A foodborne outbreak of entero-invasive E.coli (EIEC) infection in Italy, April 2012

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Background:
The signal

- 14 April 2012: The Local Public Health Service (ASL) of Milan contacted by Hospitals of the city of Milan:
  - Several persons were being admitted at the Emergency Unit presenting with severe gastro-enteric symptoms
  - All were employees of the Fire Brigade (FB) of the city of Milan
  - Faecal samples were collected, but laboratory test were on-going: causative agents still not identified

Background:
Preliminary investigation

- Primary investigation (ASL of Milan):
  - Trawling questionnaire on patients on symptoms and exposures:
    - Symptoms started between the 13 and 14 of April and were particularly severe (fever, watery and bloody diarrhoea)
    - The only factor in common was “attending the the FB canteen”
  - No other similar episodes were occurring in the city area

➡ **Hypothesis:** outbreak linked to the FB canteen

➡ **Immediate action:** the FB canteen was inspected on 15 April and the activity was suspended

Background:
Mediatic impact

la Repubblica MILANO.it

Martedì 17 Aprile 2012 – Aggiornato Alle 11.35

Milano, d
i Nas chiu

Sessanta vigili del fu
alimentare: tutti ave
di FRANCO VANNI

Sessanta vigili del fuoco intossicati dopo la mensa

I carabinieri del Nas mettono i sigilli alla cucina

(Fotogramma)

MILANO - Almeno sessanta vigili del fuoco intossicati dopo avere consumato un pasto, nella giornata di venerdì, nella caserma di via Messina (o proveniente da lì, visto che la mensa fornisce il cibo anche agli altri presidi milanesi dei pompieri). I sintomi? Febbre superiore ai 40, vomito,

94

consiglia

PIÙletti

OGGI | set

The investigation

- **Investigation:** ASL of Milan + FB of Milan:
  - To establish the magnitude of the outbreak
  - To determine the mode and vehicle of transmission
  - To identify the etiologic agent
    - **VTEC infection was suspected** because of bloody diarrhoea and **the NRL for E.coli** was contacted for support

Methods:
epidemiological investigation

- **Case finding:** on 15 April the FB invited the employees who experienced gastro enteric symptoms after 9/4 to contact the ASL of Milan

- **Collecting information:** a questionnaire on symptoms and food exposures between 9 and 14 of April was administered

- **Case-patient definition:** person who had eaten in the FB canteen between 9 and 14 April and developed diarrhoea within 6 days

Methods:
epidemiological investigation

- **Exposure to food:** case-control study
  - **Controls:** employees of the FB of Milan exposed to the canteen in the same period and did not experience symptoms
  - **Analysis:** Data were analysed separately by day of canteen attendance

Methods:
environmental investigation

- **On the 15/4 the ASL of Milan inspected the kitchen**

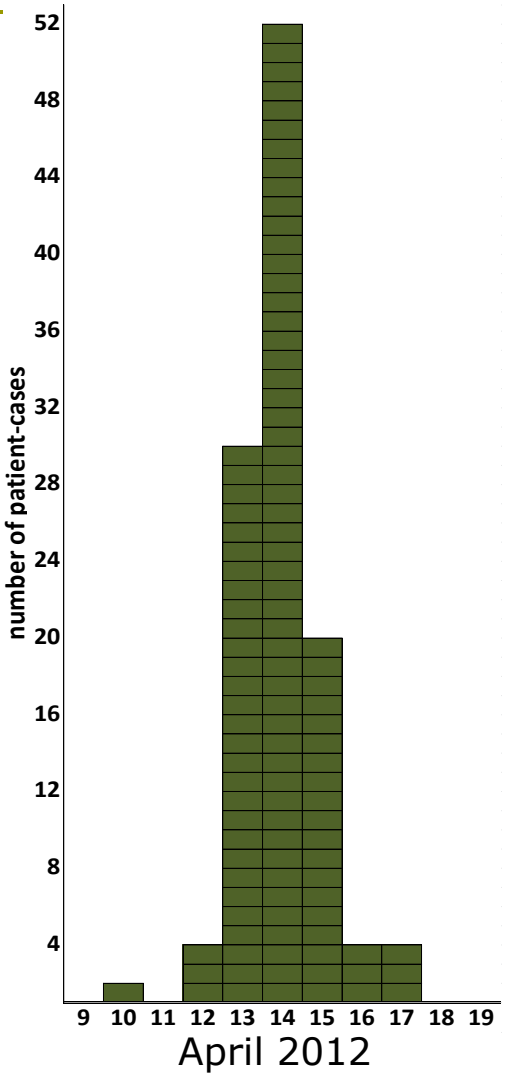
  - ASL interviewed all the 6 kitchen staff about:
    - food handling and storage procedures
  - Observation of:
    - general hygiene practices
    - functioning of the refrigerators

