Common epitopes in LPS of different *Enterobacteriaceae* are associated with an immune response against *Escherichia coli* O157 in bovine serum samples

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Epidemiological studies in both humans and animals conducted in Mexico have shown that the isolation frequency of *Escherichia coli* O157:H7 is low. In a previous study, IgG antibodies against *E. coli* O157, O7 and O116 LPS were found in serum samples from children and adults with no previous history of *E. coli* O157:H7 infection. The present study was designed to determine whether a similar immune response against *E. coli* O157:H7 and other antigenically related bacteria was present in bovine serum samples. A total of 310 serum samples from different herds in Mexico was analysed by microagglutination assays against different enterobacterial antigens, including *E. coli* O157. Microagglutination assays were positive against *E. coli* O7 (55%), O116 (76%) and O157 (36%), *Escherichia hermannii* (15%), *Salmonella enterica* serotype Urbana (14%) and *Salmonella enterica* subsp. *arizonae* (40%). These results were confirmed using a specific ELISA with purified LPS. A positive reaction was observed against the LPS of *E. coli* O7 (29%), O116 (12%) and O157 (22%), *E. hermannii* (4%), *Salmonella* Urbana (13%) and *S. enterica* subsp. *arizonae* (12%). Serum absorption studies of positive serum samples indicated the existence of at least three common epitopes shared by the LPS of *E. coli* O7, O116 and O157, and two others between *E. coli* O157 and *Salmonella* Urbana and *S. enterica* subsp. *arizonae*. A bactericidal assay against *E. coli* O157:H7 using 31 bovine serum samples was performed, and 22 (71%) of these serum samples gave positive results. The data demonstrated that bovine serum showed a response against different enterobacteria, including *E. coli* O157, and that this response could be due to the presence of shared epitopes in the LPS of these organisms.

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INTRODUCTION

Escherichia coli O157:H7 infection and the consequent associated diseases constitute an important health problem for children and the elderly, mainly in developed countries (Bitzan *et al.*, 1993; Gillespie *et al.*, 2005; Griffin & Tauxe, 1991; Rangel *et al.*, 2005). Cattle and sheep are natural reservoirs, and are also important transmission vectors for these bacteria (Chapman *et al.*, 1997; Hancock *et al.*, 1997). Isolation rates have been widely reported (Naylor *et al.*, 2005), with levels of 1.8% in Japan (Miyao *et al.*, 1998),

1.9% in Australia (Cobbold & Desmarchelier, 2000) and from 0 to 7.4% in the USA (Faith *et al.*, 1996). In Mexico, serotyping data of approximately 16 000 *E. coli* isolates collected over the past 20 years have shown an isolation frequency of 1% for *E. coli* O157 strains (Navarro *et al.*, 2003). In another study in Mexico, Callaway *et al.* (2004) showed an isolation frequency of 1.25% for *E. coli* O157 in cattle and 2.1% in swine. Studies conducted in countries with defined outbreaks of haemolytic uraemic syndrome or haemorrhagic colitis associated with the presence of *E. coli* O157:H7 strains have shown that patients infected with these strains exhibit increased levels of IgA, IgG and IgM against the O157 LPS (Chart & Rowe, 1992; Greatorex &

Abbreviations: UNAM, Universidad Nacional Autónoma de México; VTEC, Vero cytotoxin-producing *Escherichia coli*.

Thorne, 1994). In a previous study, 20 % of serum samples from 605 Mexican children and adults showed detectable levels of IgG against *E. coli* O157 LPS (Navarro *et al.*, 2003). The LPS is located in the outer membrane of Gram-negative bacteria, and usually consists of three distinct regions: lipid A, core oligosaccharide and the O antigen (Caroff & Karibian, 2003). Analysis of the LPS O antigens produced by *Salmonella* group N (Bundle *et al.*, 1986), *Escherichia hermannii*, *Brucella abortus* (Perry & Bundle, 1990), *Vibrio cholerae* O1 (Kenne *et al.*, 1982), *Yersinia enterocolitica* and *E. coli* O157 (Perry *et al.*, 1986) has revealed that a common structural epitope consisting of *N*-acetyl derivatives of 4-amino-4,6-dideoxy- α -D-mannopyranosyl residues is present in each respective LPS O antigen and that this factor is responsible for the observed serological cross-reactions. Considering our previous findings in human serum samples (Navarro *et al.*, 2003), the current study was designed to determine the immune response of bovine serum samples against *E. coli* O157:H7 and other antigenically related bacteria.

