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Isolation and characterization of enterohaemorrhagic *Escherichia coli* O157:H7 from cattle in Belgium and Poland

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SUMMARY

EHEC O157 were isolated from faeces of Belgian and Polish beef slaughter cattle. In Belgium, 1281 faecal samples were analysed by immunomagnetic separation [IMS] after enrichment in buffered peptone water from June 1998 till July 1999. Eighty-one samples (6·3%) were positive for *E. coli* O157. Phage type 8 was most frequently found. Bulls between 1 and 2 years old, slaughtered in September and October were most frequently found positive. Atypical biochemical features were observed in some isolates: 22 (27%) isolates were urease positive and 1 (1·2%) isolate was unable to ferment lactose. In Poland, 551 faecal samples, taken from January 1999 till December 1999, were examined using exactly the same techniques. Four faecal samples (0·7%) were positive for O157 EHEC, yielding seven phage type 8 isolates. All positive samples were from cattle younger than 2 years. Positive samples occurred in August, September and October.

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) produce cytotoxins identical at the genetic and protein level to the shiga toxins produced by *Shigella dysenteria* I. Enterohaemorrhagic *Escherichia coli* (EHEC) are a subset of STEC. EHEC is divided in typical and atypical EHEC strains. Typical EHEC strains cause haemorrhagic colitis and haemolytic uremic syndrome [1]. They carry several virulence factors: (i) Shiga toxins (Stx), also known as Vero cytotoxins, which can be subdivided in Stx1, Stx2 and variants of Stx2 [2], (ii) the presence of the 60-MDa EHEC plasmid [3] carrying the gene for enterohaemolysin production [4], and (iv) the ability to produce attachment-effacement lesions encoded by the *eaeA* gene [5]. Atypical EHEC strains are STEC strains that do not produce lesions and/or do not possess the EHEC plasmid. The term EHEC used in this article represents the typical EHEC strains. Serotype O157:H7 is the most frequently isolated and the most virulent Stx-producing *E. coli* and can be considered as the prototype of EHEC. Other important EHEC serotypes are O26:H11, O103:H2, O111:NM and O113:H21. *E. coli* O157 has become a significant

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worldwide cause of food-borne illness, since its first outbreak in 1982 [6]. Illness has been liked especially to the consumption of ground red meat. Ruminants, particularly cattle, are considered to be the most important reservoir of EHEC. EHEC are carried in the gastrointestinal tract of cattle and shed intermittently without causing disease in cattle [7]. EHEC strains of serotype O157:H7, and its non-motile variant O157:H-, possess a unique biotype characterized by their inability to ferment sorbitol and by the absence of β-glucuronidase, although sorbitol fermenting *E. coli* O157 have been isolated from human and cattle faeces, particularly in Europe [8].

No data are currently available on the prevalence of EHEC O157 in cattle in Belgium, although O157 EHEC has been isolated sporadically in human stool samples [9]. Until now, no outbreaks have been reported. In 1996, 47 cases of HUS were reported in Belgium. Although HUS is not notifiable in Belgium the incidence of HUS on the basis of population data from the National Institute of Statistics was 4·3 cases/100000 in children < 5 years old, 1·8 cases/100000 when all children < 15 years old were considered, and 0·42/100000 when patients of all ages were taken into account. The incidence rate that was determined in this Belgian study is closer to the North American rates, which tend to be higher than those observed in European countries [10]. Little is known about the possible reservoirs of infection in our country. In Poland, *E. coli* O157 has been isolated from clinical material, food samples [11] and healthy persons [12], but until now, no studies have been performed on bovine faeces.

The aim of this study was to determine to what extent Belgian and Polish cattle excreted O157 EHEC and to investigate the possible correlation with age and gender of the cattle as well as seasonal variations of shedding.

METHODS

Sample collection

The Belgian study was carried out between June 1998 and July 1999. Eight slaughterhouses from East and West Flanders were involved in the study. Monthly, about 100 bovine faecal samples were collected: 20 samples from each of 5 different slaughterhouses. Preferentially, samples were taken from cattle originating from different farms. Faecal samples were stored overnight at 4 °C.