Methods: laboratory investigation

- **Stool samples:**
  - case-patients + the 6 kitchen employees
  - examined for multiple enteric pathogens (bacteria, virus, parasites):
    - using commercial PCR-based screening assays
    - culture examination
- **Food items:**
  - aliquots of foods left in the kitchen (row ingredients and ready to eat foods)
  - examined for *Salmonella*, *L.monocytogenes*, *Shigella*, *Yersinia* and *E.coli* O157
- **Water:**
  - samples tested for total Coliform, *Salmonella, Shigella* and *E.coli*

Results:
epidemiological investigation

- **Case-patients:** N=109
  - 3 were kitchen workers
  - Age: median 41 years (range 25-60)
  - Gender: 85% male
  - 7 reported household members presenting diarrhoea
- **Symptoms :**
  - Onset: between 10 and 14 of April
  - Incubation period (n=10): median 21 h (10 – 96)
    - Diarrhoea 100%
    - Fever 75%
    - Abdominal pain 61%
    - Vomiting 42%
  - Severity: emergency room 74 (69%), hospitalised 32 (29%)



Results:
epidemiological investigation

- **Exposure to FB canteen:**

	All cases (n=108)	Food items served (n =119)	Information on food consumed	
			Cases : (n=83)	Controls (n=32)
09-apr	15	12	7	7
10-apr	29	17	16	5
11-apr	35	20	22	10
12-apr	69	26	50	19
13-apr	79	26	59	23
14-apr	21	18	15	4

Results:
epidemiological investigation

day of consumption	Food item	exposed cases (n cases)	exposed control (n control)	OR*	*P* value**	% cases exposed
12-apr	**Beet greens**	16 (48)	0 (16)	11.93	0.017	19%
13-apr	**Peppers**	8 (55)	0 (21)	4.80	0.098	10%
	Beet greens	7 (55)	0 (21)	4.04	0.125	8%
	Green beans	18 (55)	1 (21)	11.17	0.008	22%
	Potatoes	16 (55)	1 (21)	9.00	0.016	19%
14-apr	**peppers**	5 (15)	0 (4)	2.25	0.296	6%
	Potatoes	4 (15)	0 (4)	1.63	0.530	5%

* Calculated with exact-logistic; ** Calculated with X^2 or Fisher exact test

Overall cooked vegetables were consumed by **58 cases (70%)** and **1 control (3%)** -> **(global OR=67, (*P* <0.0001))**

Results:
environmental investigation

- Gross failures in the general hygienic condition:
  - equipment (e.g. refrigerators and freezers)
  - procedures of preparation and preservation of foods:
    - Leftover food from a meal often served in the next days

Results:
laboratory investigation

- Stool samples: collected from 62 subjects
- **All examined at the hospital and ASL Laboratories**

	Case patients (N=59)	Symptomless kitchen workers (N=3)
Molecular Screening test		
positive for the invasion plasmid antigen H **(ipaH**)	17	2
positive for other bacteria, parasites and viruses	0	0

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Molecular Screening test		
positive for the invasion plasmid antigen H **(ipaH**)	17	2
positive for other bacteria, parasites and viruses	0	0
Culture examination		
Isolation of *Shigella* spp.	0	0
Isolation of *Salmonella spp.*, *E.coli* O157 and *Yersinia enterocolitica*	0	0

