

# Survey report

## REVISED MANUSCRIPT

Survey of the contamination of foodstuffs from animal origin (beef, pork, chicken, fish) by shiga toxin producing *Escherichia coli* serotype O157:H7 in Belgium from 1999 to 2003.

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## Summary

A survey of the prevalence of shiga toxin producing *Escherichia coli* (STEC) of O157 serotype was performed in Belgium in foodstuffs of animal origin (beef, veal, pork, chicken, fish) from 1999 to 2003. STEC strains were only isolated from beef with a prevalence of 0.73%. This percentage is low in comparison with the prevalence in other countries. Among the 76 isolated STEC O157 strains, 75% belonged to the serotype O157:H7 and 25% to the serotype O157 non H7. Moreover, the most frequent pathotype was: *eae stx2 ehxA* (74%).

Keywords: *Escherichia coli*, Shiga toxin, Foodstuff, pathotype

## Introduction

Two new legal texts from 11<sup>th</sup> November 2003 published by the European parliament are dedicated to the survey and management of zoonoses and zoonotic agents in European Union: the directive 2003/99/CE [1] on the survey of zoonoses and zoonotic agents and the regulation 2160/2003/CE [2] on the control of Salmonella and other zoonotic agents present in food chain. These texts cancel the directive 92/117/CEE [3] concerning the survey of zoonoses and zoonotic agents in EU and indicate that each member country must collect relevant data concerning the major zoonotic agents and must report the data to the European commission. Among these zoonotic agents to survey, the directive mentions verocytotoxigenic *Escherichia coli* (VTEC). Moreover, at the Belgian level, public health troubles related to these bacteria are important: 46 pathologic cases associated with Shiga toxin producing *Escherichia coli* (STEC) were identified in 2002 [4].

Enterohaemorrhagic *Escherichia coli* (EHEC) are VTEC or STEC which can cause a broad spectrum of human diseases, including diarrhoea, hemorrhagic colitis, and the haemolytic uremic syndrome (HUS). The O157 serotype was responsible for numerous outbreaks worldwide involving deadly cases [5]. This justifies that EHEC O157 are subject to a very careful survey. It remains the principal serotype involved in HUS in Europe. The sensibility to EHEC infection depends of several factors. First the age, since children below 15 and people up 65 were more exposed. Second the immunity, the presence of antibodies seems to protect people from a O157 challenge. Third, gastro-intestinal modifications: a diet poor in proteins is a risk factor. Fourth, the blood group, since the shiga toxins seems to be transported by the red blood cells. The O group and people with red blood cells lacking the P antigen were more sensitive. Fifth, the ingested dose, the more bacteria were ingested the

more severe disease occurred. Sixth, a previous antibiotic treatment is a risk factor suggesting a protecting role for the intestinal flora. Moreover, an antibiotic treatment of the disease increases the risk of HUS appearance [6].

Their major virulence factors are the Shiga-toxins Stx1 and Stx2 responsible for the kidney problems and the intimin encoded by the pathogenicity island LEE and involved in diarrhoea. Moreover, EHEC O157:H7 bacteria produce an enterohaemolysin encoded by the plasmidic *ehxA* gene [7].

In Belgium, between 1994 and 2002, 398 STEC strains have been isolated from human patients. Among them, 195 (49%) were from serotype O157 but only 182 were classical EHEC (positive for *eae*, *stx* and *ehxA* PCR) [4].

The most foodstuffs from animal origin have been randomly sampled among the national production in order to evaluate the prevalence of EHEC O157 and the major incriminated pathotype.

## **Material and Methods**

The beef and pork carcasses sampling were performed in slaughterhouse by swapping on a half-carcass 2 to 4 hours postmortem. For each half-carcass, 4 surfaces were sampled corresponding to a total surface of 600 cm<sup>2</sup>: (a) the internal face of the jam (100 cm<sup>2</sup>), (b) the posterior part of the internal pelvis (100 cm<sup>2</sup>), (c) the sternum and the sternocephalic muscles (300 cm<sup>2</sup>), (d) the posterior face of the anterior member (100 cm<sup>2</sup>). For beef, a 1600 cm<sup>2</sup> surface corresponding to 4 zones of 400 cm<sup>2</sup> was swapped: (a) the postero-external face of the thigh (400 cm<sup>2</sup>), (b) the flank (400 cm<sup>2</sup>), (c) the thorax (400 cm<sup>2</sup>), (d) the posterior face of the anterior member (400 cm<sup>2</sup>).