In Poland, one slaughterhouse in Warmia and Mazury Region (northern part of Poland), contributed samples from January 1999 to December 1999. This slaughterhouse submitted 20–80 samples per month, for a total of 551 samples. Faecal samples were taken at the slaughterhouse from cattle from different farms.

Isolation of *E. coli* O157

A 25-g amount of each sample was added to 225 ml sterile buffered peptone water (Oxoid, Basingstoke, UK). After homogenization in a stomacher blender for 1 min at normal speed, the samples were incubated for 6 h at 37 °C in a warm waterbath. Next, 1 ml preenrichment was added to 20 μl magnetic beads coated with specific antibody against O157 (Dynal, Oslo, Norway). Immunomagnetic separation (IMS) was performed according to the manufacturer's instructions. The final 0·1 ml suspension obtained after IMS was plated onto sorbitol-MacConkey agar (Oxoid) (SMAC) supplemented with cefixime (0·05 mg/l) and potassium tellurite (2·5 mg/l) (Dynal, Oslo, Norway). Plates were incubated for 20–22 h at 42 °C.

Confirmation of EHEC isolates by agglutination and biochemical tests

Two to four sorbitol non-fermenting colonies were selected for confirmation. The suspected colonies were purified on plate count agar (PCA) (Oxoid) for 20–22 h at 37 °C and were tested for agglutination with an *E. coli* O157 latex test kit (Oxoid). Isolates found positive by this method were identified biochemically as *E. coli* using an API 20E-test strip (bioMérieux, Lyon, France). Each isolate was tested with β-glucuronidase (PGUA) chromogenic diagnostic tablets (Rosco, Taastrup, Denmark). Each O157 isolate was tested on the H7 antiserum-sorbitol fermentation medium [13].

Characterization of EHEC virulence factors

Isolates were further tested for Stx genes by PCR using consensus primer pair amplifying Stx1, Stx2 and its variants [14]. The isolates were then further characterized by using the primers for Stx1, Stx2, intimin (*eaeA*) and the plasmid-encoded enterohaemolysin (*E-Hly*) in separate reactions, in order to make sure that competition did not result in false negative reactions for some of the genes [15]. In each

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Stx2-positive isolate, the Stx2-subunit genes were further subtyped using a PCR–RFLP scheme described previously [16].

Phage typing

E. coli O157:H7 or O157:NM strains were plated on nutrient agar and incubated for 18 h at 37 °C. A single smooth colony was selected and inoculated into 4·5 ml of Difco phage broth (pH 6·8) and incubated while shaking for 1–2 h at 37 °C. The culture was then inoculated by flooding on a Difco phage agar plate and 16 typing phages (obtained from LCDC, Ottawa, Ontario) at their routine test dilution, each in 10 μl quantities, were spotted on the plate. Plates were allowed to dry until no spots were visible, then they were incubated at 37 °C for 18 h, before examination [17].

Statistical analysis

Analysis of variance was performed using the software packet Statistical Package for the Social Science (SPSS 6.0). The χ^2 test was used to determine if the prevalence of *E. coli* O157 was significantly different by age and gender of the animals, and to determine whether characteristics of the isolated *E. coli* O157 were significantly associated with one another, at a level of $P < 0{\cdot}05$.

RESULTS

In Belgium, a total of 167 *E. coli* O157 latex agglutinating positive isolates, were detected in 81 out of 1281 (6·3%) different bovine faecal samples from 62 ($n = 776$, 8·0%) different farms in Belgium. All 167 *E. coli* O157 isolated strains carried the enterohaemolysin (*E-Hly*) and the *eaeA* gene. All were β-glucuronidase negative. With respect to the pathogenicity and virulence characteristics, only one isolate per animal was selected. In animals harbouring colonies with different characteristics one of each was selected. Thus, 82 isolates were compared. Seventy-nine isolates (96%) harboured *stx* genes. Three isolates (3·7%) did not carry *stx* genes but showed all other characteristics of O157 EHEC strains: they were positive for *eaeA* and *E-HlyA* genes and were negative for sorbitol fermentation and β-glucuronidase; two were motile and belonged to phage type (PT) 8 and 54, while the third isolate was non-motile and was PT8. Fifty-six (68%) O157 EHEC isolates were immobilized in the H7 antiserum-sorbitol fermentation medium and belonged thus to the serotype O157:H7; the other 26 (32%) were non-motile (serotype O157:H-). Urease type was significantly associated with the serotype of the isolates ($P = 0{\cdot}001$). Twenty-two isolates (27%), comprising 21 O157:H7 and one O157:H- isolates were urease positive.