Results:
laboratory investigation

- **15 *ipaH*-positive stool samples examined at the NRL *E.coli***

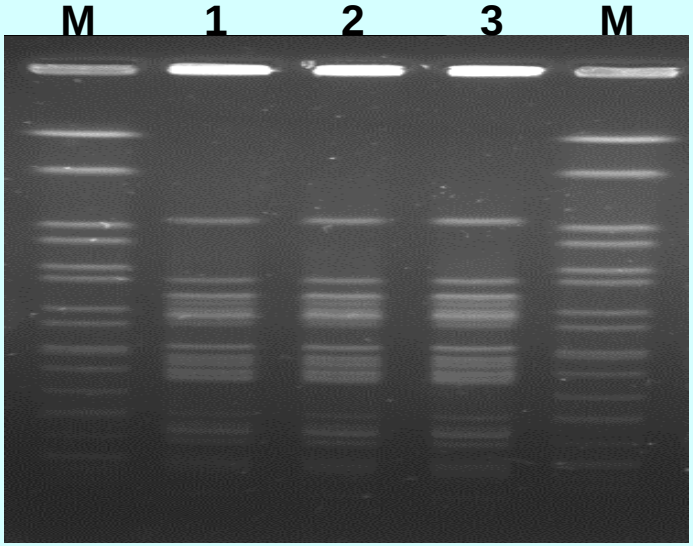
	Case patients (N=14)	Symptomless kitchen workers (N=1)
Detection of *ipaH* in enrichment cultures	14	1
Isolation of *ipaH-positive E. coli* (EIEC)	6	0
Detection of other diarrheagenic *E.coli* (VTEC, EAggEC, ETEC)	0	0

Results:
laboratory investigation

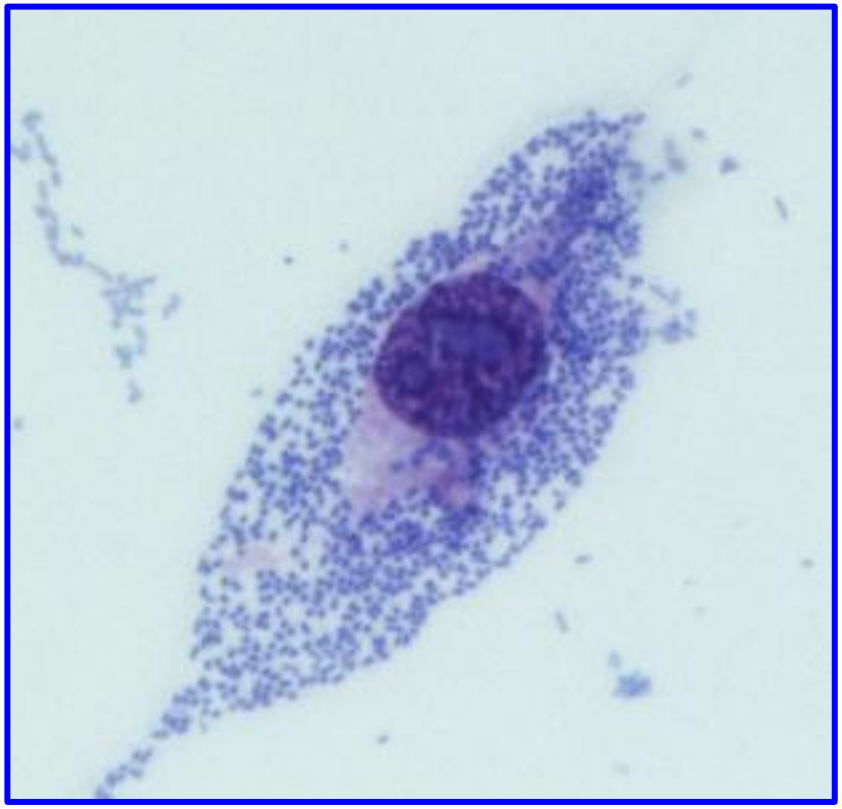
- Characteristics of the strains:
  - Same PFGE pattern
  - Serotyping (F. Scheutz, SSI, Copenhagen): ***E.coli* O96:H19**
  - All strains were:
    - motile
    - lactose-fermenting
    - Lysine decarboxylase-positive
    - Negative at the Indole test

