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Detection and Characterization of Enterohaemorrhagic *Escherichia coli* in Slaughtered Cattle

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With 3 tables

Summary

Fecal samples from slaughtered cattle were studied for enterohaemorrhagic *Escherichia coli* (EHEC) by DNA hybridization with biotin-labelled DNA probes specific for the EHEC virulence plasmid, Shiga-like toxin I (SLT I), Shiga-like toxin II (SLT II) and *eae* gene. Among 136 animals analysed, 47 (34.5%) were found to carry EHEC. The cytotoxic genotypes observed for EHEC strains were: 60.4% SLT I, 12.5% SLT II and 10.4% SLT I+SLT II; 16.7% resulted SLT I and SLT II negative. A total of 14 out of 48 EHEC strains (29.2%) hybridized with a fimbrial probe and 14 of 48 strains with an *eae* probe. An important number of strains (18 out of 48) belonged to serogroups O157, O26 and O111, serogroups also commonly isolated from haemolytic uremic syndrome cases in Chile. While EHEC isolates from the same animal were usually of the same serogroup, one animal was found to carry two EHEC strains of different serogroups. A total of 50% of EHEC strains were sorbitol negative, irrespective of the O serogroup or EHEC genotypic profile. Results obtained in this study strongly suggest that cattle in Chile are a reservoir of EHEC associated with disease in humans.

Introduction

Enterohaemorrhagic *Escherichia coli* (EHEC) produces powerful cytotoxins named verocytotoxin or Shiga-like toxin I (SLT I) and II (SLT II) (LEVINE, 1987). Enterohaemorrhagic *E. coli* of different serogroups are increasingly being isolated from cases of human diseases (GRIFFIN and TAUXE, 1991). Most of these strains belong to serotype O157:H7, which represent the major cause of haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) in humans (LEVINE et al., 1987; GRIFFIN and TAUXE, 1991; CORDOVÉZ et al., 1992; FOOD SAFETY UPDATE, 1993).

Information on sources and reservoirs of EHEC in nature has been limited. The first reported HC and HUS cases were associated with consumption of undercooked hamburger meat, raw milk, contaminated water, mayonnaise and vegetables grown on land where bovine manure is abundant. Recently, it has been shown that contaminated cantaloupe, dry sausages (salami), and yoghurt produced from milk obtained from cattle that carried EHEC, have transmitted infection to humans (RILEY et al., 1983; BORCZYK et al., 1987; GRIFFIN and TAUXE, 1991; MORGAN et al., 1993). Enterohaemorrhagic *E. coli* is widely distributed in the intestinal tract of animals and a majority of outbreaks have resulted from transmission through contaminated food of bovine origin (GRIFFIN and TAUXE, 1991; CAPRIOLI et al., 1993; FOOD SAFETY UPDATE, 1993; MORGAN et al., 1993; RENWICK et al., 1993). Healthy animals carry

these bacteria which can be transmitted directly from animal to animal and animal to man, or indirectly from animal to man through contaminated food. Person to person transmission through close contact and through human fecal contamination of food has also been reported (GRIFFIN and TAUXE, 1991). In Berlin, BEUTIN et al. (1993) isolated EHEC from sheep (66.6%), goats (56.1%), cattle (21.1%), pigs (7.5%), cats (13.8%) and dogs (4.8%). In that study none of the EHEC strains were O157:H7 but nearly 60% corresponded to other serotypes implicated in HC and HUS cases. A wide range of EHEC serotypes have been identified from different animals and have been recognized as potential human pathogens (MONTENEGRO et al., 1990; BÜLTE, 1991; BEUTIN et al., 1993). Reported rates of EHEC isolation from heifers and calves have reached 5% in different countries, with most of the isolates related to serotype O157:H7 (MONTENEGRO et al., 1990; CHAPMAN et al., 1992; WIELER et al., 1992; BEUTIN et al., 1993; CAPRIOLI et al., 1993; WRAY et al., 1993; WELLS et al., 1995). These bacteria have been isolated from both healthy and sick cattle (WIELER et al., 1992; BLANCO et al., 1993; CAPRIOLI et al., 1993; ZAMORA et al., 1994).

