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Occurrence and virulence patterns of *E. coli* O26, O103, O111 and O145 in slaughter cattle

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Abstract

The study attempted to investigate the occurrence of non-O157 *E. coli* serogroups O26, O103, O111 and O145 in cattle at slaughter and to determine the virulence potential of these isolates. A total of 399 fecal samples were analyzed by selective plating and *E. coli* isolates were characterized by polymerase chain reaction (PCR) for the genes *vtx1, vtx2, eae* and EHEC *hlyA.* Immunomagnetic separation (IMS) is required to increase the efficiency of the isolation procedure. *E. coli* O26, O103, O111 and O145 were recovered from 24 (6%) fecal samples. *E. coli* O26 and O103 seemed to be more abundant in slaughter cattle than *E. coli* O111 and O145. Sixteen out of the 24 isolates harbored *vtx* genes. All *vtx*-positive isolates harbored one or more additional virulence factors. Six out of the 8 *vtx*-negative isolates harbored *eae* and/or EHEC *hlyA,* whereas 2 strains harbored none of the tested virulence genes.

Keywords: non-O157 *E. coli*, VTEC, slaughter cattle, feces

Introduction

Verocytotoxigenic *Escherichia coli* (VTEC) constitute a major public health concern, because of the wide range of diseases they can cause, such as uncomplicated watery diarrhea, hemorrhagic colitis and hemolytic uremic syndrome. Cattle are considered to be the main reservoir and the transmission of the pathogen to humans is possible by contaminated foodstuffs, contact with infected animals or

contaminated environment (Chapman et al., 1993). *E. coli* O157:H7 represents the major cause of human illness. However VTEC non-O157 have been increasingly isolated from clinical cases. In Australia (Goldwater et al., 1996) and Argentina (Lopez et al., 1989) VTEC non-O157 infections appear to be more common than *E. coli* O157:H7 infections. In Germany VTEC non-O157 have even replaced VTEC O157 as the most commonly isolated VTEC from patients with HUS (Huppertz et al., 1996). While the epidemiology of VTEC O157 is well established, epidemiological studies on VTEC non-O157 are limited. Karmali et al. (2004) classified VTEC strains into 5 seropathotypes according to their incidence and association with HUS and outbreaks. Seropathotypes A and B include strains belonging to the most pathogenic serotypes i.e. O157:H7, O157:NM, O26:H11, O103:H2, O111:NM, O121:H19, and O145:NM. Apart from the capacity to produce verocytoxins, VTEC may express other virulence factors such as intimin and enterohemolysin.

In view of the increasing incidence of seropathotype B, the aim of this study was i) to determine the occurrence of *E. coli* serogroups O26, O103, O111 and O145 in cattle at slaughter and ii) to characterize the virulence profiles of the isolates.

Material and methods

Between December 2007 and June 2009, a total of 399 fecal samples was collected in 3 Belgian abattoirs. Each sampling day, rectal

samples from cattle originating from different farms were taken. The isolation method described by Possé (2008) was used. Briefly, a 25-g amount of each sample was enriched selectively in modified TSB. After 6 and 24 hours of enrichment, 100 µl of the enrichment broth was plated onto the novel differential agar medium for detection of *E. coli* O26, O103, O111 and O145 serogroups (Possé et al., 2008). After 24 hours of enrichment, serogroup-specific IMSs were also applied on the enrichment broth prior to plating onto the differential agar medium. For the serogroups O26 and O103, Dynabeads (Invitrogen, Paisley, UK) were used, whereas for the serogroups O111 and O145 Captivate beads (Lab M, Lancs, UK) were applied. After incubation, suspected colonies were transferred to serogroup-specific confirmation media. Isolates with a suspected morphology on both differential and confirmation media were examined using serogroup-specific PCR methods (Posse et al., 2007). One isolate per sample was further examined for the presence of virulence genes by a multiplex PCR, applying the primers for *vtx1*, *eae* and EHEC *hlyA* described by Fagan et. al. (1999) and for *vtx2* described by Paton (1998) to identify VTEC strains.

