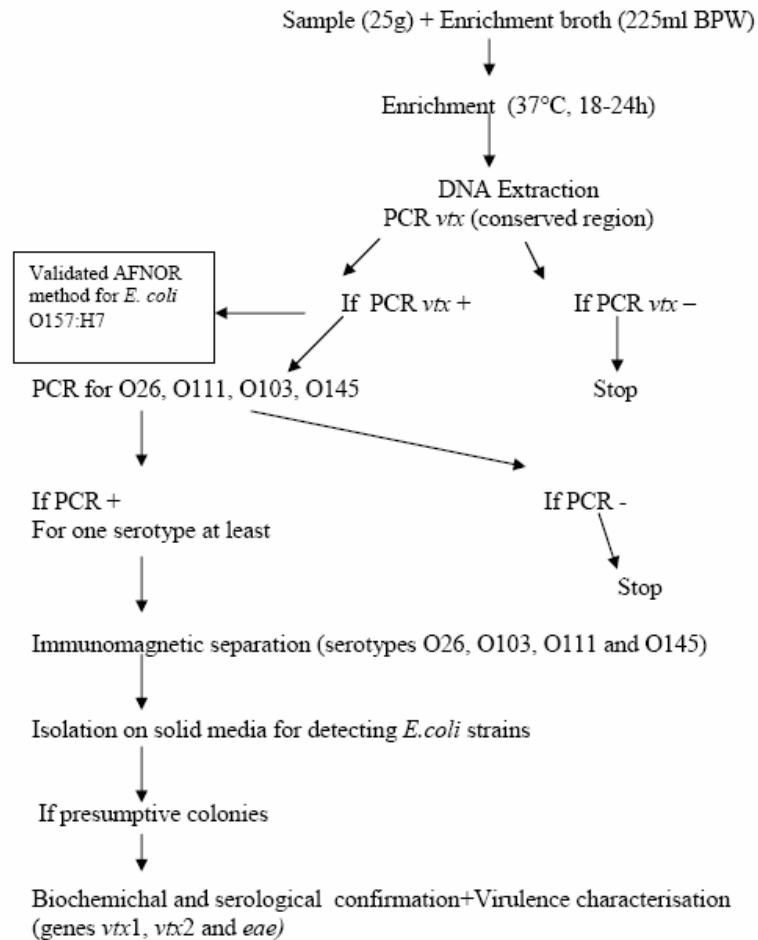


# *Prevalence of Shigatoxin producing *Escherichia coli* (STEC) in FRENCH industrial frozen minced beef*



Pr Christine ROZAND

AFSSA method for detecting pathogenic VTEC in foods





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**Istituto Superiore di Sanità**



## **A real-time PCR method for detection of Shiga toxin-producing *Escherichia coli* in food**

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\* *The “STEC ad hoc Group” is a designated group of experts appointed by CEN to define a consensus protocol aimed at the detection of STEC in foodstuffs to be proposed as a standard to ISO. The “ad hoc group” members are listed in the next page. The activities of the group are co-ordinated by Stefano Morabito and the group co-chaired by Jeppe Boel*



# PREVALENCE OF SHIGA TOXIN PRODUCING *ESCHERICHIA COLI* (STEC) IN FROZEN MINCED BEEF IN FRANCE.

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**ABSTRACT**

**Background:** Shiga toxin producing *Escherichia coli* (STEC) are important food-borne pathogens in humans. The aims of the present study were to determine STEC prevalence in frozen minced beef in France and to characterize the STEC strains isolated (virulence genes and serotype).

**Methods:** 3354 minced beef samples were analyzed. The detection of *stx* genes was performed by polymerase chain reaction (PCR). In addition, a second PCR specific for *E. coli* O26 or O103 or O111 or O145 detection was carried out on the *stx*-positive samples. STEC strains were recovered from positive samples by immuno-concentration (IMS). Then the strains isolated were genetically characterized (*stx1*, *stx2*, *ese* and *hlye*). An automated enzyme-linked fluorescence immunoassay (ELFA), the VIDAS *E. coli* O297 method, was used to detect *E. coli* O297 serogroup from the *stx*-positive samples. The isolation of *E. coli* O157:H7 from positive sample with the VIDAS was performed using an automated immunoconcentration (ICE) system. Then the strains isolated were genetically characterized (*stx1*, *stx2*, *ese* and *hlye*).

**Results:** 964 out of 3354 (28.7%) minced beef samples analyzed were PCR *stx* + and 172 were both PCR *ese* + and *stx* +. Among these 172 samples, 121 samples were proved to be positive by PCR for the research of the serogroups: 62 positive for O103, 21 positive for O157, 16 positive for O26, 14 positive for O145 and 8 positive for O111.