The gender of the cattle was associated with the isolation of *E. coli* O157. Bulls were significantly more positive for *E. coli* O157 than cows ($P < 0{\cdot}00001$). Subdividing of the animals in different age-groups gave no significant differences (Table 1).

With regard to the Stx types, 44 isolates (54%) had Stx2vh-a type; 23% had Stx1+Stx2vh-a, 15% had Stx2 type. Stx1 and Stx2+Stx2vh-a were each detected in 2 strains or 2·4%. Stx-type was associated to urease type: 18 isolates (82%) with the Stx2vh-a type and only 4 isolates (18%) with other Stx-types were urease-positive ($P = 0{\cdot}04$). Stx-type was also linked to the serotype ($P = 0{\cdot}00001$). Of the 26 isolates with serotype O157:H-, 16 had genotype Stx1+Stx2vh-a and 25 (96%) of them were urease-negative.

Although 14 different biotypes were represented, 47 of 81 isolates (58%) fell into a single biotype, and there was no significant difference between the distribution of biotypes within flagellar antigen types.

Eleven different phage types were identified. Phage type 8 was most frequently isolated ($n = 21$; 26%) followed by PT34 ($n = 12$; 15%). Fourteen isolates (17%) reacted with the phages but the patterns did not conform to the standard types and were classified as reacts but does not confirm (RDNC). RDNC isolates were more frequently found to be urease positive ($P = 0{\cdot}0007$) than strains belonging to known phage types. Phage type was also significantly related to serotype ($P < 0{\cdot}00001$).

Cattle positive for O157 EHEC were found throughout the year, with a moderate seasonal variation ($P = 0{\cdot}01$). During 2 months, September and October, 33% of the cattle positive for *E. coli* O157 were found. The isolation rate of O157 EHEC by month of sampling is reported in Figure 1.

Polish cattle positive for O157 EHEC were detected in August ($n = 1$), September ($n = 1$) and October ($n = 2$); three bulls and one cow. All isolates were serotype O157:H-, and belonged to phage type 8. None of them produced β-glucuronidase. All isolates gave the API20E profile 5144172. All isolates carried the *E-hly* and *eaeA* gene. They were urease negative and all were isolated from cattle younger than 2 years. Only two different VT-types were detected. Two

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Table 1. *Percent of cattle positive for* E. coli *O157 according to gender and age in Belgium. Gender was not known for 64 samples* [*95% confidence interval*]

		Male		Female	
	Total	Positive %	Total	Positive %	Total
< 1 year	27	0 %	17	0 %	10
1–2 years	408	10·7 % [9·9, 11·5]	393	13·3 % [10·8, 15·8]	15
2–3 years	293	10·7 % [7·4, 14]	178	4·3 % [4·0, 4·6]	115
> 3 years	489	0 % [−0·2, 0·2]	27	2·8 % [2·6, 3·0]	462
Total	1217	9·9 % [9·8, 10]	615	3·3 % [3·1, 3·5]	602

