

# ***Escherichia coli* O157: emerging issues on virulence and epidemiology**

**Alfredo Caprioli**

Community Reference Laboratory for *E.coli*  
Dipartimento di Sanità Alimentare e Animale  
Istituto Superiore di Sanità, Roma



**[www.iss.it](http://www.iss.it)**

**1982** *E.coli* O157 recognised as a pathogen

**1982, USA and Canada**

**Outbreaks of severe bloody diarrhea associated with consumption of hamburgers in fast food restaurants**



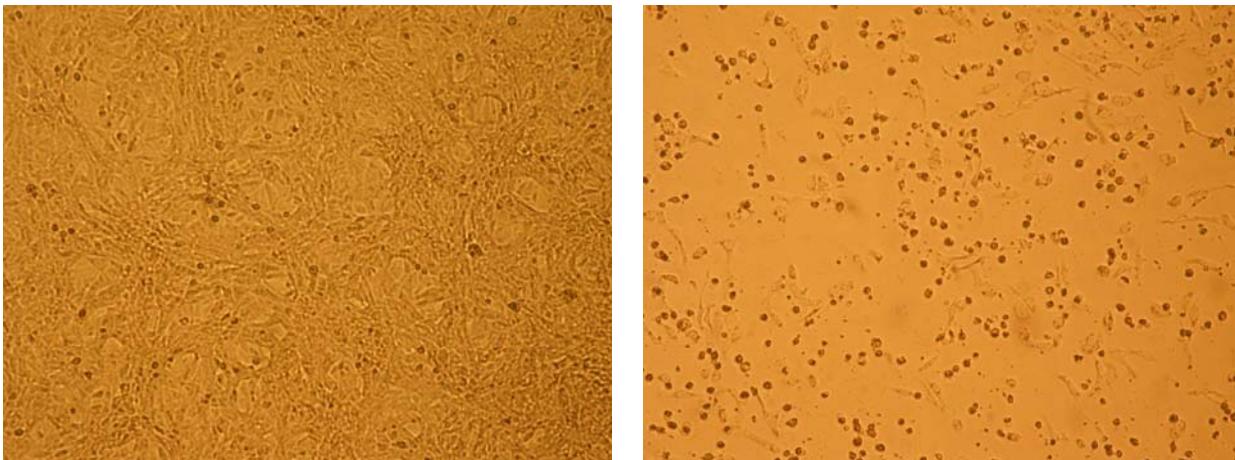
**No common pathogens, *E.coli* O157:H7 isolated from cases and from the hamburgers**

***E.coli* O157:H7: uncommon serotype never associated with human disease before**

# 1983 *E.coli* O157 produces Verocytotoxin

## Cytopathic effect on Vero cells

Konowalchuk et al. Vero response to a cytotoxin of *E.coli*. Infect. Immun. 1977;18:775-9.



Nearly identical to the toxin of *Shigella dysenteriae tipo 1* (**Shiga toxin**)

O'Brien et al. *E.coli* O157:H7 strains associated with haemorrhagic colitis in the United States produce a *Shigella dysenteriae* 1 (SHIGA) like cytotoxin. Lancet. 1983 Mar 26;1(8326 Pt 1):702.

# 1983 *E.coli* O157 and HUS

***E.coli* O157 (and other STEC) is the etiologic agent of hemolytic-uremic syndrome (HUS), the leading cause of acute renal failure in childhood.**

Karmali, et al. Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools.  
*Lancet.* 1983 Mar 19;1(8325):619-20.

# 1987 *E.coli* O157 is a zoonotic pathogen

**Healthy ruminants, and in particular cattle, are the natural reservoir of *E.coli* O157 (and other STEC)**

Orskov , et al. Cattle as reservoir of verotoxin-producing Escherichia coli O157:H7. Lancet. 1987 Aug 1;2(8553):276.

