ASSESSMENT OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* CONTAMINATION IN BELGIAN BEEF MINCED MEAT

A. Chahed[1], L. De Zutter[2], B. China[3], D. Piérard[4], Y. Ghafir[1], G. Daube[1]

[1] *Food microbiology, Faculty of veterinary medicine, University of Liege, Liege, Belgium*
[2] *Food microbiology, Faculty of veterinary medicine, University of Ghent, Ghent, Belgium*
[2] *Bacteriology , Faculty of veterinary medicine, University of Liege, Liege, Belgium*
[4] *Microbiology, Faculteit geneeskunde en farmacie, Vrije Universiteit Brussel, Brussel*

Conclusions

- This study shows that less than 1%, and probably less than 1‰, of minced beef meat is contaminated in Belgium; it can then be assessed that minced beef does not seem to be an important source of contamination by EHEC in Belgium.

Introduction

- Enterohemorrhagic *Escherichia coli* (EHEC) is a serious health problem in various countries. In Belgium, all cases are sporadic and no outbreak has been detected. In USA and UK, the consumption of poorly cooked minced beef is an essential risk factor. In order to evaluate the contamination rate of Belgian minced meat, samples were randomly investigated for EHEC.

Material and Methods

- A total of 627 samples of minced meat coming from 4 big Belgium enterprises was tested between August 1996 and July 1997 for the research of enterohemorrhagic *Escherichia coli* (E. coli O157 or others EHEC).
- The detection of serotypeO157 was carried out through a short step of non-selective pre-enrichment (7 hours, 37°C), an immunomagnetic separation with Dynabeads (Dynal), an enrichment in mEC + novobiocine (17 hours, 42°C) and an antigen detection with the immunoenzymatic Vidas ECO (bioMérieux). An agglutination with anti-O157 latex allowed the detection from Mac Conkey medium with sorbitol.
- The detection of other EHEC was based on Polymerase Chain Reaction (PCR) of genes coding for verocytotoxins and/or for intimin (Table 1).
- A DNA/DNA hybridisation assay allowed to isolate and characterise the positive isolates from the positive samples from PCR (Table 2).

Table 1 : Primers used for amplification of the *VT* and *eae*A genes

	Primers for *eae*A		Primers for *VT*	
	Upper	Lower	Upper	Lower
Sequence	AGGCTTCGTC ACAGTTG	CCATCGTCAC CACAGGA	GTTATCCATG GAACTA	CATAGGTATC CAGTTC
Amplification product	*eae*A (570 bp)		*VT* (227 bp)	
Reference	China et al. 1995		Piérard et al. 1994	
Cycles				
Start	94°C 5'		94°C 5'	
Denaturation	94°C 1'		94°C 90"	
Hybridisation	55°C 1'		43°C 1'	
Polymerisation	72°C 2'		72°C 1'	
Number of cycles	30		30	

Table 2 : Probes used for DNA-DNA hybridisation

Gene detected	Plasmid	Restriction endonuclease	Size (bp)	References
eae A	*p CDV 434*	*Sal* 1 - *Kpn* 1	1.000	Jerse et al. 1990
VT1	*p JN 37-19*	*Bam* H1	1.142	Newland et al. 1988
VT2	*p NN 11-19*	*Pst* 1	842	Newland et al. 1988

Results and Discussion

- No O157 isolates or o ther EHEC was detected during the present study. Two O157 isolates negative for *eae*A and *VT* were isolated.
- From the 45 (7,1 %) positive samples for VT and/or *eae* A, 5 VTEC *eae* A- isolates and 2 EPEC were isolated (Table 3 and 4).

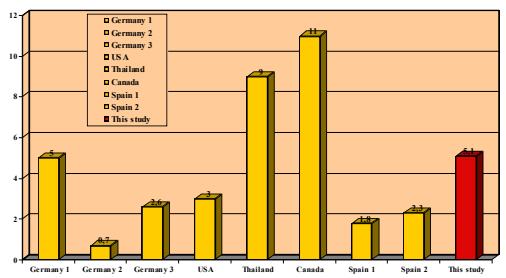
Tableau 3 : Results of PCR screening

	Total	*VT* + et *eae*A+	*VT* + et *eae*A-	*VT*- et *eae*A+	*VT*- et *eae*A-
Number	627	5	27	13	582
%		0,79%	4,3%	2,07%	92,7%

Tableau 4 : EPEC or VTEC isolates

	VT1-, VT2+, eae A-	*VT1+, VT2-, eae A-*	*VT1-, VT2-, eaeA+*
Number	2	3	2
Genotype	VT2, VT2Vh-b	VT1	
Serotype	O91	untypable	O128

Figure 1.
Prevalence of VTEC in food for different countries



This study was financially supported by the Institute of Veterinary Expertise.

References

CHINA B. et al. Appl. and Environ. Microbiol.; 1996, Vol 62; **9**; 3462-3465.
GRIFFIN P. M. and TAUXE R. V. Epidemiol. Rev.; 1991; **13**; 60-97.
JERSE A. E et al. Proc. Natl. Acad. Sci.; 1990, **87**; 7839-7843.
NEWLAND J. W., NEILL R. G. J. Clin. Microbiol.; 1988, **26** ; 1292-1297.
PIERARD D. et al. Three years PCR screening for VTEC in human stools in Brussels;. In KARMALI M. A., GOGLIO. A. G. (ed), Recent Advances in Verocytotoxin *Escherichia coli* Infections. Proceedings *Escherichia coli* infections. Elsevier Science, B. V. Amsterdam; 1994; 33-36.
Report of a WHO Consultation, Prevention and control of Enterohemorrhagic *Escherichia coli* (EHEC) Infections. 28 April-1 may 1997;1-43.