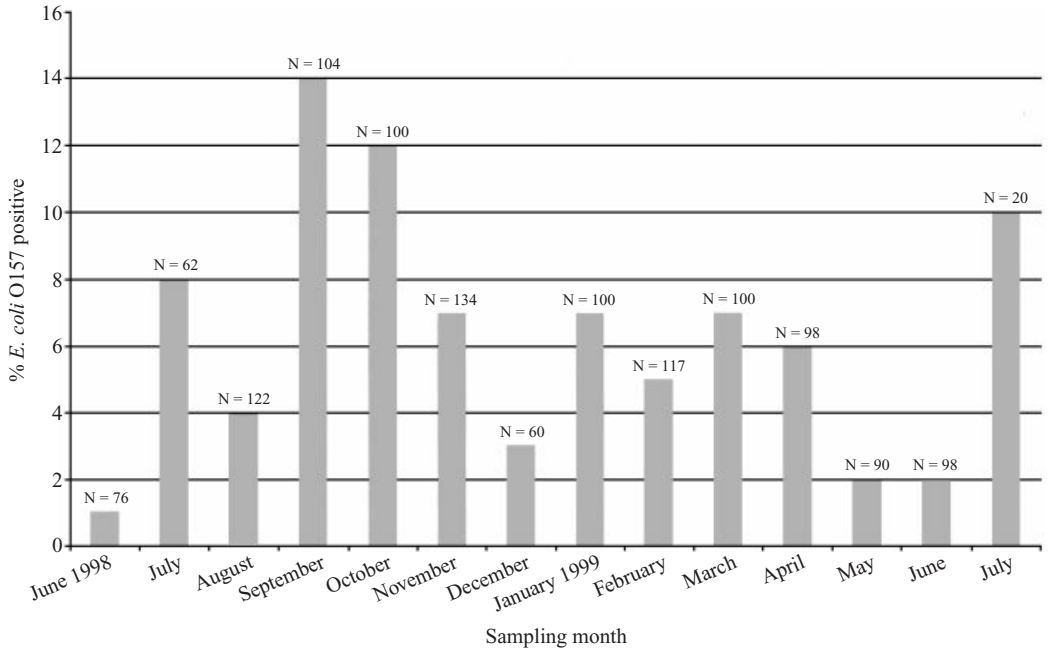

Fig 1. Number of cattle positive for *E. coli* O157 in Belgium. N = number of cattle examined during that month.

isolates showed Stx-type Stx2vh-a and two Stx2vh-a + Stx1.

DISCUSSION

This study was designed to determine the prevalence of O157 EHEC in cattle slaughtered in Flanders (Belgium) and Warmia and Mazury Region (Poland) and to compare it with data from other European countries.

Investigations using IMS enrichment yielded the following prevalence values for O157 EHEC: 0·3 % in faecal samples from cattle at herd in Norway [18]; 0·5 % in faecal samples from veal calves slaughtered in the Netherlands [19]; 1·1 % in faecal samples of calves and adult beef cattle at slaughter in Sweden [20]; 10 % in faecal matter of adult cattle slaughtered in the Netherlands [19], 4·7 % in faecal matter from cattle at the slaughterhouse in France [21], 1·3 % in bovine faecal samples [22] and 16 % in rectal swabs of cattle at slaughter in United Kingdom [23]. No German studies were performed on the prevalence of O157 EHEC in cattle at the slaughterhouse. The O157 EHEC prevalence in healthy German cattle was estimated at 0 % [24] and 0·8 % [25].

EHEC O157 isolates were found in 6·3 % of the examined Belgian cattle and in 0·7 % of the Polish cattle. Because the same isolation and detection protocol was used, it can be concluded that O157 EHEC is more prevalent in Belgian than in Polish cattle. The number of positive cattle in Poland is comparable to the numbers found in Norway, 0·3 % [12] and Sweden 1·1 % [22].

In the Netherlands, 10 % faecal matter of adult cattle but only 0·5 % faecal matter from veal calves at slaughter were found positive for O157 EHEC [19]. In

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Table 2. *List of all* E. coli *O157 strains isolated in Belgium and Poland. Biotype is the number received doing an API 20E test, RDNC; reacts but does not confirm*