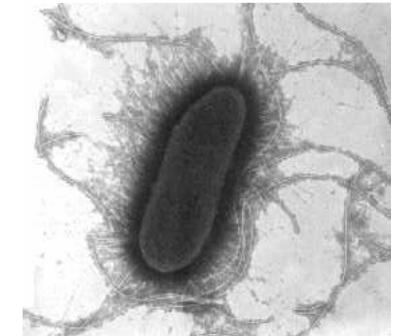


# *E.coli* O157 is a nasty bug !!

**Life-threatening clinical manifestation**

**Extremely low infectious dose**

**Very large community outbreaks**



**One of the most dangerous foodborne  
pathogen !**

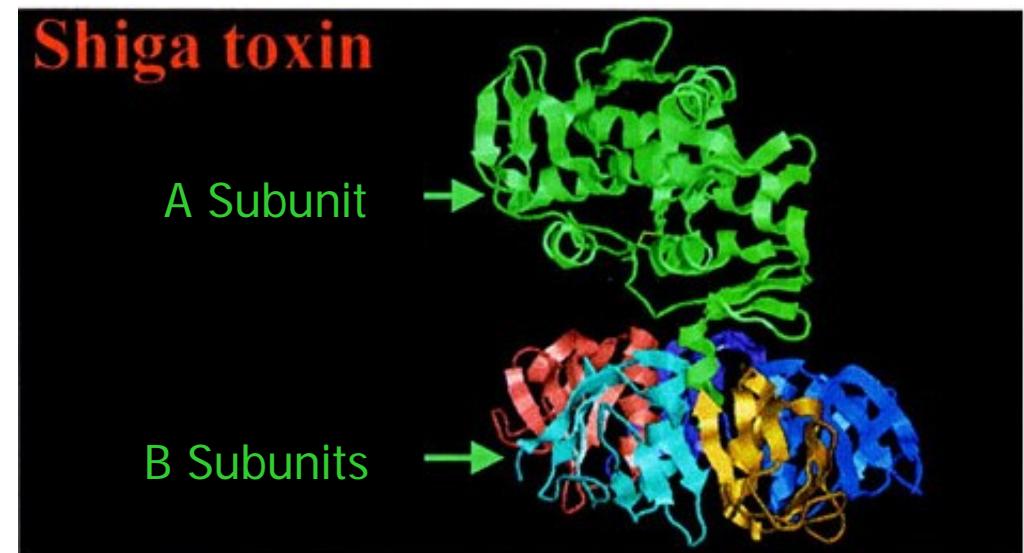
# Shiga-toxins

Inhibition of protein synthesis

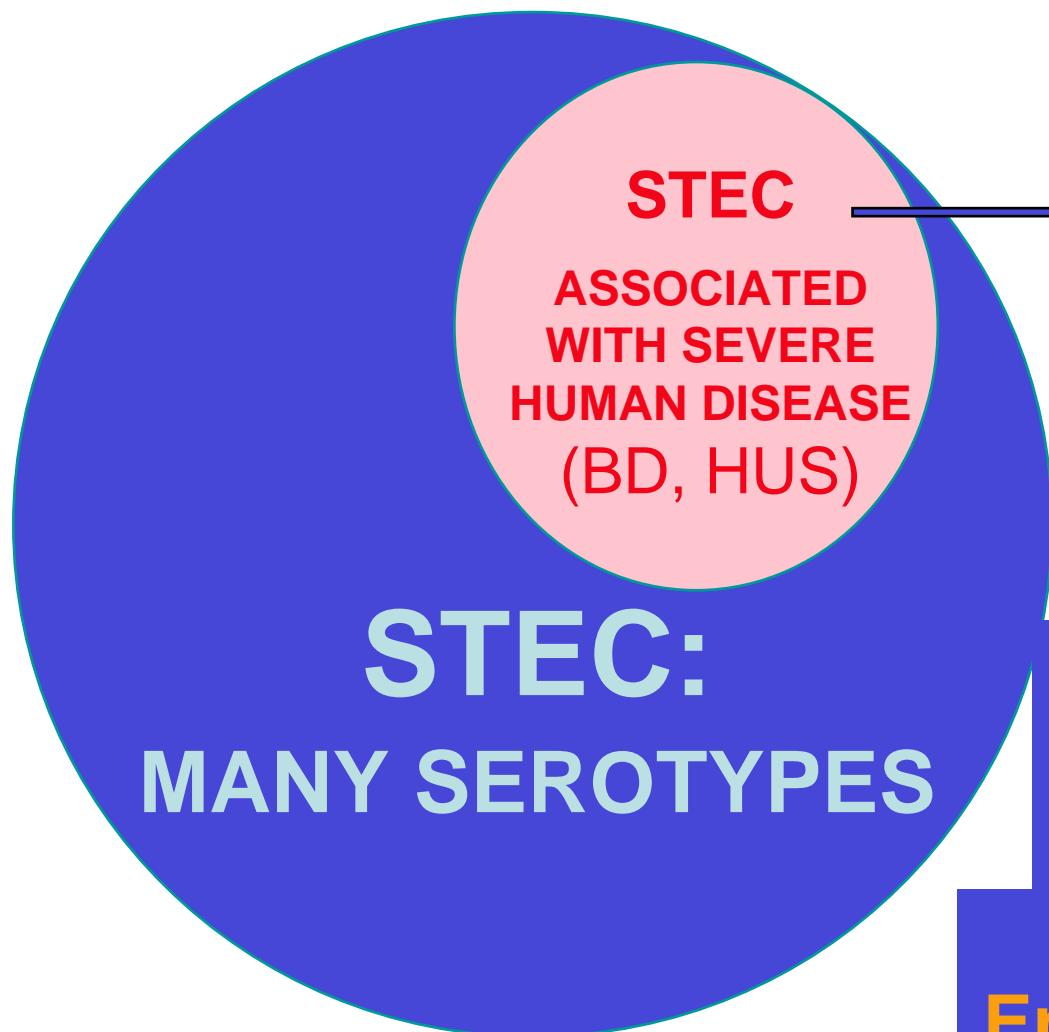
Lethal for laboratory animals

Cytopathic effect on many cell lines

Particularly active on endothelial cells



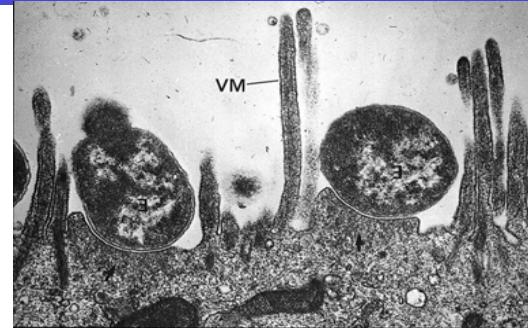
# Stx is not sufficient to cause disease



**STEC**

ASSOCIATED  
WITH SEVERE  
HUMAN DISEASE  
(BD, HUS)

Attaching/Effacing  
adhesion to the  
intestinal mucosa



Belong to a restricted  
number of serogroups:  
O157, O26, O111, O103 ....

