Occurrence of E. coli O157 in food from animal origin in Belgium since 1997

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Introduction

Enterohemorrhagic *Escherichia coli* (EHEC), implicated in aqueous diarrhoea, hemorrhagic colitis or hemolytic uremic syndrome (HUS), is a serious health problem in developed countries. In Belgium, all cases are sporadic and only one outbreak has been suspected in 2001. In USA and UK, the consumption of poorly cooked minced beef is an essential risk factor. The knowledge of the rate and the level of contamination are essential for an efficient risk assessment program.

Material and Methods

In 1997, a large number of matrixes have been investigated for the presence of *E. coli* O157. These matrixes were: cattle and pork (carcasses, livers, retail cuts and minced meat); broiler (carcasses, livers and breasts); layer, turkey and rabbit (carcasses). All samples were 400cm^2 (carcasses of beef and veal), or 600cm^2 swabs, or 25g of product.

In 1998, priority was given to cattle for further investigations. In order to increase the number of samples, 6000 carcasses of beef, 1% of the annual beef's slaughtering in Belgium were swabbed. In four zones of 100cm^2 (10x10cm each for a total sample size of 400cm^2) These carcasses were collected from the 40 greatest Belgian establishments and were pooled 5 by 5. If a pool was detected positive, each individual sample was assessed in order to know the exact prevalence rate.

In 1999, 1984 carcasses were swabbed in four zones of 400cm^2 (20x20cm each for a total sample size of 1600cm^2) and processed individually. Minced meat was also investigated by the analyse of 974 samples directly taken in the butcheries.

In 2000, 1501 beef carcasses, 487 minced meat of beef, 157 veal carcasses and 467 pork samples (carcasses, retail cuts and minced meat) were investigated (1600 cm^2 for beef and veal, 600 cm^2 for pork and 25g for the other matrixes).

In 2001, 1388 beef carcasses, 298 minced meat of beef, and 576 samples of poultry were investigated (1600 cm^2 for beef carcasses and 25g for the other matrixes).

In 1997, the method used was a double method with a pre-enrichment in buffered peptone water during 7h at 37°C followed by an immuno-magnetic separation (Dynabeads anti-E. coli O157, Dynal, Norway). The first half of the concentrate was then plated onto sorbitol MacConkey agar incubated during 18h at 42°C, the other half was cultured into 10ml of modified E. coli broth with novobiocin and incubated 18h at 37°C. After the incubation period, an ELISA test was performed (Vidas ECO, bio-Mérieux, France) and in case of positive result, agglutination test (E. coli latex test, Oxoïd, United Kingdom) was performed on several colonies and then tested for biochemical characterisation (Api20E, bio-Mérieux, France). All isolates were then tested for virulence factors.

Since 1998, the official method from the Ministry of Public Health (SP-VG M001) was used. This method is based on a short pre-enrichment in modified trypticase soy broth with novobiocin, incubated during 6-7h at 42°C. The incubation was followed by the enrichment of 1ml in 9ml of MacConkey broth with cefixime and tellurite, incubated during 18h at 37°C and then tested with an ELISA test as described above (Vidas ECO, bio-Mérieux, France). From the positive broth, an immuno-magnetic separation was performed and the concentrate was plated onto SMAC and SMAC with cefixime and tellurite. Further detection and confirmation were realised as described above.

Results and discussion

In 1997, no E. coli O 157 was detected but some matrixes have given a lot of false positive results with the ELISA test, which suggested to change the used protocol.

In 1998, enterohemorragic *E. coli* O157 were detected in 10 of the 1202 pooled carcasses but it was nearly impossible to find which individual sample was positive and the estimated rate of contamination was supposed to be 0,2% (10 positive results from 6010 carcasses). New changes in the sampling strategy were necessary.

Since 1999, the prevalence in beef minced meat (0,1% in 1999; 0,2% in 2000 and 0,0% in 2001) and on beef carcasses (1,3% in 1999; 0,5% in 2000 and 0,9% in 2001) are stable but no positive enterohemorragic *E. coli* O157 was found in the other matrixes. The EHEC isolates were eae positives, enterohemorragic and VT positives. Three isolates were eae positives, enterohemorragic but VT negatives, which should mean a loss of this virulence characteristic (Table 1).

Table 1 : Pathotypes of enterohemorragic *E. coli* O157 isolates (2001)

Pathotype	Number of EHEC isolates
eaeA+, enterohem., VT1&2+	6
eaeA+, enterohem., VT2+	6
eaeA+, enterohem., VT-	3

Conclusion

- These studies show that the prevalence of beef carcasses is stable until 1999.
- Hygienic measures should be taken in slaughterhouses in which highly faecal contaminated carcasses should be reserved for cooked meat.
- The Belgian diet contains raw or undercooked beef meat, which could endanger the risk population, children and elderly, if they eat contaminated meat, even at a low level of contamination. Consumer information must be reinforced.
- Because of the repercussions for public health, surveillance must be maintained in Belgium and quantitative risk assessment performed.

References

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