Biotype	Serotype	Phage type	Urease	Stx-type	Number isolated strains
1154172	O157:H7	54	+	Stx2 + Stx2vh-a	1
1154172	O157:H7	RDNC	+	Stx2vh-a	3
5044172	O157:H7	2	−	Stx1 + Stx-2vh-a	1
5144112	O157:H7	54	−	Stx2vh-a	1
5144142	O157:H-	8	−	Stx1 + Stx2vh-a	1
5144142	O157:H-	RDNC	−	Stx1 + Stx2vh-a	1
5144142	O157:H7	34	−	Stx1 + Stx2vh-a	1
5144142	O157:H7	34	−	Stx2vh-a	2
5144152	O157:H-	34	−	Stx2vh-a	1
5144152	O157:H7	2	+	Stx2vh-a	1
5144152	O157:H7	34	+	Stx2vh-a	3
5144152	O157:H7	71	−	Stx2vh-a	2
5144162	O157:H-	8	−	Stx1 + Stx2vh-a	1
5144162	O157:H7	34	−	Stx2vh-a	1
5144162	O157:H7	RDNC	−	Stx2vh-a	1
5144172	O157:H-	8	−	Stx negative	1
5144172	O157:H-	8	−	Stx1 + Stx2vh-a	9*
5144172	O157:H-	8	−	Stx2vh-a	4*
5144172	O157:H-	21	−	Stx1 + Stx2vh-a	1
5144172	O157:H-	34	−	Stx2vh-a	1
5144172	O157:H-	49	−	Stx1 + Stx2vh-a	1
5144172	O157:H-	21/28	−	Stx2	2
5144172	O157:H7	2	−	Stx2	2
5144172	O157:H7	2	−	Stx2 + Stx2vh-a	1
5144172	O157:H7	2	+	Stx2vh-a	1
5144172	O157:H7	4	+	Stx2	2
5144172	O157:H7	8	−	Stx negative	1
5144172	O157:H7	8	−	Stx2	2
5144172	O157:H7	23	−	Stx2vh-a	1
5144172	O157:H7	32	−	Stx1 + Stx2vh-a	1
5144172	O157:H7	34	−	Stx2vh-a	3
5144172	O157:H7	54	−	Stx-negative	1
5144172	O157:H7	54	−	Stx2vh-a	5
5144172	O157:H7	21/28	−	Stx1	1
5144172	O157:H7	RDNC	−	Stx2vh-a	1
5144373	O157:H7	32	−	Stx2vh-a	1
5144572	O157:H-	8	−	Stx1 + Stx2vh-a	2
5144572	O157:H7	32	−	Stx2	1
5154172	O157:H-	32	+	Stx1 + Stx2vh-a	1
5154172	O157:H7	4	−	Stx2	1
5154172	O157:H7	4	−	Stx2	2
5154172	O157:H7	32	−	Stx1	1
5154172	O157:H7	32	+	Stx2vh-a	1
5154172	O157:H7	RDNC	+	Stx2vh-a	7
5154572	O157:H7	2	+	Stx2vh-a	1
5154572	O157:H7	RDNC	+	Stx2vh-a	1
7144142	O157:H-	8	−	Stx2vh-a	1
7144172	O157:H-	8	−	Stx1 + Stx2vh-a	1
7144172	O157:H-	8	−	Stx2vh-a	2
7144172	O157:H7	2	−	Stx2	1
7144572	O157:H7	2	−	Stx2vh-a	1

* Contains two Polish strains.

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Belgium, none of the cattle younger than 1 year was positive ($n = 27$). Also in Italy [26] it was reported that veal calves rarely carry O157 EHEC. The latter investigation concluded that this may be due to the peculiar nutritional status of these animals. Calves are usually fed with liquid milk replacers until slaughtering. Due to the diet, the fermentative activity in the rumen has not yet developed. The very low pH in the abomasum may hinder the survival of *E. coli*.

Similar to other countries, there is also a seasonal variation in the *E. coli* O157 shedding in Belgium. Most O157-positive cattle were found in late summer – early autumn namely September ($n = 15$) and October ($n = 12$). In total, 33% of the positive samples were isolated during these months. In Poland, one positive cow was found in August, one in September and two in October. The same seasonal peak was also seen in Sweden [20].