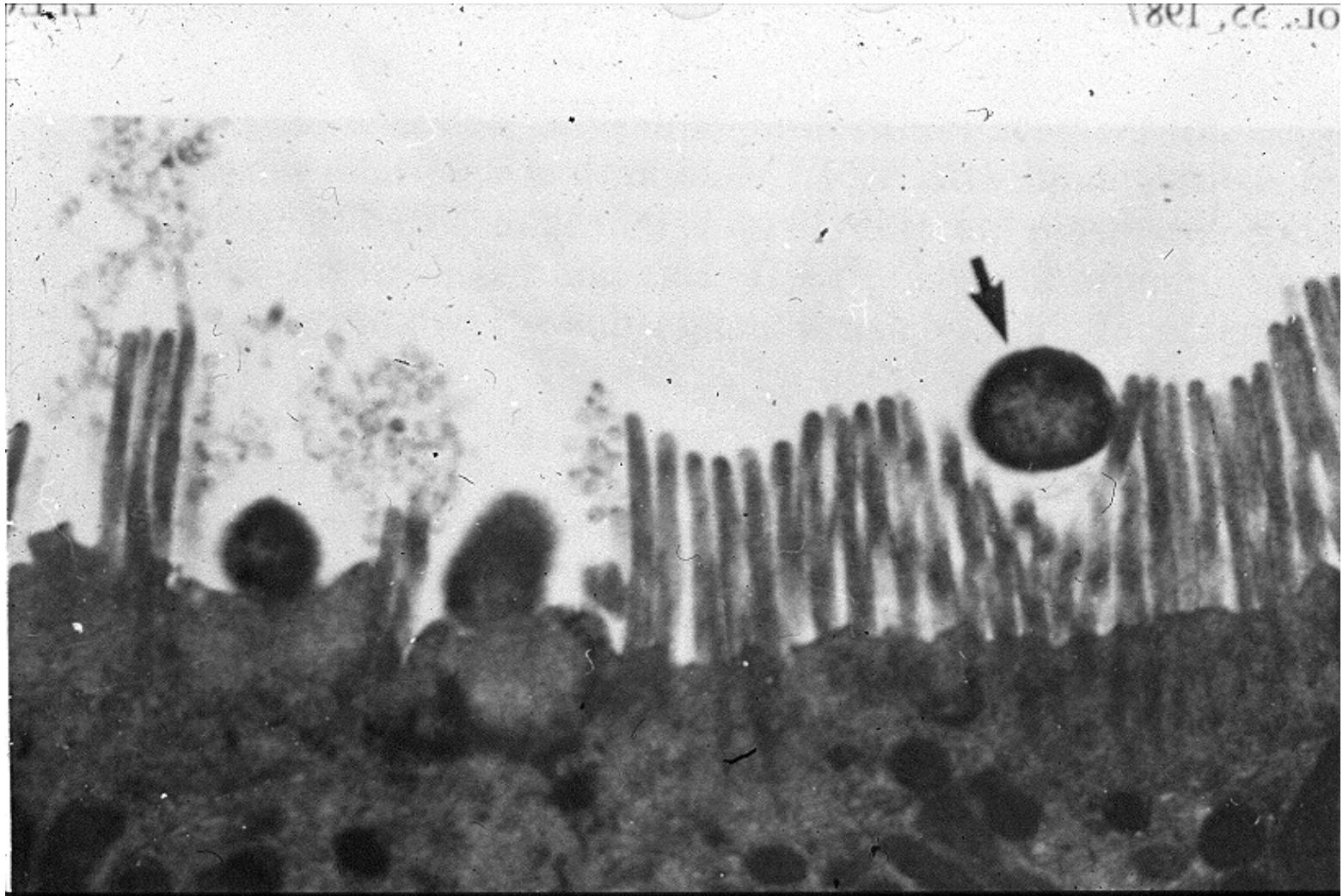
Are defined as  
**Enterohemorrhagic *E.coli***

# *E.coli* O157 bloody diarrhea



Istituto Superiore di Sanità - Dipartimento Sanità Alimentare e Animale  
Community Reference Laboratory for *E.coli*

