

# Shiga-toxin producing *Escherichia coli* O26 infection and unpasteurised cows cheese, France, 2005

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## Introduction

- In France, no routine screening for Shiga-toxin producing *E. coli* (STEC) in clinical laboratories
  - Surveillance of STEC infections based on surveillance of hemolytic uremic syndrome (HUS) in France
- Nation-wide HUS surveillance since 1996
  - STEC related HUS cases
  - Children under 15 years
  - Voluntary network of 31 pediatric nephrology services
- HUS in France
  - Incidence per year: 0.70 / 100 000 children under 15 years
  - Most sporadic cases
  - Majority of cases due to *E. coli* O157:H7 (84% of confirmed cases)
  - 2005: an regional outbreak of *E. coli* O157:H7 infections
- Outbreak alert** on the 25 Nov 2005
  - Three HUS cases were notified to the National public health institute by one hospital in Northern part of France
  - These cases were hospitalized over a period of one week and resident in 2 neighbouring districts
  - No evidence of *E. coli* O157:H7 infection
- Prompted outbreak investigation to identify causal agent, vehicle of transmission and implement control measures

## Method

### EPIDEMIOLOGICAL INVESTIGATION

- Case definition**
  - HUS case with
    - isolation of O26 or undetermined serotype Shiga-toxin producing *E. coli* (STEC) in stools
    - or positive antibody response to O26 STEC lipopolysaccharide
  - Resident in France
  - Date of onset of symptoms after October 1, 2005
- Retrospective and prospective case finding**
  - National surveillance network of HUS
  - Pediatric services of all hospitals of Northern part of France
- Trawling questionnaire**
  - Food and drink consumption, contact with diarrhea cases, contact with animals, activities, travel

### VETERINARY AND ENVIRONMENTAL INVESTIGATIONS

- Trace back investigation of the suspected food vehicle
- Physical inspection of production plant
- Inspection of quality control procedures
- Sampling of production line and environment
- Identification and examination of the milk producing herds

### MICROBIOLOGICAL INVESTIGATION

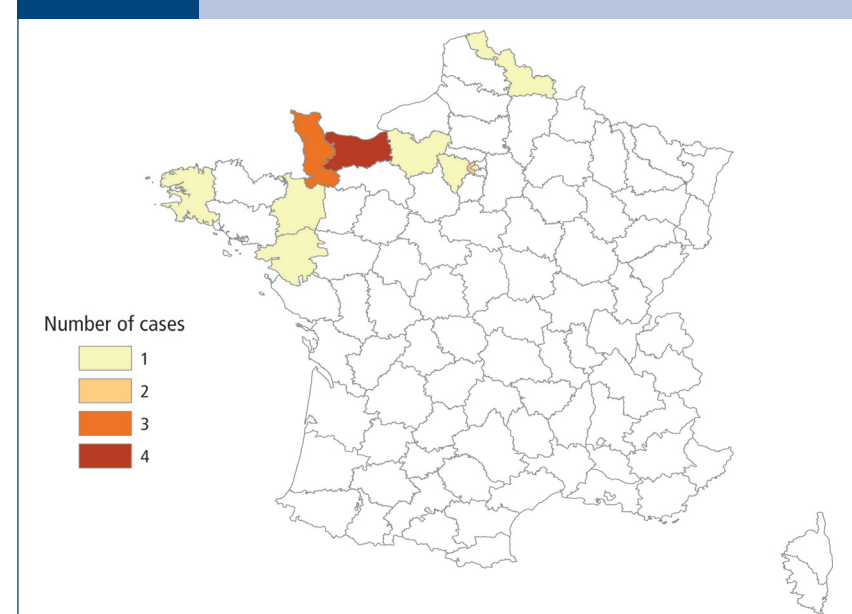
- Cases**
  - Serology (O26, O55, O91, O103, O111, O128, O145, O157)
  - Stool sample: culture, serotyping, PCR *stx* and *eae* and PFGE
- Food samples**
  - Cheeses from cases' homes and production plant
  - Milk of each herd
  - Culture, serotyping, PCR *E. coli* O26, PCR *stx* and *eae* and PFGE
- Environmental samples at the farm**
  - Feed (ensilage) and drinking water (through)
  - Faeces of starlings
  - Culture, serotyping, PCR *E. coli* O26, PCR *stx* and *eae* and PFGE

## Results

### CASE CHARACTERISTICS

- 16 cases between October 14 and December 30 2005
  - 12 females and 4 males
  - from 10 months to 6 years
- Clinical symptoms**
  - HUS with 13 bloody diarrhea and 3 non bloody diarrhea
  - One neurological sequelae
  - No death
- 9/16 (56%) of cases consumed unpasteurized cows cheese 'Camembert' from a single producer