METHODS

Bacterial strains. The bacterial strains used in this study are listed in Table 1. Prior to use, they were analysed for purity and then conserved in Dorset's medium until required.

Bovine serum samples. A total of 310 bovine serum samples obtained from two adult cattle herds in Mexico were included in the study. Of these, 100 samples were obtained from farms belonging to the School of Veterinary Medicine, National Autonomous University of Mexico (UNAM) and 210 samples from cattle raised in the State of Mexico, adjoining Mexico City. UNAM cattle were raised for veterinary purposes, whilst cattle in the State of Mexico was raised for milk and meat production.

Rabbit serum samples. Anti-LPS sera against bacterial antigens were raised in rabbits using the procedures described by Ewing (1986).

Table 1. Bacterial strains used in this study

Strain no.	Identification	Serotype
Bi7509-41*	<i>E. coli</i>	O7:NM
28w*	<i>E. coli</i>	O116:H10
A2*	<i>E. coli</i>	O157:H19
EDL 933 (ATCC 700927)	<i>E. coli</i>	O157:H7
FMU 110538†	<i>E. hermannii</i>	
FMU 108459‡	<i>S. enterica</i> serotype Urbana	
FMU 108308†	<i>S. enterica</i> subsp. <i>arizonae</i>	

*Strains provided by the Division of Enteric Pathogens, Health Protection Agency, London, UK.

†Strains isolated and characterized in the Public Health Laboratory, Medicine School, UNAM, and used for the different assays.

‡Strain provided by the Laboratory of Microbiology, National Institute of Reference and Epidemiological Diagnosis (INDRE), Mexico.

Microagglutination assay. The serum response to bacterial surface antigens was determined according to Ørskov & Ørskov (1984). Strain purity was verified on both blood and MacConkey agar following overnight incubation at 37 °C. Smooth colonies were then taken from the blood agar culture, plated onto tryptic soy agar (TSA; Difco) and incubated overnight at 37 °C. Developing colonies from each plate were harvested and diluted in 0.15 M saline solution. The suspension was then boiled at 100 °C for 1 h and adjusted with 0.6 % (v/v) formaldehyde/saline solution to a McFarland no. 3 standard to give a concentration of 3.0×10^8 bacteria ml⁻¹.

For the agglutination assays, 50 µl of serial dilutions of the bovine serum samples (from 1:10 to 1:1280) were dispensed into 96-well microtitre plates (Nunc) containing 50 µl of the adjusted bacterial suspension, and incubated for between 18 and 24 h at 50 °C, as described by Ewing (1986). Serum samples capable of agglutinating the bacterial suspensions at a dilution of 1:160 or more were considered positive. Differences in the titres between serum samples were compared using a χ^2 test.

LPS purification. Bacterial LPS was obtained using the phenol/water method described by Westphal & Jann (1965). The purity of LPS samples was enhanced by treatment with DNase, RNase and proteinase K (Sigma).