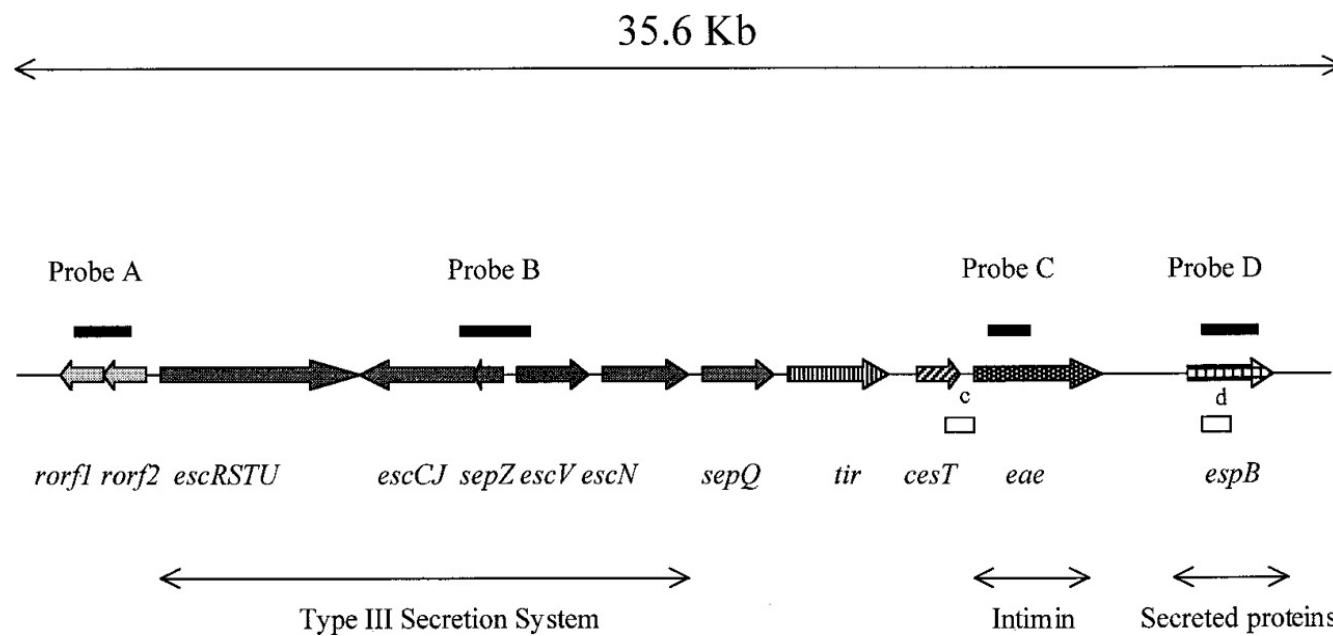
# Attaching/effacing lesion on the intestinal mucosa



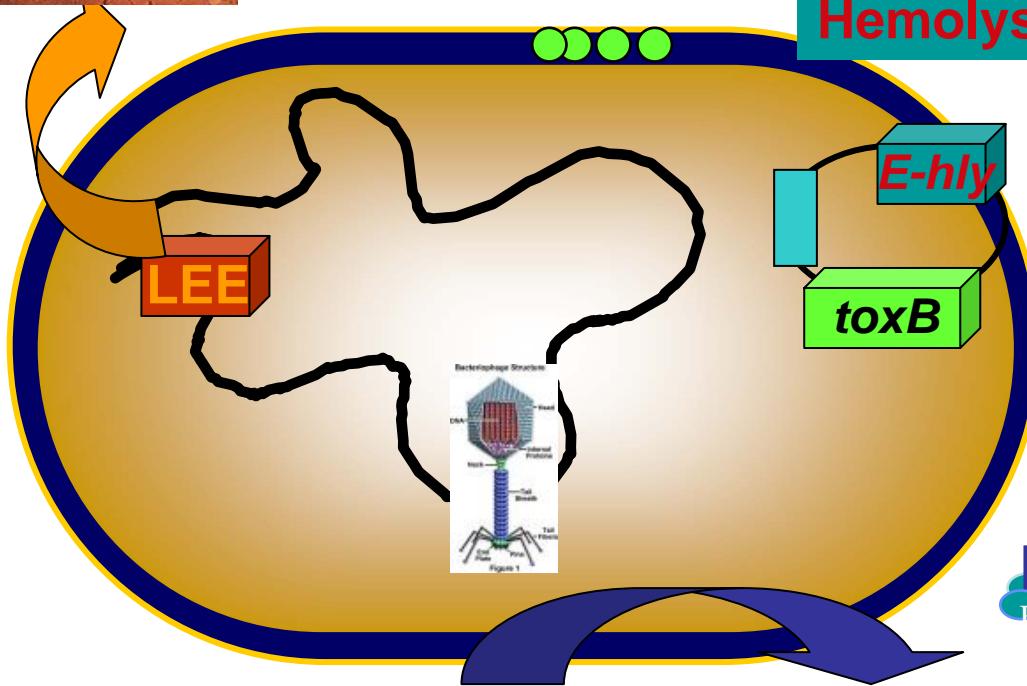
# Attaching/effacing lesion on the intestinal mucosa