Results

A total of 399 fecal samples from 50 female and 349 male beef cattle were investigated for the presence of *E. coli* serogroups O26, O103, O111 and O145. Samples were taken from cattle originating from 199

different farms, respectively 1 animal from 122 farms, 2 animals from 31 farms and more than 2 animals from 44 farms. Non-O157 *E. coli* strains were recovered from 6 (12%) cows and 18 (5.2%) bulls originating from 21 (11%) of 199 herds examined (Table1). From the one and the two farms, from which two positive animals, carrying strains belonging to the same serogroup and with identical virulence profiles, were detected, 2 and 3 animals were sampled, respectively. The herd specific types were not investigated in this survey. Of the 24 fecal samples in which *E. coli* O26, O103, O111 and O145 was detected, 16 harbored VTEC isolates (Table 2). The serogroups O26 and O103 were detected most frequently, respectively in 9 and 10 samples. Serogroups O111 and O145 were detected in respectively 2 and 3 samples.

Direct plating after 6h resulted in 7 positive samples, while a prolonged enrichment period until 24h yielded only 4 positive samples. Application of IMS after 24 hours of enrichment resulted in 13 (54%) of the 24 positive samples (Table 3). Regarding to direct plating versus IMS, no differences were observed in the proportion of *vtx*-positive and *vtx*-negative *E. coli* isolates depending on the methodologies used. As only a limited number of positive samples were obtained, no definite conclusions could be drawn regarding the isolation efficiency of IMS prior plating for the different serogroups.

Although, the obtained results may suggest that serogroup O26 was detected more efficiently after the application of IMS prior plating.

Virulence profiles of the isolated *E. coli* strains are shown in Table 4. Of the 24 isolates, all originating from different animals, 16 (66.5%) isolates harbored *vtx* genes. Seven (29%) strains harbored only the *vtx1* gene, 7 (29%) only the *vtx2* gene and 2 (8.5%) possessed the *vtx1* as well as the *vtx2* gene. All *vtx*-positive isolates harbored one or more additional virulence factors, i.e. 11 (46%) isolates possessed both *eae* and EHEC *hlyA*, whereas respectively 1 and 4 isolates only harbored *eae* or EHEC *hlyA*. Of the strains, which do not carry *vtx* genes, 6 strains of 8 harbored *eae* and/or EHEC *hlyA*. Two strains possessed none of the tested virulence genes.

Discussion

This study was designed to determine the occurrence of *E. coli* serogroups O26, O103, O111 and O145 in cattle at slaughter. To our knowledge, a set of novel differential and confirmation media for non-O157 *E. coli* strains was used for the first time on naturally contaminated fecal samples. The obtained results indicate that only a combination of direct plating after two enrichment times (6 hours and 24 hours) and the application of IMSs after 24 hours of enrichment can yield the highest number of positive samples. However such a protocol is laborious, expensive and time consuming. This finding is in contrast with the results obtained by Verstraete et al. (2010) on

artificially contaminated fecal samples where most of the fecal samples tested positive both by direct plating and IMS prior to plating. This difference may be explained by the fact that results obtained from artificially contaminated fecal samples can differ from those obtained from naturally contaminated fecal samples (Tutenel et al., 2003).

The second objective was to characterize the isolated strains for their virulence factors and hence their possible virulence capacity for humans. Since VTEC are characterized by the presence of at least 1 *vtx* gene, 66.5% of the isolates belong to this group by definition (Blanco et al., 2004). However, the *vtx*-lacking strains cannot be neglected because they have occasionally been isolated from patients with HUS (Bielaszewska et al., 2008). It is rather unclear if these strains are inherently *vtx*-negative or whether they have lost their *vtx*-genes during culturing. The *vtx*-negative strains possessing genes encoding for intimin (*eae)* and enterohemolysin (EHEC *hlyA)* are generally regarded as enteropathogenic *E. coli* (EPEC). In comparison with VTEC, such strains are less likely to cause severe human disease. Nevertheless EPEC have emerged in recent years as a cause of diarrhea in humans (Ramachandran et al., 2003).