Although *E. coli* is typically described as being unable to produce urease, 26·8% of the Belgian isolates were urease-positive. In Italy, 11·7% were urease-positive [26] and it was proposed that production of urease could serve as a marker in epidemiological surveys. From this investigation it can be concluded that urease production of O157 EHEC is linked to the phage type, serotype and Stx-type. All Polish isolates were urease negative.

From October 1990 to December 1995, 17296 human stool samples were analysed for STEC by PCR in Belgium [9]. Stx genes were detected in 177 (1·0%) stool samples. Twenty-nine (20·3%) of all 143 STEC isolates belonged to serogroup O157 (0·2% of all stool samples analysed). As in the bovine isolates, phage type 8 was most frequently isolated ($n = 12$). Although most urease positive isolates from bovine were reported as RDNC's, 6 of the 7 human urease-positive isolates belonged to phage type 50, which was not found in cattle in the present study.

In Poland, the prevalence of *E. coli* O157 in cattle is much lower. This can be correlated with a lower incidence of EHEC O157 infections in humans. This organism could not be isolated from 1005 human faecal samples. Only six (0·6%) O157 *E. coli* were isolated but none of the isolates were enterohaemorrhagic: they did not produce Stx's and fermented sorbitol in contrast with the bovine isolates [12].

Reasons why Polish cattle carry less frequent *E. coli* O157 can be that Polish farms are not as big as in Belgium. Most Polish farms have between 3 and 10 animals. In Belgium, more cattle are kept at shed. The feeding conditions are also different. In Poland, during the winter cattle are fed with grains and dried grass. Polish farmers do not give additional 'force feeding'. In summer, cattle are kept out-side and only eat grass. In Belgium, bulls are kept inside and eat concentrated feeds during the whole year. In most Belgian farms, cattle have the ability to eat *ad libitum*. In Poland, cattle are fed twice a day during winter.

It can be concluded that similar to other Western (European) countries, cattle in Belgium serve as a natural host of *E. coli* O157, which are pathogenic for humans. When compared to Poland, where isolation rates are much lower, farm size and feeding conditions may contribute to the higher isolation rate of *E. coli* O157 in Belgium.

The prevalence of *E. coli* O157 in cattle makes highly controlled slaughter hygiene crucial for the production of safe food.

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REFERENCES

1. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. Clin Microbiol Reviews 1998; **11**: 142–201.
2. Karmali MA, Lingwood CA, Petric M, Bruton J, Gyles C. Maintaining the existing phenotype nomenclatures for *E. coli* cytotoxins. Am Soc Microbiol News 1996; **62**: 167–9.
3. Karch H, Heesemann J, Laufs R, O'Brien AD, Tacket CO, Levine MM. A plasmid of *Escherichia coli* O157: H7 is required for expression of a new fimbral antigen and for adhesion to epithelial cells. Infect Immun 1987; **55**: 455–61.
4. Beutin L, Montenegro MA, Orskov I, et al. Close association of verocytotoxin (Shiga-like toxin) production with enterohemolysin production in strains of *Escherichia coli*. J Clin Microbiol 1989; **27**: 2559–64.
5. Yu J, Kaper JB. Cloning and characterization of the *eae* gene of enterohaemorrhagic *Escherichia coli* O157:H7. Mol Microbiol 1992; **6**: 411–7.
6. Riley LWR, Remis S, Helgerson SD, et al. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N Engl J Med 1983; **308**: 681–5.
7. Cizek A, Alexa P, Literak I, Hamrik I, Novak P, Smola J. Shiga toxin-producing *Escherichia coli* O157 in feedlot cattle and Norwegian rats from large-scale farm. Lett App Microbiol 1999; **28**: 435–9.
8. Bielaszewska M, Schmidt H, Liesegang A, et al. Cattle can be a reservoir of sorbitol-fermenting Shiga toxin-