ELISA. The presence of IgG antibodies against the bacterial LPS (Table 1) in the bovine serum samples was determined using an ELISA test as described by Chart *et al.* (1989). Briefly, 96-well flat-bottomed plates (Immulon) were coated with 1 µg each LPS in 100 µl coating buffer (1.59 g NaCO₃ l⁻¹, 2.93 g NaCHO₃ l⁻¹, pH 9.6). After washing with PBS containing 0.5 % (v/v) Tween 20 (PBS-Tween), non-specific binding sites were blocked by the addition of 200 µl 1 % (w/v) BSA in PBS-Tween (30 min, 37 °C). Aliquots of 100 µl of the diluted bovine serum samples (1:1000) were added to plates and incubated at 37 °C for a further 2 h. The plates were then washed three times with PBS-Tween, and 100 µl goat anti-bovine IgG (diluted 1:1000) labelled with alkaline phosphatase (Zymed Laboratories) was added to each well. Plates were incubated at 37 °C for 2 h and washed three times with PBS-Tween. To visualize the reaction, 200 µl *p*-nitrophenyl phosphate (1 mg ml⁻¹; Sigma) in diethanolamine buffer (pH 9.8; Sigma) was added. The plates were incubated at room temperature for 30 min and the reaction was stopped by adding 25 µl 3 M NaOH. A₄₀₅ was measured in an ELISA microplate reader (MR 580; Dynatech Instruments). The assays were processed in duplicate and the mean value obtained from the two tests was used for the statistical analysis. A cut-off point of 0.7 at a 1:1000 serum dilution was considered a positive ELISA result, as described previously (Navarro *et al.*, 2003).

Antigenic relationships. To determine the existence of common epitopes among *E. coli* O157, O7 and O116, and between *E. coli* O157 and *Salmonella enterica* serotype Urbana and *Salmonella enterica* subsp. *arizonae*, seven bovine serum samples were selected for further study. Five of these showed a response to *E. coli* (O7, O116 and O157), and two responded to the O157 and *Salmonella* Urbana boiled antigens. Serum samples from rabbits immunized individually against *E. coli* O157, *Salmonella* Urbana or *S. enterica* subsp. *arizonae* were also analysed. The serum absorption studies were conducted according to the method of Ewing (1986). In brief, bacteria were grown overnight on blood agar plates at 37 °C and smooth colonies were selected and inoculated onto TSA plates. The cultures were incubated overnight at 37 °C and the bacteria were harvested in 10 ml 0.15 M saline solution, boiled at 100 °C for 1 h and adjusted to a McFarland no. 10 standard to give a concentration of 9×10^8 bacteria ml⁻¹. The bacterial suspension was centrifuged (6000 g, 10 min, 4 °C), and the pellets were mixed with 0.5 ml of each selected serum sample and then incubated for 2 h at 50 °C. The samples were

centrifuged (6000 g, 10 min, 4 °C) to separate the supernatant, and then tested for agglutination against bacterial suspensions of *E. coli* O157, O7 and O116, *Salmonella* Urbana and *S. enterica* subsp. *arizonae* as described previously to determine whether the cross-reactions present in the original serum sample had been eliminated after each procedure.

Serum bactericidal activity. The serum bactericidal activity against a prototype *E. coli* O157:H7 strain (EDL 933, ATCC 700927) was determined in 31 of the 310 serum samples using a method described by Qadri *et al.* (1995). Samples were selected according to their ELISA results: serum samples that had a response against all three *E. coli* LPS antigens (O7, O116 and O157), serum samples that had a response against two of these antigens together (*E. coli* O7 and O157 or O116 and O157), serum samples that had a response against a single antigen (*E. coli* O7 or O116 or O157) or serum samples without a response to the three LPS antigens. In brief, *E. coli* O157:H7 was inoculated onto brain heart infusion (BHI) agar plates (Difco) and incubated at 37 °C for 18 h. Samples of the resulting cultures were plated onto BHI plates and incubated at 37 °C for 4 h. Suspensions from these cultures containing 1.5×10^8 bacteria ml^{-1} were prepared in 0.15 M saline solution. Bactericidal assays were performed by mixing 1 ml bacterial suspension with 2 ml guinea pig complement (Sigma) and 17 ml cold saline solution. Aliquots of 25 μl were mixed with 25 μl of positive (against O157 LPS) or negative bovine serum samples initially diluted 1:2, and then diluted twofold in 96-well microplates and incubated at 37 °C for 1 h. One of the microplate wells containing all assay components except serum was used as a control for bacterial growth. BHI broth (150 μl) was added to the wells and the mixture was incubated at 37 °C for a further 3 h, followed by 18 h incubation at 4 °C. The bactericidal effect of the serum samples was determined by determining the number of c.f.u. The prevalence of ELISA-positive and -negative serum samples was determined by comparing the mean numbers of c.f.u. in three separate assays. For statistical analysis, the mean c.f.u. values of the three independent assays were compared by one-tailed analysis of variance with a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Bovine serum response to LPS antigens

