	0	2	1	0
O168	1	(1/0/1)	1	0	0	0
O171	1	(0/1/1)	0	1	0	0
NT†	23	(16/6/16)	6	12	5	3
Total	83	(52/16/27)	17 (20%)	43 (52%)	23 (28%)	8 (10%)

* Number of cows, calves and farms yielding VTEC strains belonging to indicated serogroups.
† Not typeable strains.

Table 5. *Type of VT,* eae *gene and EHEC in bovine VTEC strains*

	Number of VTEC strains from	
Characteristics of VTEC strains	Cows ($n = 60$)	Calves ($n = 23$)
VT1$^+$	8 (13%)	9 (39%)
VT2$^+$	35 (58%)	8 (35%)
VT1$^+$VT2$^+$	17 (28%)	6 (26%)
eae$^+$	2 (3%)	6 (26%)
EHEC*	0 (0%)	2 (9%)

* Enterohaemorrhagic *E. coli* serotypes were recovered in two cases: one strain O26:H11 VT1$^+$*eae*$^+$ and one strain O157:H7 VT2$^+$*eae*$^+$.

not shown). Little is known about the putative pathogenic role of VTEC in cattle. Although VTEC strains have been isolated from a high proportion of both healthy and diarrhoeic calves [5–13, 23–28], recent studies suggest that strains that produce VT1 only, and possess the *eae* gene, in addition may be involved in calf diarrhoea [24, 25, 29]. Interestingly we found the *eae* genes significantly more frequently in VTEC isolated from diarrhoeic calves (59%) (23 of 39) than in those from healthy cattle (10%) ($P < 0{\cdot}001$). Dorn and co-workers [25] suggests that the higher prevalence of VT2-producing *E. coli* than VT1 *E. coli* infections among adult cattle might result in a greater passive transfer of antibodies to VT2 than VT1, resulting in lower incidence of clinical illness due to VT2-producing VTEC strains in calves. This interesting hypothesis requires further investigations.

The production of VT by *E. coli* strains of bovine origin has been detected in 79 O serogroups, although 20 (O4, O8, O22, O25, O32, O45, O82, O84, O103, O111, O113, O116, O121, O136, O146, O153, O157,

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O171, O172 and OX3) are more prevalent among VTEC strains isolated from healthy cattle [1–13, 30]. Twenty-five VTEC serogroups were identified in this study and included four serogroups (O77, O96, O140, O162) not previously described. Fourteen (O2, O4, O7, O8, O9, O15, O20, O22, O26, O110, O113, O126, O157 and O168) of 25 serogroups have been associated with human disease (CH and HUS) [1–4, 30] and this is in agreement with previous reports [9, 10, 11]. Only 2 (2%) strains of the 83 VTEC belonged to EHEC serotypes. In contrast, EHEC serotypes were observed in 11 (28%) of 39 VTEC strains isolated from calves with diarrhoea [5, 26, 27]. The results of this study, and others [5, 8, 23, 27], indicate therefore, that EHEC strains are isolated sporadically from faeces of cattle but appear more prevalent in animals with diarrhoea than in healthy animals.

The number of VTEC serotypes associated with human disease is increasing, although EHEC O157:H7 continues to be the main cause of HC and HUS [31, 32]. We detected this enteropathogen in only 1 (0·3%) of the 328 animals investigated. This is consistent with other surveys of VTEC in cattle, which suggests that EHEC O157:H7 is uncommon in cattle [6, 7, 11, 12]. On the other hand, the prevalence of EHEC O157:H7 infection among cattle is higher on farms linked epidemiologically to cases of O157:H7 associated with illness in humans [7, 9]. The methods used for isolating EHEC O157:H7 greatly influence the isolation rates. Recently, Zhao and co-workers [13], using a sensitive ELISA procedure, detected EHEC O157:H7 in 18 (28%) of 64 herds investigated in 15 states of USA. This, and previous studies [9], revealed that EHEC O157:H7 is more frequently carried by calves and heifers than by adult cattle.