In Chile, EHEC has been associated with HUS in children from Santiago, the capital, and two southern cities. The EHEC serogroups isolated included O157, O26 and O111 (CORDO-VÉZ et al., 1992; PRADO et al., 1995). Studies of EHEC infection in animals are limited. In Valdivia, a southern city, ZAMORA et al. (1994) isolated two EHEC strains from neonate lambs and one strain from a calf with diarrhoea. Studies with healthy animals are lacking. The aim of this study was to determine the role of cattle as a possible reservoir of EHEC in Chile.

Materials and Methods

Specimen collection

From January to March 1994, steers recently arrived at one of the four abattoirs in Santiago, Chile, were studied. A rectal swab specimen was obtained from 136 randomly selected cattle. Fecal samples were immediately transported in Cary-Blair medium (EDWARDS and EWING, 1972) to the laboratory for further processing.

Isolation and identification of E. coli strains

Fecal material was streaked on McConkey agar and the plates incubated for 24 h at 37°C. From each fecal specimen, 10 lactose positive colonies with morphological appearance resembling E. coli were randomly selected. Escherichia coli isolates were identified by standard biochemical methods (EDWARDS and EWING, 1972) and stored frozen at −40°C in Tripticase Soy broth (BBL) with 30% of glycerol (Merck, Darmstadt, Germany), for future testing.

Detection of EHEC by DNA probes

Escherichia coli colonies were transferred to Whatman filter papers for colony blot hybridization with biotinylated probes specific for EHEC virulence plasmid associated with adherence fimbrial expression (LEVINE et al., 1987), SLT I and SLT II genes (NEWLAND and NEILL, 1988) and eae gen (JERSE et al., 1990). Methods for filters and biotinylated probe preparation have been previously described in detail (GICQUELAIS et al., 1990). Briefly, filters were treated with NaOH and heat to release DNA from bacterial colonies, render it single stranded, and fix the DNA to the solid phase. Filters were then exposed to a solution containing 1.5 mg/100 ml of lysozyme (Sigma®,St Louis, MO, USA) and 25 g/100 ml of sucrose for 30 min, washed, and treated with proteinase K (Sigma: 250 µg/ml) for 30 min at 37°C. The pCVD 419 EHEC probe was a 3.4 kb Hind III fragment of the EHEC plasmid of prototype strain 933, a highly sensitive and specific probe to detect EHEC (LEVINE et al., 1987). The SLT I probe consisted of a 1.1 kb phage derived Hind III fragment that encoded 98% of the A subunit and the complete B subunit (NEWLAND and NEILL, 1988). The SLT II probe consisted of an 0.84 kb Sma-Pst I fragment that encoded 95% of the A subunit (NEWLAND and NEILL, 1988). All EHEC strains identified were also tested with the eae probe, a 1 kbp Kpn I-Sal I fragment (JERSE et al., 1990), responsible for the attaching and effacing lesion in the intestinal cells. For the purposes of this study, E. coli strains were defined as EHEC when they hybridized with at least one of the probes for virulence plasmid and/or cytotoxins.

Serogrouping and sorbitol phenotype of EHEC

Enterohaemorrhagic *E. coli* selected by DNA probes were serogrouped by agglutination with O157, O26 and O111 monovalent commercial antisera (ProBac®, Sao Paulo, Brazil).

All EHEC strains were subcultured in Trypticase Soy agar (BBL) containing 1% sorbitol and examined for the presence of clear colonies (non-fermentors) after an 18 h incubation at 37°C (OJEDA et al., 1995).

Results

Prevalence of EHEC in slaughtered cattle

Overall, 4–10 *E. coli* colonies could be identified by morphology (mean eight colonies) from each of 136 stool samples studied. All these strains were characterized by gene profile. The EHEC strains were isolated from 47 (34.5%) of the 136 animals.

Detection of fimbrial, SLT I, SLT II and eae specific DNA sequences

Results of the genotypic characterization of strains isolated in this study are summarized in Table 1.