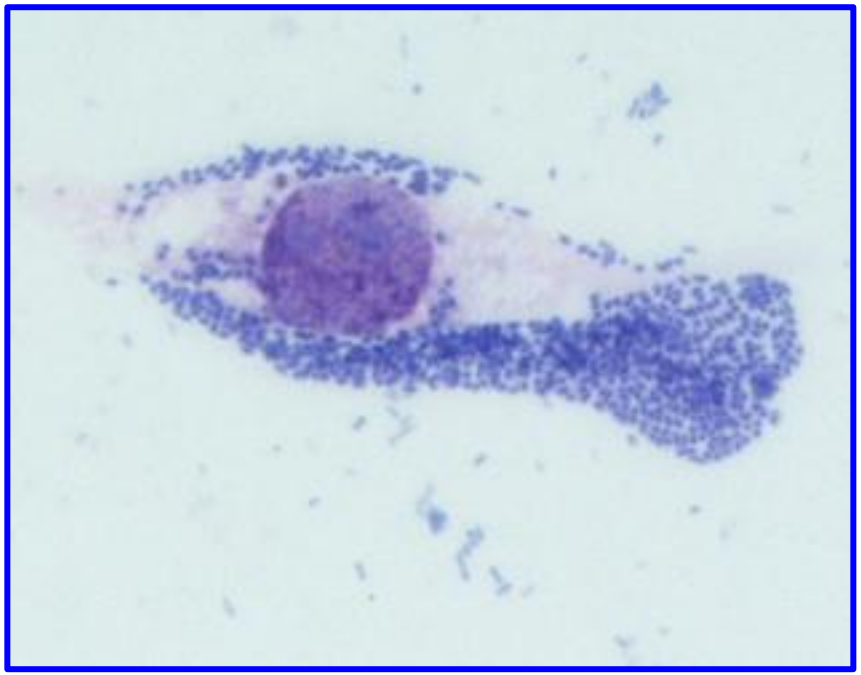
and …..

    - did not agglutinate with Shigella antisera
    - R-type: SSu



Results:
HEp-2 cells adhesion/invasion assay





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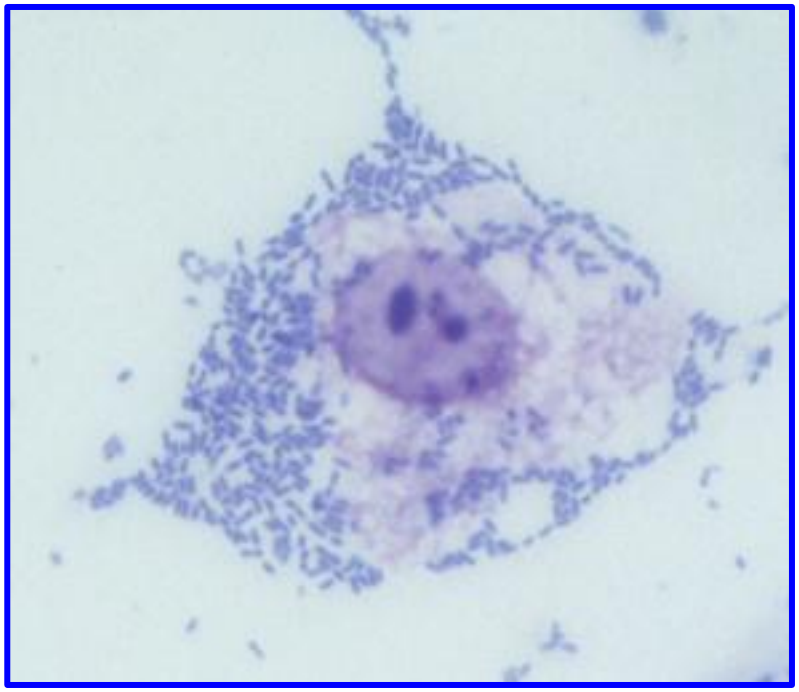