Serum samples obtained from 310 cows from two different herds were analysed by agglutination assays and ELISA. Microagglutination assays showed that 169 (55 %) of the samples were positive for *E. coli* O7, 237 (76 %) for *E. coli*

O116 and 112 (36 %) for *E. coli* O157 (Table 2). In addition, 47 samples (15 %) reacted with *E. hermannii*, 43 (14 %) with *Salmonella* Urbana and 125 (40 %) with *S. enterica* subsp. *arizonae*. Analysis by ELISA showed that 29 % of the serum samples reacted with *E. coli* O7 LPS, 12 % with O116 LPS and 22 % with O157 LPS (Table 3). The serum response against other bacteria showed that 4 % of samples reacted with *E. hermannii* LPS, 13 % with *Salmonella* Urbana LPS and 12 % with *S. enterica* subsp. *arizonae* LPS (Table 3). Location or the conditions under which the animals were kept appeared to be important determinants for the type of immune response against the different antigens tested. Cattle kept in open farms in the State of Mexico showed a higher response to *E. coli* O157 than cows that had been bred at the UNAM farms (45 vs 17 % in the agglutination assay and 27 vs 12 % by ELISA, $P < 0.05$). Other significant differences between the UNAM and State of Mexico herds was the serum response to *E. hermannii* (23 vs 11 %, $P < 0.05$), *Salmonella* Urbana (22 vs 10 %, $P < 0.05$) and *S. enterica* subsp. *arizonae* (55 % vs 33 %, $P < 0.05$). Furthermore, the ELISA results showed that the response to *Salmonella* Urbana LPS was significantly higher in serum samples from animals kept in farms in the State of Mexico ($P < 0.05$). The differences in the immune response were similar regardless of the evaluation method employed. Differences observed in the serum responses between the two farms could therefore be due to factors other than the sensitivity of the test employed or the characteristics of the antigens used, and could include climatic and dietary conditions (Caprioli *et al.*, 2005), differences in the cows themselves or differences in the conditions of the feedlot pen (Smith *et al.*, 2001). These latter factors were not analysed as part of the current study.

The observed immune response against *E. coli* O157 in Mexican cattle serum samples was lower (22 %) than that reported for beef calves at weaning raised in the USA, in which 60–100 % of serum samples were positive (Laegreid *et al.*, 1999). It is important to note that, in this last study, up to 20 % of the animals were found to be shedding *E. coli* O157:H7 in their faeces. A recent study analysing beef

Table 2. Agglutination assays against different enterobacterial antigens

Results of agglutination assays using dilutions of between 1:160 and 1:1280, showing the number of positive samples. Percentages are shown in parentheses.

Serum sample origin	<i>E. coli</i>			<i>E. hermannii</i>	<i>Salmonella</i> Urbana	<i>S. enterica</i> subsp. <i>arizonae</i>
	O7	O116	O157			
Cattle herd (UNAM) ($n=100$)	87 (87)	73 (73)	17 (17)	23 (23)	22 (22)	55 (55)
Cattle herd (Estado de Mexico) ($n=210$)	82 (39)	164 (78)	95 (45)	24 (11)	21 (10)	70 (33)
Total ($n=310$)	169 (55)	237 (76)	112 (36)	47 (15)	43 (14)	125 (40)
<i>P</i> value*	<0.05	>0.05	<0.05	<0.05	<0.05	<0.05

*Statistical difference between the two herds using a χ^2 test.