FIGURE 1 GEOGRAPHICAL DISTRIBUTION OF HUS CASES (N=16), FRANCE 2005



### VETERINARY AND ENVIRONMENTAL INVESTIGATION

- At the production plant**
  - Production of raw milk cheeses, pasteurized cream and butter
  - Inspection: no deficiencies in the hygienic practices
  - Routine controls for *E. coli* O157:H7 (food and environment) negative
- At the farms**
  - 48 herds for a daily production of 3 600 liters of milk
  - An unusual high number of starlings present

### MICROBIOLOGICAL RESULTS

- Patients**
  - Serology:
    - E. coli* O26: 10 cases
  - Stools:
    - E. coli* O26:H11 strains (4 were *stx2+* *eae+*): 7 cases
    - STEC strains with undetermined serotype (*stx2+* *eae+*): 9 cases (results available at the time of the investigation, but 6 strains were later identified as *E. coli* O80:H2)

TABLE 1 CHARACTERISATION OF HUMAN *E. COLI* ISOLATES WITH RESPECT TO O SEROTYPE, VIRULENCE FACTORS AND SEROLOGY RESULTS, FRANCE 2005

No of case	Age	Sex	STEC strains	Serology	PFGE
1	6 y	M	- <sup>a</sup>	O26	- <sup>a</sup>
2	15 m	F	O26:H11 <i>stx2+</i> <i>eae+</i>	O26	1
3	13 m	M	- <sup>a</sup>	O26	- <sup>a</sup>
4	10 m	F	NT* <i>stx2+</i> <i>eae+</i>	negative	3
5	21 m	F	O80:H2 <i>stx2+</i> <i>eae+</i>	negative	2
6	14 m	F	O80:H2 <i>stx2+</i> <i>eae+</i>	O26	2
7	15 m	F	O26:H11 <i>stx2+</i> <i>eae+</i>	negative	1
			O80:H2 <i>stx2+</i> <i>eae+</i>		2
8	15 m	F	O26: H11 <i>eae+</i>	O26	1
			O80: H2 <i>stx2+</i> <i>eae+</i>		2
9	16 m	F	O26: H11 <i>eae+</i>	O26	1
10	17 m	F	O26: H11 <i>eae+</i>	-	1
11	2 y	F	O80:H2 <i>stx2+</i> <i>eae+</i>	O26	2
12	16 m	M	O26:H11 <i>stx2+</i> <i>eae+</i>	O26	1
13	15 m	F	O26:H11 <i>stx2+</i> <i>eae+</i>	O26	1
14	17 m	M	NT* <i>stx2+</i> <i>eae+</i>	negative	4
15	3 y	F	O80:H2 <i>stx2+</i> <i>eae+</i>	O26	2
16	8 m	F	NT* <i>stx2+</i> <i>eae+</i>	negative	5

<sup>a</sup>No analyse; NT\*: undetermined serotype

### MICROBIOLOGICAL RESULTS

- Food samples at the production plant**
  - Cows cheeses:
    - E. coli* O26:H11 (*stx2+* *eae+*) strains
    - E. coli* O26:H11 (*eae+*) strains
  - Pooled milk:
    - E. coli* O26:H11 (*eae+*) strains

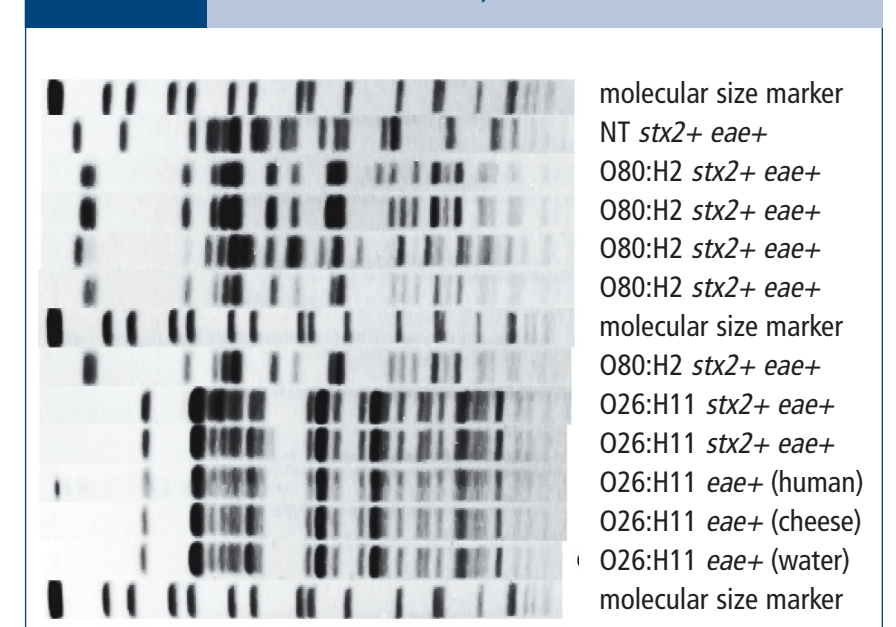
### Environmental samples at the farms

- Faeces of starlings, feed and water tested PCR positive for *E. coli* O26
- No STEC strains