For pork, the number of analysed carcasses was 163 in 2000 representing 0.015‰ of the Belgian annual production. For beef, the number of studied carcasses was, 1984 in 1999, 1501

in 2000, 1388 in 2001, 1215 in 2002 and 1479 in 2003 representing 0.35 %, 0.25%, 0.19%, 0.26 % of the annual Belgian bee production, respectively. For calves, 157 carcasses samples were analysed in 2000.

The chicken carcasses were sampled at the slaughterhouse exit or at the distribution level. Twenty-five grams of carcasses skin were removed at the neck and the front neck level. 243 carcasses samples were analysed in 2001 representing 0.007% of the annual Belgian chicken production. Minced meat and cuts of beef and pork were sampled at the production stage or at the distribution stage. The raw chicken minced meat preparations were sampled at the distribution level with a minimum sample of 100 g. The chicken fillets (without skin and bones) were sampled at the exit of the production chain or at the consumer distribution level. For fish, 25g of flesh and skin were sampled on the all fish at the abattoir exit.

The isolation protocol for *E. coli* O157:H7 involved a pre-enrichment at 42°C in mTSB broth supplemented with novobiocin during 6 at 7 hours, followed by an enrichment in MacConkey broth supplemented with cefixime-tellurite and incubated at 37°C for 18 hours. An O157 immunoassay (VIDAS ECO) and an immuno-concentration if the immunoassay was positive (VIDAS ICE or Dynabeads O157) were performed. In the case of a positive immuno-assay, a plating on sorbitol-Mac Conkey agar supplemented with cefixime-tellurite was performed and incubated for 18 hours at 42°C, followed by a confirmation by latex agglutination (Oxoid) and by biochemical gallery (Api20E, Biomérieux) [8]. If this confirmation step was not concluding, the result was considered as negative. The presence of the H7 antigen was investigated using H7 antiserum-sorbitol fermentation medium [9] Finally the presence and of the virulence genes (*eae*, *stx1*, *stx2*, *ehxA*) were investigated by PCR[10].

The statistics (contingency table,  $\chi^2$  calculation) were performed using the InStat2.01 software.

## Results

In pork, 163 carcasses have been analysed in 2000 with 145 cut samples and 159 minced meat samples. All these samples were negative for the presence of EHEC O157.

For chicken, 243 broiler skin samples, 181 fillets samples, 152 hen skin samples were analysed in 2001. All samples were negative.

For calf, the 157 carcasses samples analysed in 200 were negative. Among the 153-aquaculture fish samples analysed 1999, all were negative.

In opposite, in beef, among the 7567 carcasses samples analysed from 1999 to 2003, 67 (0.89%) were positive (Table 1). Among the 520 beef cuts analysed in 2002 and 2003, 5 (0.96%) were positive. Among the 2341 beef minced meat samples analysed between 1999 and 2003, 4 (0.17%) were positive. A statistical analysis indicated the prevalence between carcasses and cuts was not significant but the differences of prevalence between minced meat and the two other matrixes were significant. Moreover, there was no significant difference between years for a particular matrix or for all the matrixes taken together.

The 76 STEC O157 strains isolated from bovine samples were analysed for the presence of the H7 antigen and for the presence of virulence genes (Table 2). Among these strains, 75% expressed the H7 antigen and 25% not. Moreover, 74% harboured the *stx2* gene, 20% the *stx1* and *stx2* genes, and 6% the *stx1* gene (only for strains isolated in 1999). Finally, the *ehxA* and the *eae* genes were present in all strains. The more frequent pathotype was: *eae stx2 ehxA* (62%).

## Discussion

The survey plans institute in Belgium to follow the prevalence of EHEC O157:H7 in the major foodstuffs of animal origin indicate that only beef samples were positive.

Nevertheless, the sample number for other groups (calves, pork, chicken, fish) was low. Nevertheless, foodborne diseases due to EHEC in pork meat were rare. Actually, in pork meat, several studies indicated that the *E. coli* O157:H7 prevalence in fresh pork raw meat was lower than 2% [11]. This study confirms that the prevalence of STEC O157:H7 in pork meat is low. Indeed, Bouvet et al. (2002) showed that 15% of the examined carcasses swabs contained STEC but that none of these STEC were from serotype O157:H7 [12].