The other objective of this study was to characterize VTEC from healthy animals for their virulence factors and thereby for a possible relationship with types of *E. coli* known to be pathogenic to humans since is not known whether all variants of VTEC are equally pathogenic for humans. However, the relatively low incidence of non-O157 VTEC disease in humans suggests that verotoxin production alone may not be sufficient for VTEC to cause disease in humans [15]. Since intimate adherence to intestinal epithelial cells and effacing of microvilli are also virulence determinants of human pathogenic EHEC strains. The *eae* genes are essential for causing attaching and effacing lesions and are present in most human EHEC strains [15–17]. In contrast, only 8 (10%) of 83 bovine VTEC investigated in this study were positive for *eae*. The low prevalence corresponds to recently published data [29, 33]. The *eae* genes were found frequently in VTEC from cattle and humans with diarrhoea, whereas *eae*-negative VTEC were more common in healthy cattle [15, 29, 33]. Our results indicate that *eae*-positive VTEC are more commonly found among calves than among adult cows, confirming that young animals are a more important reservoir of pathogenic VTEC strains.

Having confirmed that the *eae* gene is an essential virulence factor in EHEC, it is probably the majority of bovine VTEC strains isolated from healthy cattle in this study would not be pathogenic for humans. This would explain, in part, why VTEC are rarely isolated in Spain from the stools of diarrhoeic patients in spite of being so prevalent in cattle. Further studies of VTEC strains isolated from cattle are required to assess their role as human pathogens.

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REFERENCES

1. Blanco J, Blanco M, Blanco JE, Alonso MP, Escribano A. Patogénesis, epidemiología y diagnóstico microbiológico de las infecciones producidas por los *Escherichia coli* enterohemorrágicos productores de verotoxinas. Enferm Infecc Microbiol Clin 1993; **11**: 324–34.
2. Griffin PM, Tauxe RV. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol Rev 1991; **13**: 60–8.
3. Karmali MA. Infection by verocytotoxin-producing *Escherichia coli*. Clin Microbiol Rev 1989; **2**: 15–38.
4. Smith HR, Scotland SM. Vero cytotoxin-producing strains of *Escherichia coli*. J. Med Microbiol 1988; **26**: 77–85.
5. Blanco M, Blanco J, Blanco JE, Ramos J. Enterotoxigenic, verotoxigenic and necrotoxigenic *Escherichia coli* isolated from cattle in Spain. Am J Vet Res 1993; **54**: 1446–51.
6. Beutin L, Geier D, Steinrück H, Zimmermann S, Scheutz F. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven

different species of healthy domestic animals. J Clin Microbiol 1993; **31**: 2483–8.
7. Chapman PA, Wright DJ, Siddons CA. A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O157 from bovine faeces. J Med Microbiol 1994; **40**: 424–7.
8. Pohl P. Les *Escherichia coli* verotoxinogènes isolées des bovins. Ann Méd Vét 1991; **135**: 569–76.
9. Wells JG, Shipman LD, Greene KD, et al. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like-toxin-producing *Escherichia coli* from dairy cattle. J Clin Microbiol 1991; **29**: 985–9.
10. Willshaw GA, Cheasty T, Jiggle B, Rowe B, Gibbons D, Hutchinson DN. Vero cytotoxin-producing *Escherichia coli* in a herd of dairy cattle. Vet Rec 1993; **132**: 96.
11. Montenegro MA, Bülte M, Trumpf T, et al. Detection and characterization of fecal verotoxin-producing *Escherichia coli* from healthy cattle. J Clin Microbiol 1990; **28**: 1417–21.
12. Wilson JB, McEwen SA, Clarke RC, et al. Distribution and characteristics of verocytotoxigenic *Escherichia coli* isolated from Ontario dairy cattle. Epidemiol Infect 1992; **108**: 423–39.
13. Zhao T, Doyle MP, Shere J, Garber L. Prevalence of enterohemorrhagic O157:H7 in a survey of dairy herds. Appl Environ Microbiol 1995; **61**: 1290–3.
14. Levine MM. *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. J Infect Dis 1987; **155**: 377–89.
15. Barret TJ, Kaper JB, Jerse AE, Wachsmuth IK. Virulence factors in Shiga-like toxin-producing *Escherichia coli* isolated from humans and cattle. J Infect Dis 1992; **165**: 979–80.
16. Beutin L, Geier D, Zimmermann S, Karch H. Virulence markers of Shiga-like toxin-producing *Escherichia coli* strains originating from healthy domestic animals of different species. J Clin Microbiol 1995; **33**: 631–5.
17. Jerse AE, Yu J, Tall BD, Karper JB. A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. Proc Natl Acad Sci USA 1990; **87**: 7839–43.
18. Blanco J, Blanco M, González EA, Alonso MP, Garabal JI. Comparative evaluation of three test for the detection of *Escherichia coli* cytotoxic necrotizing factors (CNF1 and CNF2) using filtrates of cultures treated with mitomycin C. FEMS Microbiol Lett 1990; **69**: 311–6.
19. Gannon VPJ, Rashed M, King RK, Golsteyn Thomas EJ. Detection and characterization of the *eae* gene of Shiga-like toxin-producing *Escherichia coli* using polymerase chain reaction. J Clin Microbiol 1993; **31**: 1268–74.
20. Olsvik Ø., Strockbine NA. PCR detection of heat-stable, heat-labile, and Shiga-like toxin genes in *Escherichia coli*. In: Persing DH, Smith TF, Tenover FC, White, TJ, eds. Diagnostic molecular microbiology. Washington: American Society for Microbiology, 1993; 271–6.
21. Blanco J, Blanco M, Alonso MP, Blanco JE, Garabal JI, González EA. Serogroups of *Escherichia coli* strains producing cytotoxic necrotizing factors CNF1 and CNF2. FEMS Microbiol Lett 1992; **96**: 155–60.
22. Guinée PAM, Jansen HW, Wadström T, Sellwood R. *Escherichia coli* associated with neonatal diarrhoea in piglets and calves. In: de Leeww PW, Guinée PAM, eds. Laboratory diagnosis in neonatal calf and pig diarrhoea: Current topics in veterinary and animal science. The Netherlands: Martinus Nijhoff Publishers, 1981; **13**: 126–62.
23. Pohl P, Daube G, Lintermans P, Kaeckenbeeck A, Mainil J. Description de 70 souches d'*Escherichia coli* d'origine bovine possédant les gènes des vérotoxines. Ann Méd Vét 1991; **135**: 267–72.
24. Wieler LH, Bauerfeind R, Baljer G. Characterization of Shiga-like toxin producing *Escherichia coli* (SLTEC) isolated from calves with and without diarrhoea. Zbl Bakt 1992; **276**: 243–53.
25. Dorn CR, Francis DH, Angrick EJ, et al. Characteristics of Vero cytotoxin producing *Escherichia coli* associated with intestinal colonization and diarrhea in calves. Vet Microbiol 1993; **36**: 149–59.
26. Blanco J, González EA, García S, Blanco M, Regueiro B, Bernázdez I. Production of toxins by *Escherichia coli* strains isolated from calves with diarrhoea in Galicia (North-western Spain). Vet Microbiol 1988; **18**: 297–311.
27. Blanco M, Blanco J, Blanco JE, et al. Genes coding for Shiga-like toxins in bovine verotoxin-producing *Escherichia coli* (VTEC) strains belonging to different O:K:H serotypes. Vet Microbiol 1994; **42**: 105–10.
28. Mohammad A, Peiris JSM, Wijewanta EA, Mahalingam S, Gunasekara G. Role of verocytotoxigenic *Escherichia coli* in cattle and buffalo calf diarrhoea. FEMS Microbiol Lett 1985; **26**: 281–3.
29. Mainil JG, Jacquemin ER, Kaeckenbeeck AE, Pohl PH. Association between the effacing (EAE) gene and the Shiga-like toxin-encoding genes in *Escherichia coli* isolates from cattle. Am J Vet Res 1993; **54**: 1064–8.
30. Blanco J, Blanco M, Blanco JE, Alonso MP, Garabal JI, González EA. *Escherichia coli* enterotoxigénicos, necrotoxigénicos y verotoxigénicos de origen humano y bovino. Patogénesis, epidemiología y diagnóstico microbiológico. Servicio de Publicaciones de la Diputación Provincial de Lugo. 1993: 361.
31. Neill MA. Pathogenesis of *Escherichia coli* O157:H7 infection. Curr Opin Infect Dis 1994; **7**: 295–303.
32. Sharp JCM, Coia JE, Curnow J, Reilly WJ. *Escherichia coli* O157 infections in Scotland. J Med Microbiol 1994; **40**: 3–9.
33. Louie M, de Azavedo J, Clarke R, et al. Sequence heterogeneity of the *eae* gene and detection of verotoxin-producing *Escherichia coli* using serotype-specific primers. Epidemiol Infect 1994; **112**: 449–61.