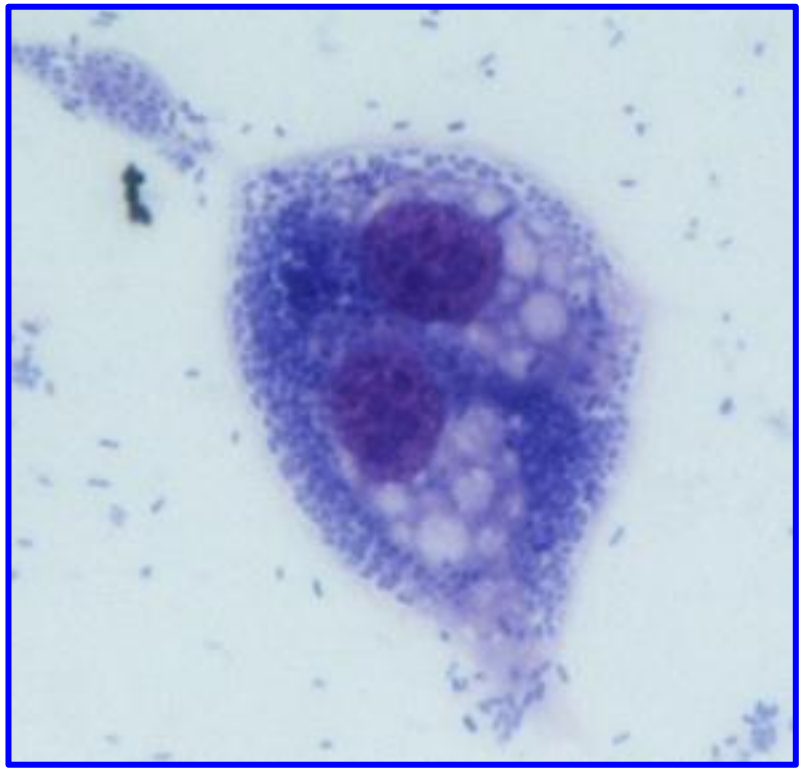

Results:
laboratory investigation

- Summary
  - 19 laboratory confirmed (*ipaH*- positive*)* cases:
    - 17 case patients
    - 2 asymptomatic kitchen employees (one just came back from a travel in Vietnam)
  - Duration of carriage was up to 45 days in case-patients
  - A healthy carrier was positive for 45 days !!!
  - Negative correlation between interval from onset of symptom - collection of the stool samples and *ipaH* positivity (p= 0.0014):
    - interval for positive samples: mean 12 days (6 - 18)
    - interval for negative samples: mean 25 days (20 – 30)

- Food and water samples: all samples were negative for EIEC or other enteric pathogens.

Discussion

- EIEC O96:H19 was the causative agent of this outbreak:
  - EIEC are human pathogens, uncommon in industrialized countries
  - this is the first outbreak detected in Italy and possibly in Western Europe
- The epidemiological characteristics were similar to other EIEC outbreaks, but symptoms were more severe

Discussion

- Cooked vegetables were identified as the most likely vehicle of infection. Hypothesis:

kitchen-workers were the primary EIEC source

vegetables' contamination occurred during preparation

Contamination was amplified by incorrect storage procedure;

- Person-to-person transmission?

Lesson learned

- EIEC can cause serious outbreaks also in countries with high sanitation and socio-economic status:
  - their presence should not be overlooked in laboratory testing
  - The *ipaH* gene is **not only** a marker of Shigella
  - good hygiene practices by food handlers should be encouraged because they represent the key stage in preventing outbreaks

Thanks to the investigation team

- Azienda Sanitaria Locale di Milano, Milan, Italy :
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.....and to you for your attention