For chicken, no foodborne disease involved STEC O157:H7 in chicken meat or eggs was reported. Nevertheless, a French study indicated that 4 chicken meat samples on 110 were positive for *E. coli* O157 but that these strains did not produce shiga toxins. An American study indicated that 4% carcasses were contaminated EHEC O157:H7 [11]. But other studies did not show any positive samples in chicken [11]. Concerning fish, an O157:H7 strain was isolated from an outbreak in Japan in 1998. Moreover, consumption of fish appeared to be a risk factor for EHEC infection in Belgium [13].

For beef meat, the average prevalence for the 5 years was 0.73% whereas the calf samples were negative. The data from the literature indicate the absence of STEC O157:H7 in carcasses of calves analysed in the United-States and in Europe whereas the prevalence in adult bovine was 4% [11]. It is difficult to compare our data with the data of the literature since the sampling methods and the isolation methods are different from one study to another. Nevertheless, for bovine carcasses, the prevalence in our study was 0.89%. A Danish, a Czech and an English studies showed a similar prevalence of 0.7%, 1% and 1.4%, respectively [14, 15, 16]. In opposite, a Irish study showed a prevalence of 11% of STEC O157: H7, an Italian study showed a prevalence of 12% of STEC O157 and a French study showed a STEC O157 prevalence of 10.7% [17, 18, 19]. Some studies showed an intermediate STEC O157:H7 proportion such as in Turkey with a prevalence of 3.6% [20]. Interestingly, An American study indicated that the prevalence of STEC O157 decreased on

carcasses at the slaughterhouse during the processing: 87% positive in preevisceration step, 57% in postevisceration step and 17% at postprocessing step [21]. Since our samples were taken at the postprocessing level, it is maybe one explanation for the low contamination level observed. The prevalence of STEC O157 in beef minced meat observed in our study was 0.17%. Such a low prevalence was also observed in an English and a French studies with 0.35% and 0.11%, respectively [16, 11]. For beef cuts, no at lot of data are available, a Danish study performed in 2001 indicated that none of the examined cuts (543) was positive [14]. An American study performed in 2002 Indicated that 0.2% of the examined beef cuts (1014) were positive for STEC O157:H7 [22].

Most of the isolated strains belonged to the O157:H7 serotype with a higher prevalence for strains harbouring the *stx2* gene in comparison to the strains harbouring *stx1* and *stx2* genes or to the strains with only the *stx1* genes. Similar results were obtained in France [19].

Moreover, the *stx2* positive strains are the most virulent EHEC O157:H7 strains [23]. In consequences, even with a low prevalence, the potential implication of these EHEC strains in human pathology must be monitored.

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**Table1. Prevalence of STEC O157 in foodstuffs of bovine origin**

Matrix	Analysed amount	Year	1999	2000	2001	2002	2003	Total	Statistics ( $\chi^2$ )*			
Beef carcass	1600 cm <sup>2</sup>	n	198	150	138			7567	Significant	Significant		Non significant
		p	4	1	8	1215	1479	67				
		%	25	6	13	13	10	0.89				
			1.3	0.5	0.9	1.1	0.68					
Statistics ( $\chi^2$ )*			Non significant							Significant		
Minced meat	25 g	n	974	487	298	297	285	2341				
		p	1	1	0	0	2	4				
		%	0.1	0.2	0	0	0.7	0.17				
Statistics ( $\chi^2$ )*			Non significant							Significant		Non significant
Cut	25 g	n	NT	NT	NT	222	298	520				
		p				0	5	5				
		%				0	1.68	0.96				
Statistics ( $\chi^2$ )*						Non significant						
Isolated strains		n	295	198	168	1734	2062	1042				
		p	8	8	6	13	17	8				
		%	26	7	13	0.74	0.82	76				
			0.88	0.35	0.77			0.73				
Statistics ( $\chi^2$ )*			Non significant									

n=number of analysed samples \* : (p<0.05)

p: pnumber of positive samples NT : non tested

**Table 2. Characteristics of isolated STEC strains**

serotype	pathotype	1999	2000	2001	2002	2003	Total
O157:H7	<i>eae stx2 ehxA</i>	19	0	6	12	10	47*
O157 :H7	<i>eae stx1 stx2 ehxA</i>	0	0	2	0	6	8
O157:H7	<i>eae stx1 ehxA</i>	2	0	0	0	0	2
O157 non H7	<i>eae stx1 stx2 ehxA</i>	0	1	4	1	1	7
O157 non H7?	<i>eae stx2 ehxA</i>	2	6	1	0	0	9
O157 non H7	<i>eae stx1 ehxA</i>	3	0	0	0	0	3
Total		26	7	13	13	17	76

\* : This pathotype is significantly more frequent than the other (p<0.01)