Genetically governed by a Patogenicity Island

## Locus of Enterocyte Effacement (LEE)



# *E.coli* O157 virulence factors



## Mobile genetic elements:

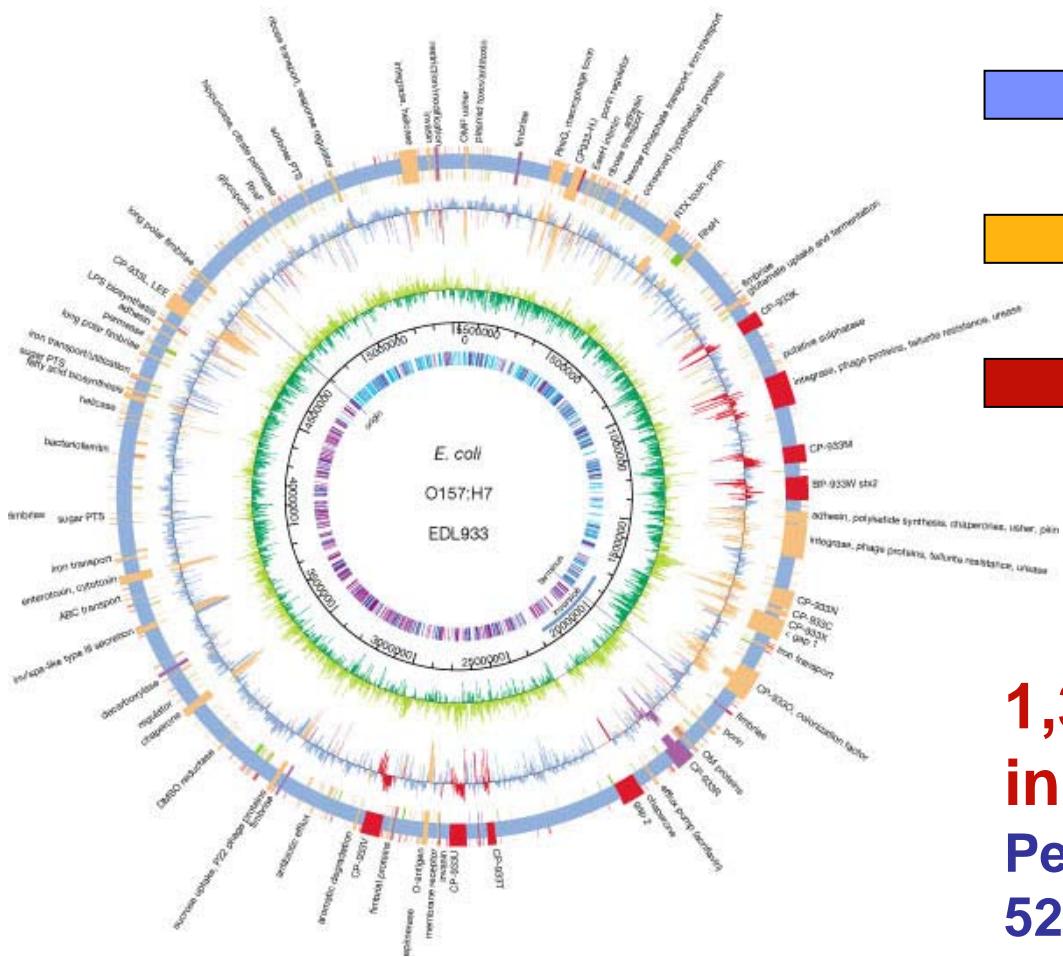
- Phages
- Genomic islands
- Plasmid



## Shiga toxin-Production



# *E.coli* O157 chromosome



*E.coli* K12 sequences

Genomic islands

Integrated prophages

1,387 genes absent  
in *E.coli* K12  
Perna et al. Nature, 409:  
529-33, 2001

# **Evolution of *E.coli* O157:H7**

**Evolutionary analysis by multilocus enzyme  
electrophoresis:**

