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

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11 **Abstract**

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

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

27 **Introduction**

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

34 contaminated environment (Chapman et al., 1993). *E. coli* O157:H7
 35 represents the major cause of human illness. However VTEC non-
 36 O157 have been increasingly isolated from clinical cases. In Australia
 37 (Goldwater et al., 1996) and Argentina (Lopez et al., 1989) VTEC
 38 non-O157 infections appear to be more common than *E. coli* O157:H7
 39 infections. In Germany VTEC non-O157 have even replaced VTEC
 40 O157 as the most commonly isolated VTEC from patients with HUS
 41 (Huppertz et al., 1996). While the epidemiology of VTEC O157 is
 42 well established, epidemiological studies on VTEC non-O157 are
 43 limited. Karmali et al. (2004) classified VTEC strains into 5
 44 seropathotypes according to their incidence and association with HUS
 45 and outbreaks. Seropathotypes A and B include strains belonging to
 46 the most pathogenic serotypes i.e. O157:H7, O157:NM, O26:H11,
 47 O103:H2, O111:NM, O121:H19, and O145:NM. Apart from the
 48 capacity to produce verocytotoxins, VTEC may express other virulence
 49 factors such as intimin and enterohemolysin.
 50 In view of the increasing incidence of seropathotype B, the aim of this
 51 study was i) to determine the occurrence of *E. coli* serogroups O26,
 52 O103, O111 and O145 in cattle at slaughter and ii) to characterize the
 53 virulence profiles of the isolates.

54 **Material and methods**

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

57 samples from cattle originating from different farms were taken. The
58 isolation method described by Possé (2008) was used. Briefly, a 25-g
59 amount of each sample was enriched selectively in modified TSB.
60 After 6 and 24 hours of enrichment, 100 µl of the enrichment broth
61 was plated onto the novel differential agar medium for detection of *E.*
62 *coli* O26, O103, O111 and O145 serogroups (Possé et al., 2008). After
63 24 hours of enrichment, serogroup-specific IMSs were also applied on
64 the enrichment broth prior to plating onto the differential agar
65 medium. For the serogroups O26 and O103, Dynabeads (Invitrogen,
66 Paisley, UK) were used, whereas for the serogroups O111 and O145
67 Captivate beads (Lab M, Lancs, UK) were applied. After incubation,
68 suspected colonies were transferred to serogroup-specific confirmation
69 media. Isolates with a suspected morphology on both differential and
70 confirmation media were examined using serogroup-specific PCR
71 methods (Posse et al., 2007). One isolate per sample was further
72 examined for the presence of virulence genes by a multiplex PCR,
73 applying the primers for *vtx1*, *eae* and EHEC *hlyA* described by Fagan
74 et. al. (1999) and for *vtx2* described by Paton (1998) to identify
75 VTEC strains.

76 **Results**

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

80 different farms, respectively 1 animal from 122 farms, 2 animals from
81 31 farms and more than 2 animals from 44 farms. Non-O157 *E. coli*
82 strains were recovered from 6 (12%) cows and 18 (5.2%) bulls
83 originating from 21 (11%) of 199 herds examined (Table1). From the
84 one and the two farms, from which two positive animals, carrying
85 strains belonging to the same serogroup and with identical virulence
86 profiles, were detected, 2 and 3 animals were sampled, respectively.
87 The herd specific types were not investigated in this survey. Of the 24
88 fecal samples in which *E. coli* O26, O103, O111 and O145 was
89 detected, 16 harbored VTEC isolates (Table 2). The serogroups O26
90 and O103 were detected most frequently, respectively in 9 and 10
91 samples. Serogroups O111 and O145 were detected in respectively 2
92 and 3 samples.

93 Direct plating after 6h resulted in 7 positive samples, while a
94 prolonged enrichment period until 24h yielded only 4 positive
95 samples. Application of IMS after 24 hours of enrichment resulted in
96 13 (54%) of the 24 positive samples (Table 3). Regarding to direct
97 plating versus IMS, no differences were observed in the proportion of
98 *vtx*-positive and *vtx*-negative *E. coli* isolates depending on the
99 methodologies used. As only a limited number of positive samples
100 were obtained, no definite conclusions could be drawn regarding the
101 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

125 artificially contaminated fecal samples where most of the fecal
126 samples tested positive both by direct plating and IMS prior to plating.
127 This difference may be explained by the fact that results obtained from
128 artificially contaminated fecal samples can differ from those obtained
129 from naturally contaminated fecal samples (Tutenel et al., 2003).
130 The second objective was to characterize the isolated strains for their
131 virulence factors and hence their possible virulence capacity for
132 humans. Since VTEC are characterized by the presence of at least 1
133 *vtx* gene, 66.5% of the isolates belong to this group by definition
134 (Blanco et al., 2004). However, the *vtx*-lacking strains cannot be
135 neglected because they have occasionally been isolated from patients
136 with HUS (Bielaszewska et al., 2008). It is rather unclear if these
137 strains are inherently *vtx*-negative or whether they have lost their *vtx*-
138 genes during culturing. The *vtx*-negative strains possessing genes
139 encoding for intimin (*eae*) and enterohemolysin (EHEC *hlyA*) are
140 generally regarded as enteropathogenic *E. coli* (EPEC). In comparison
141 with VTEC, such strains are less likely to cause severe human disease.
142 Nevertheless EPEC have emerged in recent years as a cause of
143 diarrhea in humans (Ramachandran et al., 2003).
144 There is a wide range of reported values on the occurrence of non-
145 O157 *E. coli* in cattle. There are limitations in comparing the results of
146 the conducted studies due to the isolation procedure discrepancies as
147 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

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

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

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180 Health, Food Chain Safety and Environment (RT-07/8-FOODZOOM).
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182 **Conflict of interest statement**

183 None

184 **Table legends**

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

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

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

192 Table 4: Virulence patterns of *E. coli* serogroups O26, O103, O111
 193 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%)
<i>Total</i>	18	6	24(6%)
N° farms sampled	174	25	199
Vtx-negative farms*	4	2	6(3%)
Vtx-positive farms**	12	3	15(7%)
<i>Total</i>	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
	<i>vtx1</i>	<i>vtx2</i>	<i>eaeA</i>	EHEC <i>hlyA</i>	
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