There is a wide range of reported values on the occurrence of non-O157 *E. coli* in cattle. There are limitations in comparing the results of the conducted studies due to the isolation procedure discrepancies as there is no international standard isolation procedure. According to the

EFSA report (EFSA, 2010), the proportion of positive samples ranges from 0% to 30% with an average of 2.2%. However, high rates of occurrence of VTEC (O157 and non-O157) were detected by Pradel et al. (2000) and Rahn et al. (1996) using PCR screening for verocytotoxin-encoding genes. In a study of slaughter cattle in Italy, VTEC non-O157 were isolated in 3.6% of 247 animals (Bonardi et al., 2004). Investigations for the presence of the different non-O157 *E. coli* serogroups revealed that in Korea (Jeon et al., 2006) 6.67 and 4.57% of the cattle harbored *E. coli* O26 and O111, respectively and in Germany (Wieler et al., 1996) 1.2%, 0.75% and 0.5% of the cattle were carrier of *E. coli* O26, O111 and O145, respectively. On farm-level investigations for the occurrence of *E. coli* serogroups O26, O103, O111 and O145 in cattle in Scotland, revealed that the weighted mean prevalence in fecal pats were 4.6% for O26, 2.7% for O103, 0.7% for O145 and 0% for O111 (Pearce et al., 2006). Thus, regarded to these studies we have detected a comparable proportion (4%) of VTEC carrier animals at the slaughterhouse level. The proportion of female cattle considered as non-O157 *E. coli* carrier was slightly higher than that of male cattle. However, due to the minor proportion of female animals included in this survey, the significance was not statistically determined. For O157, Aslantas (2006) demonstrated that the percentage of the male cattle determined as *E. coli* O157 positive was not significantly higher than that of female cattle. The present

study also revealed that 7% of the cattle farms house VTEC carrier animals.

Therefore, results obtained in this study indicate that cattle may be an important source of VTEC non-O157. As VTEC can enter the food chain through many cattle-related transmission ways, further research is indispensable to reveal prevention strategies on farm, as well as in the slaughterhouse and food processing industries.

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Conflict of interest statement

None

Table legends

Table 1: Occurrence of *vtx*-positive and *vtx*-negative *E. coli* belonging to the serogroups O26, O103, O111 and O145 in cattle at slaughter.

Table 2: Number of animals positive for *vtx*-positive and *vtx*-negative *E. coli* belonging to the serogroups O26, O103, O111 and O145

Table 3: Effect of enrichment times and the application of IMS on the isolation rate of *E. coli* serogroups O26, O103, O111 and O145 from cattle at slaughter

Table 4: Virulence patterns of *E. coli* serogroups O26, O103, O111 and O145 strains isolated from cattle at slaughter.

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	Male	Female	Total
N° animals sampled	**349**	**50**	**399**
***Vtx*-negative animals**	**5**	**3**	**8(2%)**
***Vtx*-positive animals**	**13**	**3**	**16(4%)**
Total	**18**	**6**	**24(6%)**
N° farms sampled	**174**	**25**	**199**
Vtx*-negative farms	**4**	**2**	**6(3%)**
Vtx*-positive farms*	**12**	**3**	**15(7%)**
Total	**16**	**5**	**21 (10%)**

* At least 1 *vtx*-negative animal was delivered

** At least 1 *vtx*-positive animal was delivered

Serogroup	*vtx*	Male	Female	Total
O26	-	3	0	3
	+	3	3	6
O103	-	0	3	3
	+	7	0	7
O111	-	0	0	0
	+	2	0	2
O145	-	2	0	2
	+	1	0	1
Total	-	5	3	8
	+	13	3	16

Serogroup	Enrichment time and IMS			Total
	6h	24h		
	Direct plating	IMS prior plating	Direct plating	
O26	2	6	1	9
O103	4	4	2	10
O111	1	1	0	2
O145	0	2	1	3
Total	7	13	4	24

Serogroup	Virulence determinants				N° of isolates
	vtx1	*vtx2*	*eaeA*	EHEC *hlyA*	
O145	-	-	+	+	**2**
O145	-	+	+	+	**1**
O103	-	-	-	-	**2**
O103	+	-	+	+	**2**
O103	+	+	+	+	**1**
O103	-	+	+	+	**3**
O103	-	+	-	+	**1**
O103	-	-	+	-	**1**
O111	+	-	+	+	**1**
O111	-	+	+	-	**1**
O26	-	-	+	-	**3**
O26	+	+	+	+	**1**
O26	+	-	+	+	**2**
O26	-	+	-	+	**1**
O26	+	-	-	+	**2**
Total	**9**	**9**	**18**	**17**	**24**