All *E. coli* strains isolated from the same animal had the same genetic profile. The most frequent cytotoxic genotypes of EHEC strains were SLT I positive (60.4%, mostly fimbria negative); SLT II and SLT I–SLT II cytotoxic genotypes were observed in 12.5% and 10.4% of strains, respectively. As shown in Table 1, the most common *E. coli* trait was the sole presence of SLT I (56.2%). Eight EHEC strains (16.7%) hybridized exclusively with the fimbrial probe and three (6.2%) exclusively with the SLT II probe. The *eae* probe hybridized with only 29% of EHEC strains without a significant predominance for any specific cytotoxic genotype (data not shown) or serogroups (Table 2).

Serogrouping and sorbitol phenotype of EHEC strains

All strains hybridizing with one or more probes were serogrouped with antibodies for the three serogroups most commonly associated with HUS in Chile: O157, O26 and O111. A total of 37.5% of EHEC strains could be serogrouped with the three antisera used, with a light predominance of O26 serogroup (16.7%) over O157 (10.4%) and O111 (10.4%) serogroups (Table 3). Only one animal harboured two different serogroups of EHEC: O111 and O26.

Altogether 43 EHEC were tested for sorbitol phenotype. Of these, 24 (55.8%) were sorbitol negative and 19 (44.2%) sorbitol positive. Sorbitol phenotype of EHEC strains was not associated with any specific cytotoxic genotype (data not shown). Only three of five O157 EHEC strains were sorbitol negative.

Table 1. Genotypic profile of 48 EHEC strains isolated from 47 slaughtered cattle

DNA probes positive for:	Number of strains	%
SLT I only	27	56.2
Fimbrial EHEC only	8	16.7
SLT I and SLT II	4	8.3
Fimbrial EHEC/SLT II	3	6.2
SLT II only	3	6.2
Fimbrial EHEC/SLT I	2	4.2
Fimbrial EHEC/SLT I and SLT II	1	2.1
TOTAL	48	100.0

Table 2. Genotypic profile from 18 EHEC strains belonging to three different serogroups

Serogroup	Number of strains	DNA probe for			
		Fimbria	SLT I	SLT II	eae
O157	5	+	–	–	–
		+	+	–	–
		–	+	–	–
		–	+	–	–
		–	+	–	–
O111	5	–	+	+	+
		+	–	+	–
		–	+	+	+
		–	+	–	–
		–	+	–	–
O26	8	–	+	+	+
		–	+	–	–
		–	+	–	–
		+	–	–	–
		+	–	–	–
		+	–	–	–
		–	+	–	+
		+	+	–	–

Table 3. Serogroups of 48 EHEC strains isolated from 47 slaughtered cattle

Serogroup	Number of strains	%
O 157	5	10.4
O 111	5	10.4
O 26	8	16.7
NT	30	62.5

NT, non-typable with the three antisera used

Discussion

Many of the first reported outbreaks of O157:H7 EHEC infections in humans were associated with ingestion of contaminated food of bovine origin (GRIFFIN and TAUXE, 1991). Since then, worldwide isolation of EHEC from cattle have stressed the importance of these animals as a main reservoir of *E. coli* O157:H7 and non-O157:H7 strains (MONTENEGRO et al., 1990; BEUTIN et al., 1993; FOOD SAFETY UPDATE, 1993; WELLS et al., 1995).

This study showed that slaughtered cattle in Chile form an important reservoir of EHEC strains. The prevalence of EHEC carriage in the intestinal contents of cattle detected in this study is higher than that reported in surveys carried out in Italy (CAPRIOLI et al., 1993), England (WRAY et al., 1993), Germany (MONTENEGRO et al., 1990; BEUTIN et al., 1993), the USA (WELLS et al., 1995), Spain (BLANCO et al., 1993) and Canada (BORCZYK et al., 1987). This difference may be partly explained by the methodology used, specifically by the number of colonies studied in each survey. The proposal of MONTENEGRO et al. (1990) was followed, with more than five colonies of *E. coli* studied per animal, in order to increase the probability of EHEC isolation. In this study a mean of eight *E. coli* isolates per animal were analysed. *Escherichia coli* isolates from the same animal had the same virulence markers indicating that in

a great majority of animals they corresponded to the same strain. In only one animal were two different *E. coli* serogroups concomitantly detected in stools.