TABLE 2 CHARACTERISATION OF NON HUMAN *E. COLI* ISOLATES WITH RESPECT TO O SEROTYPE AND VIRULENCE FACTORS, FRANCE 2005

	No of samples tested	PCR O26 positive	Serotype	Virulence profile	PFGE
Pooled milk	48	5	<i>E. coli</i> O26:H11	<i>eae+</i>	1
Unpasteurised cows' cheeses	54	20	<i>E. coli</i> O26:H11 <i>E. coli</i> O26:H11	<i>stx2+</i> <i>eae+</i> <i>eae+</i>	1 1
Farm drinking water	18	3	<i>E. coli</i> O26:H11	<i>eae+</i>	1
Feed (ensilage)	7	1	<i>E. coli</i> O26:H11	<i>stx2+</i>	1
Faeces of starlings	9	9	-	-	-

FIGURE 2 PULSED-FIELD GEL ELECTROPHORESIS PATTERNS OF XBAI DIGESTED-GENOMIC DNA FROM *E. COLI* O26:H11 AND *E. COLI* O80: H2 STRAINS, FRANCE 2005

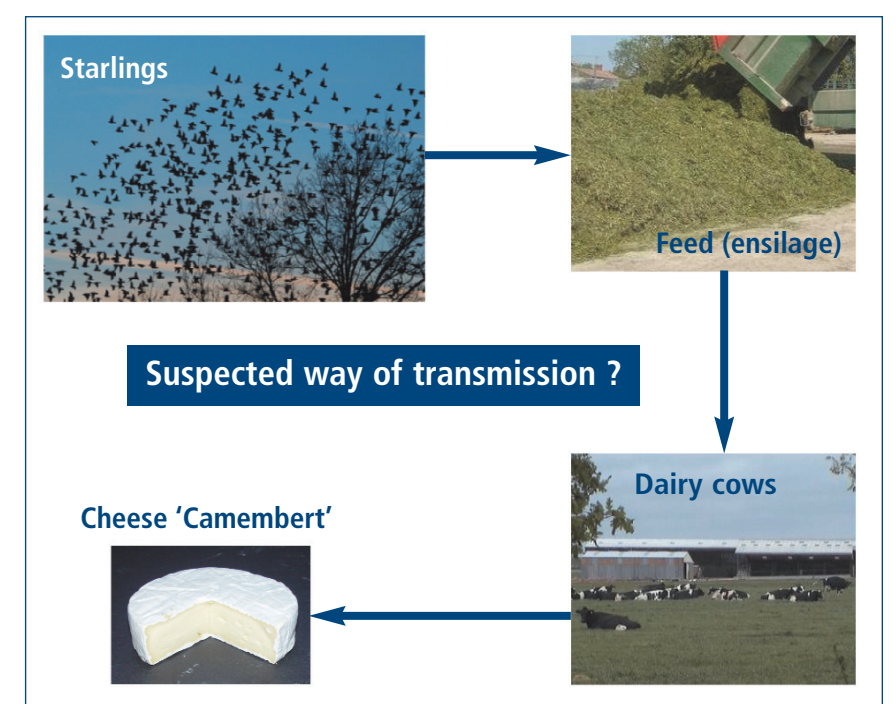


### CONTROL MEASURES

- National and international recall of entire production of cows cheeses: 17 December 2005
- Public alerted through media and press
- International alert: no cases identified in other countries
- Cheese production halted during one week
- Extensive controls of milk and cheeses: daily *E. coli* enumeration and PCR *Stx*

## Conclusion

- First outbreak of STEC non O157 in unpasteurised cows' cheese in France
- Suspected origin: environmental contamination with a possible role of starlings



- Multiple contamination by at least two different serotypes of STEC (*E. coli* O26:H11, *E. coli* O80:H2 and undetermined serotype?)
- Interpretation of the results of STEC O26 research
  - Extensive genetic diversity
  - Recent toxigenic evolution, including changes in *Stx* genotypes
- Need for survey of prevalence of STEC in food, animals and environment
  - Survey studies of cheeses made with unpasteurised cows' milk in 2007 and 2008