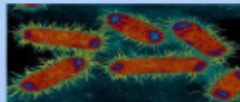
Eleven strains of STEC were isolated: 5 *E. coli* O157:H7, 3 *E. coli* O103, 2 *E. coli* O26 and 1 *E. coli* O111. Twenty other strains were isolated: 13 *E. coli* O103, 8 *E. coli* O26, 1 *E. coli* O145. All of them were not pathogenic according to the definition given by the French Food Safety Agency.

**Conclusion:** The results of this study did not differ greatly from those reported in other countries with regard to the prevalence of O157 in minced beef. However we must keep in mind that serotypes different from O157:H7 also played an important role in minced beef contamination.

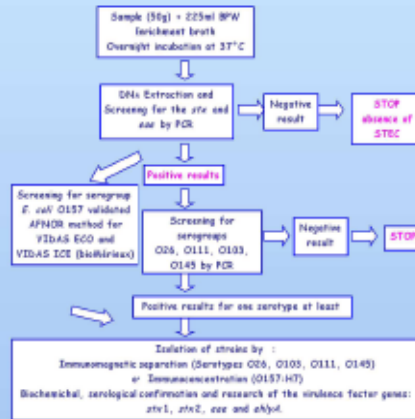
**INTRODUCTION**



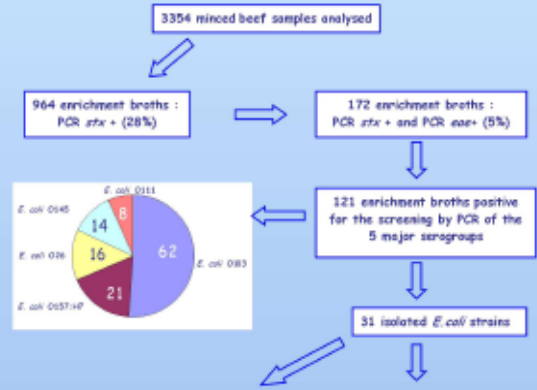
The aims of the present study were to determine STEC prevalence in frozen minced beef in France in the particular context of a surveillance plan launched by the French Ministry of Agriculture and Fisheries and to characterize the STEC strains isolated (virulence genes and serotype).



**PROTOCOL**

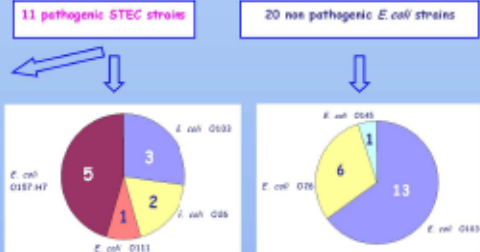


**RESULTS AND DISCUSSION**



**Serogroup and profil of virulence of 11 pathogenic STEC strains isolated**

Sample	Serogroup	Virulence factors	Sample	Serogroup	Virulence factors
		<i>stx</i> 1 <i>stx</i> 2 <i>ese</i> <i>hlye</i>			<i>stx</i> 1 <i>stx</i> 2 <i>ese</i> <i>hlye</i>
83-245	O103	+ - - +	71-81	O157:H7	+ - - +
89-440	O103	+ + + +	72-370	O103	+ - - +
43-03	O157:H7	- - + +	85-07	O157:H7	+ + + +
43-07	O157:H7	- - - -	89-08	O26	+ - - +
84-126	O26	+ - - +	88-17	O157:H7	+ - - +
84-159	O111	+ - - +			



**CONCLUSION**

In accordance with the AFSSA expert committee: a STEC strain can be considered as pathogenic if the strain is *stx*⊕ and *ese*⊕ and it belongs to one of the next serogroups: O26, O103, O111, O145 or O157:H7.

In comparison to the high number of samples analysed (3354), only eleven minced beef samples allowed the isolation of pathogenic STEC strains that is to say a minced beef prevalence of contamination of 3 ‰.

In summary, these data demonstrated that French industrial frozen minced beef is a *low potential danger* for humans. However it must be kept in mind that Good Manufacturing Practises and the implementation of a HACCP (Hazard Analysis Critical Control Point) program all through food chain, from abattoirs to markets, in order to reduce contamination and consequently, illnesses related to minced beef can be held to control STEC.

In addition, consumers should reduce their risk for STEC foodborne diseases by following safe food-handling recommendations and by avoiding consumption of raw or undercooked meat products.