Table 3. ELISA against different enterobacterial lipopolysaccharides

The ELISA used a cut-off point of >0.7 at A_{405} at a serum dilution of 1:1000. Results are shown as the number of positive samples, with the percentage in parentheses.

Serum sample origin	<i>E. coli</i>			<i>E. hermannii</i>	<i>Salmonella</i> Urbana	<i>S. enterica</i> subsp. <i>arizonae</i>
	O7	O116	O157			
Cattle herd (UNAM) ($n=100$)	28 (28)	9 (9)	12 (12)	6 (6)	7 (7)	12 (12)
Cattle herd (Estado de Mexico) ($n=210$)	62 (30)	29 (14)	57 (27)	5 (2)	32 (15)	26 (12)
Total ($n=310$)	90 (29)	38 (12)	69 (22)	11 (4)	39 (13)	38 (12)
<i>P</i> value*	>0.05	>0.05	<0.05	>0.05	<0.05	>0.05

*Statistical difference between the two herds using a χ^2 test.

carcasses in Mexico (Varela-Hernández *et al.*, 2007) showed frequencies of 5 % for non-motile *E. coli* O157 and 2.7 % for *E. coli* O157:H7, somewhat lower than those observed by Laegreid *et al.* (1999). In the current study, the results also demonstrated that serum samples from the two herds reacted against LPS antigens of *Salmonella*, different *E. coli* serogroups and *E. hermannii*, bacteria that have been shown to cross-react with the O157 LPS (Ewing, 1986; Perry *et al.*, 1986; Rice *et al.*, 1992). *E. coli* O157 LPS contains a tetrasaccharide repeat unit of *N*-acetyl derivatives of 4-amino-4,6-dideoxy- α -D-mannopyranosyl residues, which are also present in *Salmonella* Urbana LPS (Samuel *et al.*, 2004) and in other *Enterobacteriaceae* (Nishiuchi *et al.*, 2000; Rice *et al.*, 1992). This finding could help to explain our results. However, other surface structures including outer-membrane proteins, LPS-associated proteins and the type of core present in the LPS could be also involved in this bovine immune response. Currie *et al.* (2001) observed high levels of both systemic and local IgA against O157 LPS in patients recovering from an *E. coli* O157:H7 infection. This IgA response appeared to have been caused, at least in part, by the R3 core of the O157 LPS. Different studies (Amor *et al.*, 2000; Chart *et al.*, 2002; Currie & Poxton, 1999) have shown that *E. coli* O157 and some other Vero cytotoxin-producing *E. coli* (VTEC) belonging to serogroups O26, O55, O86, O111 and O128 carry a similar R3 LPS core. Gibbs *et al.* (2004) reported a statistically significant difference in the level of R3 core LPS in bovine commensal *E. coli* strains when compared with human strains. This suggests that there is a correlation between the elevated anti-R3 core serum response and contact with a greater number of species related to VTEC and enteropathogenic *E. coli* (EPEC) within the commensal bacterial populations of cattle. Serotyping studies of *E. coli* strains isolated in Mexico from different sources (Cravioto *et al.*, 1990) have also shown the existence of VTEC and EPEC strains in humans and animals. The long exposure of Mexican cattle to all of these environmental antigens could be related to the development of an immune response to different antigens associated with *E. coli* O157.

Antigenic relationships

Five of the bovine serum samples with the highest titres obtained in the ELISA assays against *E. coli* O7, O116 and O157 LPS were selected for absorption assays. These samples, plus two others chosen at random, were used to determine whether the existence of common epitopes was responsible for the cross-reacting immune results. Reactions against *E. coli* O7, O116 and O157 LPS antigens in these samples were completely abolished when the sera were absorbed with *E. coli* O7, O116 and O157 (Table 4). Similar negative results against the three antigens were seen when the sera were absorbed with *E. coli* O7 and O116 only, or with *E. coli* O7 and O157. Interestingly, when the same serum samples were absorbed with *E. coli* O116 and O157, the agglutination was negative against both antigens but not against the *E. coli* O7 antigen (Table 4). These results suggested the existence of at least three shared epitopes among *E. coli* O7, O116 and O157 strains.