The most common cytotoxic profile of EHEC strains isolated from slaughtered cattle in Santiago was SLT I (60.4%), either alone or in combination with fimbrial EHEC. This profile is similar to that observed among EHEC strains isolated from HUS cases in Chilean children where SLT I producing strains are more common than SLT II producing strains (CORDOVÉZ et al., 1992; PRADO et al., 1995). In studies from other countries, in contrast, the most frequent genetic profile from EHEC strains isolated from healthy cattle has been SLT II (MONTENEGRO et al., 1990; DORN and ANGRICK, 1991; POHL et al., 1992; WIELER et al., 1992; BEUTIN et al., 1993; CAPRIOLI et al., 1993). In this study only 22.8% of EHEC strains hybridized with the SLT II probe, either alone or in combination with other probes.

It is important to emphasize the low percentage (29.2%) of EHEC strains that hybridized with the fimbrial EHEC probe. The strains reported here were stored frozen at $-40°C$ for 6 months and it is possible that these genes could have been lost during storage and passage, an observation that has been previously considered (DORN and ANGRICK, 1991) and reported (ZTIPORI et al., 1986).

The *eae* gene is necessary for intimate attachment of EHEC to enterocytes (DONNENBERG et al., 1993). A study conducted by WILLSHAW et al. (1994) demonstrated that EHEC O157 strains isolated from cattle hybridized with the *eae* probe derived from the central portion of the *eae*A gene of classical enteropathogenic *E. coli* strain E2348/69, and also with the *eae* O157 probe corresponding to the C-terminal end of the *eae* gene from an EHEC strain of serotype O157:H7 (WILLSHAW et al., 1994). The presence of the *eae* gene was analysed in all EHEC strains isolated from slaughtered cattle by hybridization with the *eae* probe obtained from an enteropathogenic *E. coli* (EPEC) strain. Only a small percentage (29%) of EHEC strains isolated from steers were *eae* positive, a characteristic also observed in EHEC strains isolated from Chilean HUS patients (PRADO et al., 1995). These data are in agreement with those of LOUIE et al. (1994), who reported that 41% of EHEC strains isolated from healthy cattle were *eae* negative in contrast to 17% of EHEC *eae* negative isolated from cattle with severe diarrhoea (LOUIE et al., 1994): thus the *eae* gene may be associated with the occurrence of disease in animals.

In this study, only five strains belonged to serogroup O157, five strains were O111 and eight O26; H typing was not done. These serogroups are common in North America and Europe (CORDOVÉZ et al., 1992; POHL et al., 1992; BLANCO et al., 1993; CAPRIOLI et al., 1993) and are the most frequently isolated from Chilean HUS cases (PRADO et al., 1995). A large number of EHEC strains (62.5%) were non-typable with the three antisera used for serogrouping, not allowing the establishment of a possible association between EHEC serogroup and their genetic profile.

A well-described phenotypic characteristic of the prototype O157:H7 EHEC strain is its inability or delayed capacity to ferment sorbitol (MARCH and RATNAM, 1986). The sorbitol negative phenotype was previously analysed among EHEC, enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and enteroinvasive *E. coli* (EIEC) strains of different serotypes and sources (OJEDA et al., 1995). Sorbitol negativity was common only in EHEC, particularly among strains from severe clinical infections. All the EHEC strains from patients with HUS, irrespective of O serogroup or SLT genotype, were sorbitol negative. These results suggest that testing for sorbitol negativity may be sensitive and predictive of EHEC infections in bloody diarrhoea or HUS (OJEDA et al., 1995). In contrast, it was observed that only 50% of EHEC strains isolated from healthy cattle and three of five O157 strains were sorbitol negative. Screening for sorbitol negative coliforms may be less useful for identifyng EHEC strains in healthy animals, food products and environmental samples, than in humans with bloody diarrhoea.

In conclusion, the results of this study provide evidence that slaughtered cattle could represent an important EHEC reservoir in Chile and that the consumption of raw or under-cooked bovine meat and other bovine products may be an important risk factor for the acquisition of EHEC infection.

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