producing *Escherichia coli* O157:H- strains and a source of human diseases. J Clin Microbiol 2000; **38**: 3470–3.
9. Pierard D, Stevens D, Moriau L, Lior H, Lauwers S. Isolation and virulence factors of verocytotoxin-producing *Escherichia coli* in human stool samples. Clin Microbiol Infect 1997; **3**: 531–40.
10. Pierard D, Cornu G, Proesmans W, et al. Hemolytic uremic syndrome in Belgium: incidence and association with verocytotoxin-producing *Escherichia coli* infection. Clin Microbiol Infect 1999; **5**: 16–22.
11. Szych J, Paciorek J, Cieslik A, Kaluzewski S. Characterization of *E. coli* O157 strains isolated in Poland from clinical material and food samples. Med Dosw Mikrobiol 1998; **50**: 179–96.
12. Szych J, Paciorek J, Kaluzewski S, et al. Occurrence of *Escherichia coli* O157 in feces of healthy persons, from selected groups, in the country. Med Dosw Mikrobiol 1999; **51**: 81–90.
13. Farmer JJ III, Davis BR. H7 antiserum -sorbitol fermentation medium: a single tube screening medium for detecting *Escherichia coli* O157: H7 associated with hemorrhagic colitis. J Clin Microbiol 1985; **22**: 620–5.
14. Karch H, Meyer T. Single primer pair for amplifying segments of distinct Shiga-like toxin genes by polymerase chain reaction. J Clin Microbiol 1989; **27**: 2751–7.
15. Paton AW, Paton JC. Detection and characterisation of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx_1, stx_2, *eaeA*, enterohemorrhagic *E. coli* *hlyA*, rfb_{0111}, and rfb_{0157}. J Clin Microbiol 1998; **36**: 598–602.
16. Pierard D, Muyldermans G, Moriau L, Stevens D, Lauwers S. Identification of new verocytotoxin type 2 variant B-subunit genes in human and animal *Escherichia coli* isolates. J Clin Microbiol 1998; **36**: 3317–22.
17. Khakhria R, Duck D, Lior H. Extended phage-typing scheme for *Escherichia coli* O157:H7. Epidemiol Infect 1990; **105**: 511–20.
18. Vold L, Klungseth Johansen B, Kruse H, Skjerve E, Wasteson Y. Occurrence of shigatoxinogenic *Escherichia coli* O157 in Norwegian cattle herds. Epidemiol Infect 1998; **120**: 21–8.
19. Heuvelink AE, van den Biggelaar FLAM, et al. Isolation and characterisation of verocytotoxin-producing *Escherichia coli* O157 strains from Dutch cattle and sheep. J Clin Microbiol 1998; **36**: 878–82.
20. Albihn A, Zimmerman U, Rehbinder V, Jansson C, Tysen E, Engvall A. Enterohemorrhagic *E. coli* (EHEC) – a nation-wide Swedish survey of bovine faeces. Epidémiol Santé Animal 1997; **31–32**: 04.13.1–04.13.3.
21. Andral B, Decomble C, Ducotterd B, Lazizzera C, Perelle S, Pichoire M. Contamination of bovine chain by *Escherichia coli* O157:H7. Results of a pre-study carried out at the abattoir. Point Vet 1999; **30**: 45–7.
22. Lahti E, Keskimaki M, Rantala L, Hyvonen P, Siitonen A, Honkanen-Buzalski T. Occurrence of *Escherichia coli* O157 in Finnish cattle. Vet Microbiol 2001; **79**: 239–51.
23. Chapman PA, Siddons CA, Cerdan Malo AT, Harkin MA. A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. Epidemiol Infect 1997; **119**: 245–50.
24. Zschöck M, Hamann HP, Kloppert B, Wolter W. Shiga-toxin-producing *Escherichia coli* in faeces of healthy dairy cows, sheep and goats: prevalence and virulence properties. Lett App Microbiol 2001; **31**: 203–8.
25. Montenegro MA, Bülte M, Trumpf T, et al. Detection and characterisation of faecal verocytotoxin-producing *Escherichia coli* from healthy cattle. J Clin Microbiol 1990; **28**: 1417–21.
26. Bonardi S, Maggi E, Bottarelli A, et al. Isolation of verocytotoxin-producing *Escherichia coli* O157:H7 from cattle at slaughter in Italy. Vet Microbiol 1999; **67**: 203–11.