● 3354 minced beef samples tested:

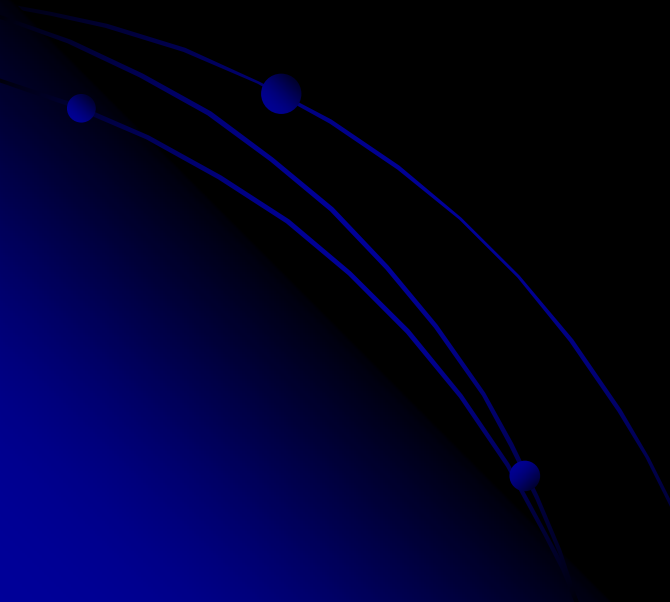
- 964 PCR stx +

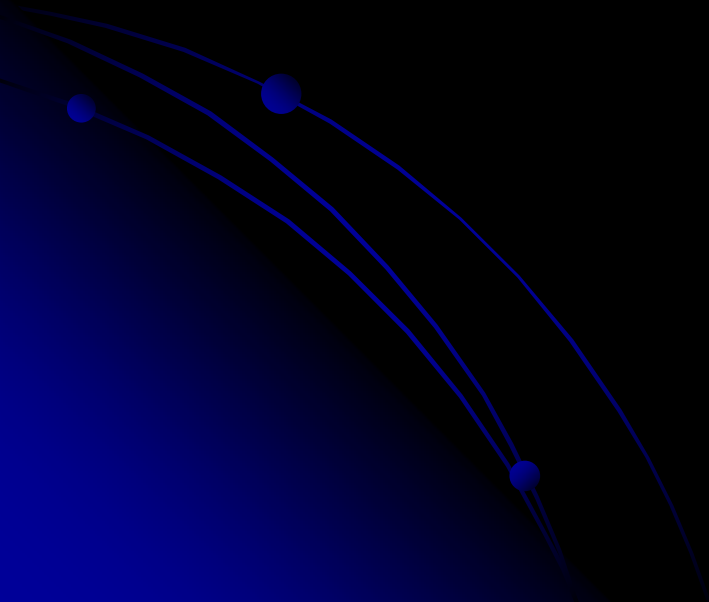
- 172 PCR stx + AND eae + (5%)

- 121 PCR stx + AND eae + AND +  
for one of the 5 major serogroups  
(3.6%)

121 PCR stx + AND eae + AND + for one of the 5 major serogroups (3.6%)

- 21 O157 : 5 *E. coli* O157:H7 isolates
- 16 O26 : 6 *E. coli* O26 isolates (2 pathogenic STEC and 4 EPEC)
- 62 O103 : 13 *E. coli* O103 isolates including 3 « pathogenic » STEC
- 14 O145 : 1 *E. coli* O145 isolate (eae+ but stx-)
- 8 O111:1 *E. coli* O111 (stx+ and eae+)

- **3%** French frozen minced beef contaminated by « pathogenic » STEC strains
  - **3.7%** « highly presumptive » samples (stx+, eae+, and + for one serogroup)
  - **Confirmation rate : only 10% !!!**
- 

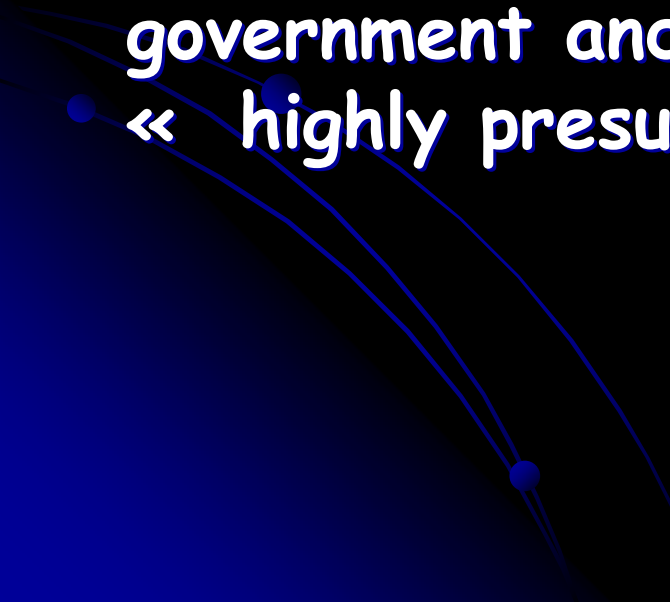




# Main drawbacks

- Meaning of positive multiplex PCR results
- Optimization of the isolation step required

What will be the decision of the government and the food industries with « highly presumptive results »???





Thank  
you



For your



Attention