Table 4. Detection of common epitopes in *E. coli* strains of different serogroups by absorption assays of bovine serum samples

Bovine serum sample* ($n=5$)	<i>E. coli</i> antigen agglutination titre		
	O7	O116	O157
Non-absorbed sera	1:320	1:160	1:160
Sera absorbed with:†			
O7 + O116 + O157	0	0	0
O7	0	1:40	1:20
O116	1:80	0	0
O157	1:40	1:40	0
O7 + O116	0	0	0
O7 + O157	0	0	0
O116 + O157	1:40	0	0

*Analysis of bovine serum samples that showed similar reactions to O7, O116 and O157 LPS.

†Serum was absorbed with boiled antigen for 1 h.

Table 5. Common epitopes between *E. coli* O157 and *Salmonella* Urbana identified in bovine serum samples by absorption assays

Bovine serum sample	Absorbed with:*	Agglutination titre	
		<i>E. coli</i> O157	<i>Salmonella</i> Urbana
B47	Non-absorbed	1:1280	1:320
B60	Non-absorbed	1:320	1:160
B47	O157	0	0
B60	O157	0	0
B47	<i>Salmonella</i> Urbana	1:160	0
B60	<i>Salmonella</i> Urbana	1:160	0
B47	O157 + <i>Salmonella</i> Urbana	0	0
B60	O157 + <i>Salmonella</i> Urbana	0	0

*Serum was absorbed with boiled antigen for 1 h.

Similar assays with bovine serum samples that reacted against both *E. coli* O157 and *Salmonella* Urbana showed that absorption with *E. coli* O157 induced a negative agglutination reaction against both antigens (Table 5). Absorption with *Salmonella* Urbana caused a negative response against the homologous antigen (*Salmonella* Urbana) and only a reduction in titre against *E. coli* O157. These results also suggested the existence of a shared epitope between the *E. coli* O157 and *Salmonella* Urbana antigens.

Three rabbit serum samples raised individually against *E. coli* O157, *Salmonella* Urbana and *S. enterica* subsp. *arizonae* were analysed by absorption assays to confirm the previous observations. *E. coli* O157 antisera showed titres of 1:3200 to the homologous antigen, and titres of 1:1600 to *Salmonella* Urbana and *S. enterica* subsp. *arizonae* (Table 6). The absorption of *E. coli* O157 serum with *Salmonella* Urbana antigen produced a negative response against the homologous and heterologous antigens. However, when the absorption of this serum was carried out with *S. enterica* subsp. *arizonae*, there was only a reduction in the titres against *E. coli* O157 and *Salmonella* Urbana antigens, whilst the response against *S. enterica* subsp. *arizonae* was completely suppressed by the procedure. The rabbit serum prepared against *Salmonella* Urbana showed the same titre (1:1600) against *E. coli* O157 and against the homologous antigens. The absorption of this serum with *E. coli* O157 induced a negative response against both the *E. coli* O157 and *S. enterica* subsp. *arizonae* antigens, in addition to a reduction in the titre against *Salmonella* Urbana.

Rabbit antiserum raised against *S. enterica* subsp. *arizonae* showed titres of 1:1600 against the homologous antigen, and titres of 1:800 and 1:200 against *E. coli* O157 and *Salmonella* Urbana, respectively (Table 6). The absorption

of this rabbit serum with *E. coli* O157 antigens led to a negative response against *E. coli* O157 and *Salmonella* Urbana antigens, and to a reduction in titre against the homologous antigen (*S. enterica* subsp. *arizonae*). Analysis of these absorption results suggested the existence of at least one shared epitope between *E. coli* O157 and *Salmonella* Urbana, and another between *E. coli* O157 and *S. enterica* subsp. *arizonae*. Although common epitopes between *E. coli* O157 and *Salmonella* Urbana have been reported (Samuel *et al.*, 2004), the existence of common epitopes between *E. coli* O157 and *S. enterica* subsp. *arizonae* has not. These observations suggested that the serum response against *E. coli* O157 observed in this study could be associated with a heterologous immune response against other more common bacteria.

The existence of core epitopes shared by different *E. coli*, including O157:H7 (Chart *et al.*, 2002; Currie & Poxton, 1999), and *Salmonella* and *Shigella* spp. (Gibb *et al.*, 1992), suggests the possibility of the production of a cross-protective immune response. Currie *et al.* (2001) observed that a group of workers constantly exposed to different organisms in a slaughterhouse showed elevated levels of IgM to the R3 LPS core. These findings suggest that a high frequency of exposure to organisms with shared epitopes could be associated with the presence of a protective immune response.

Serum bactericidal activity

The bactericidal response against *E. coli* O157 was positive in 22 of the 31 selected bovine serum samples. The bactericidal titres ranging from serum dilutions of 1:2 to 1:64 coincided with c.f.u. counts of between 1.16×10^7 and 17.20×10^7 c.f.u. ml⁻¹, compared with 28.83×10^8 c.f.u. ml⁻¹ found in the negative control; this difference was statistically significant ($P < 0.05$) (Table 7). Analysis of the reactivity of the 22 serum samples against the different LPSs used in this study indicated that six were positive against O157 LPS only, three reacted with both O157 and O7 LPS, two reacted with O7 LPS, two with O7 and O116 LPS, and nine reacted with all three LPS antigens (O7, O116 and O157). The nine serum samples without bactericidal activity against O157:H7 showed a response against O157 LPS only by ELISA, two reacted with both O7 and O157 LPS antigens, one reacted with O7 LPS only, one with both O116 and O157 LPS antigens, and three showed no response to any of the *E. coli* LPSs studied.

The neutralizing capacity of serum antibodies is important for the host to eliminate micro-organisms from mucosal surfaces. Studies carried out using O157-specific polysaccharide-protein conjugates in vaccinated adults (Konadu *et al.*, 1998), children (Ahmed *et al.*, 2006) and mice have shown that serum IgG LPS antibodies with high bactericidal activity titres have a protective immune response (Konadu *et al.*, 1994). Similarly, cattle colonized with enteric organisms that share common repeating units in

Table 6. Common epitopes between *E. coli* O157 and *Salmonella* Urbana, and *E. coli* O157 and *S. enterica* subsp. *arizonae*, identified in rabbit serum by absorption assays

Serum sample	Absorbed with:*	Agglutination titre		
		<i>E. coli</i> O157	<i>Salmonella</i> Urbana	<i>S. enterica</i> subsp. <i>arizonae</i>
<i>E. coli</i> O157	Non-absorbed	1:3200	1:1600	1:1600
<i>Salmonella</i> Urbana	Non-absorbed	1:1600	1:1600	1:800
<i>S. enterica</i> subsp. <i>arizonae</i>	Non-absorbed	1:800	1:200	1:1600
<i>E. coli</i> O157	<i>Salmonella</i> Urbana	0	0	0
<i>S. enterica</i> subsp. <i>arizonae</i>	<i>Salmonella</i> Urbana	1:100	0	1:400
<i>E. coli</i> O157	<i>S. enterica</i> subsp. <i>arizonae</i>	1:100	1:100	0
<i>Salmonella</i> Urbana	<i>S. enterica</i> subsp. <i>arizonae</i>	1:200	1:800	0
<i>Salmonella</i> Urbana	<i>E. coli</i> O157	0	1:50	0
<i>S. enterica</i> subsp. <i>arizonae</i>	<i>E. coli</i> O157	0	0	1:800
<i>E. coli</i> O157	O157 + <i>Salmonella</i> Urbana	0	0	0
<i>Salmonella</i> Urbana	O157 + <i>Salmonella</i> Urbana	0	0	1:50
<i>E. coli</i> O157	O157 + <i>S. enterica</i> subsp. <i>arizonae</i>	0	0	0
<i>S. enterica</i> subsp. <i>arizonae</i>	O157 + <i>S. enterica</i> subsp. <i>arizonae</i>	0	0	1:50

*Serum was absorbed with boiled antigen for 1 h.

their LPS carbohydrates seem to have a raised serum response against a specific LPS, in this case O157.

Different factors affect the shedding of *E. coli* O157:H7 in cattle bred in developing and developed countries. Some of

Table 7. Bactericidal activity against *E. coli* O157:H7 in 31 bovine serum samples with or without an ELISA response to combinations of O7, O116 and/or O157 *E. coli* LPS antigens

ELISA response	No. of serum samples	Bactericidal activity titre*	No. of c.f.u. ($\times 10^7$)	P value†
O157	1			
O157 + O7 + O116	1	1:2	1.16	0.005
O7 + O116	1			
O157 + O7	1	1:4	5.84	0.001
O157 + O7 + O116	3			
O157	1			
O157 + O7	2	1:8	4.40	0.005
O157 + O7 + O116	2			
O157	2			
O157 + O7 + O116	2	1:16	14.10	0.003
O7	1			
O7 + O116	1	1:32	15.50	0.002
O157	2			
O157 + O7 + O116	1	1:64	17.20	0.002
O7	1			
Total	22			

*Bactericidal activity was analysed by comparison with the mean c.f.u. obtained from the negative control serum sample (28.83×10^8 c.f.u. ml⁻¹).

†One-tail analysis of variance with a significance level of $P < 0.05$.

these are related to climatic (Chapman *et al.*, 1997; Hancock *et al.*, 1997) or dietary conditions (Diez-Gonzalez *et al.*, 1998), or to the age of the animals (Cray & Moon, 1995). The frequency of isolation of *E. coli* O157 in dairy cattle in Mexico is low, around 1.25 %, according to Callaway *et al.* (2004), with a prevalence rate for the isolation of *E. coli* O157:H7 of around 3.0 % (Navarro *et al.*, 2006); the rate was 2.7 % in a study at a slaughter plant involving beef carcasses (Varela-Hernández *et al.*, 2007). Prevalence data reported for *E. coli* O157 in other Latin American countries varies. In Colombia, *E. coli* O157 was isolated in 6.5 % of 307 rectal swabs from cattle (Mattar & Vásquez, 1998). The prevalence of *E. coli* O157 in healthy young beef steers and grazing-fed cattle in Argentina was 0.5 and 6.8 %, respectively (Meichtri *et al.*, 2004; Padola *et al.*, 2004), whilst similar studies in Brazil have shown rates of 0.6 % in dairy cattle (Irino *et al.*, 2005), and 1.5 % in beef and dairy calves (Cerqueira *et al.*, 1999). In developed countries, the prevalence rates of *E. coli* O157:H7 vary considerably from 1.8 % in Japan (Miyao *et al.*, 1998) to 23 % in the USA (Smith *et al.*, 2001). Studies conducted in England and Wales have reported prevalence rates of *E. coli* O157 ranging from 0.5 to 36 % in dairy herds (Chapman *et al.*, 1997; Hancock *et al.*, 1997), and from 1.2 to 51 % in cattle in general (Paiba *et al.*, 2003). Although some studies involving slaughtered animals have shown that only 10 % of cattle shed *E. coli* O157 in their faeces (Omisakin *et al.*, 2003), a study of cattle farms in Scotland reported that 80 % of *E. coli* transmission could arise from only 20 % of the most infectious animals (Matthews *et al.*, 2006).

The current study showed that cattle in Mexico had a serum response against *E. coli* O157:H7 in 22 % of 310

serum samples obtained from animals living under different conditions, with a prevalence of isolation of *E. coli* O157:H7 of 2.7%. The results of this study and the review of *E. coli* O157:H7 isolation data from previous studies, which tend to vary widely, lead to the conclusion that variation in prevalence rates could be associated with an immune response against common epitopes present in other enteric organisms that could in turn be protecting against intestinal colonization by *E. coli* O157:H7. Studies are currently being undertaken to further investigate this.

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