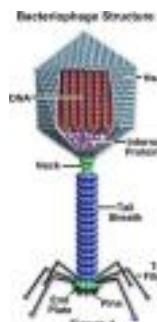
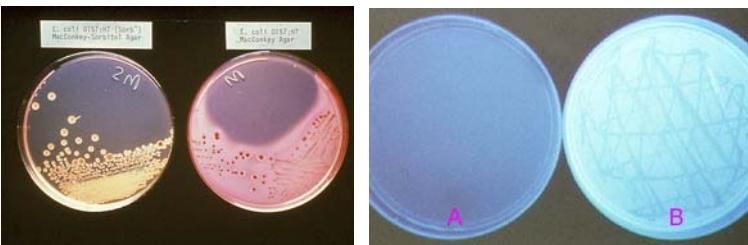
**EHEC O157:H7 is genetically related to EPEC O55:H7  
(classical human EPEC associated with worldwide  
outbreaks of infantile diarrhea)**

**Whittam et al. Infect. Immun. 61:1619-29, 1993  
Feng et al. J. Infect. Dis. 177:1750-3, 1998**

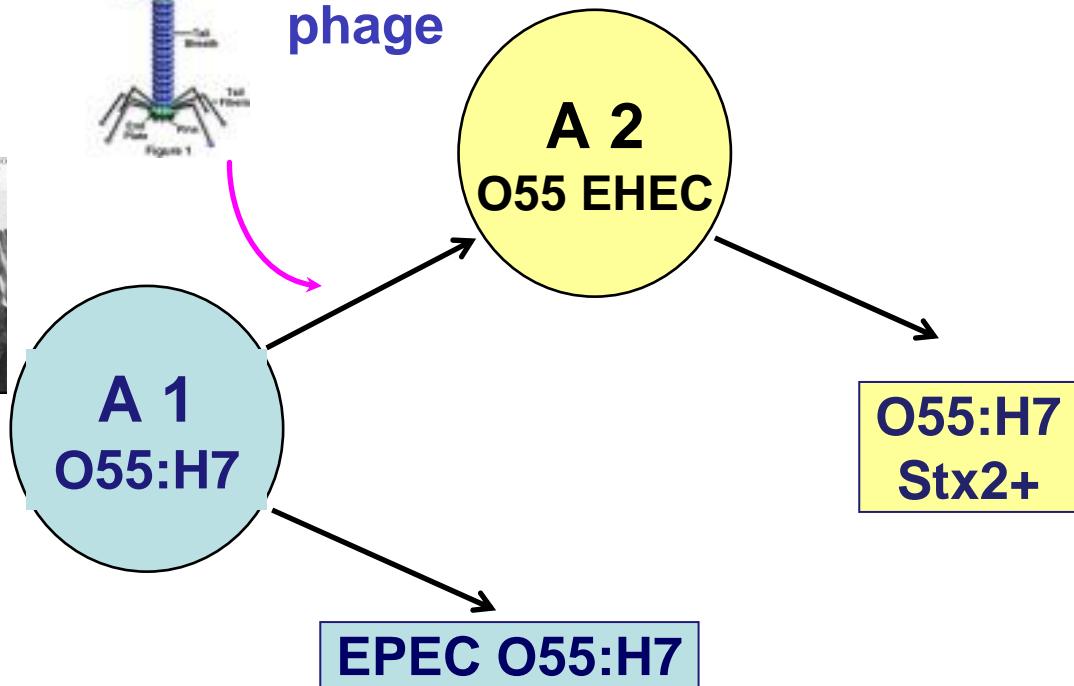
# Stepwise evolution of *E.coli* O157

EPEC-like ancestor  
O55:H7

LEE +  
(and many  
other PAIs)  
  
Sorbitol +  
 $\beta$ -glucuronidase +



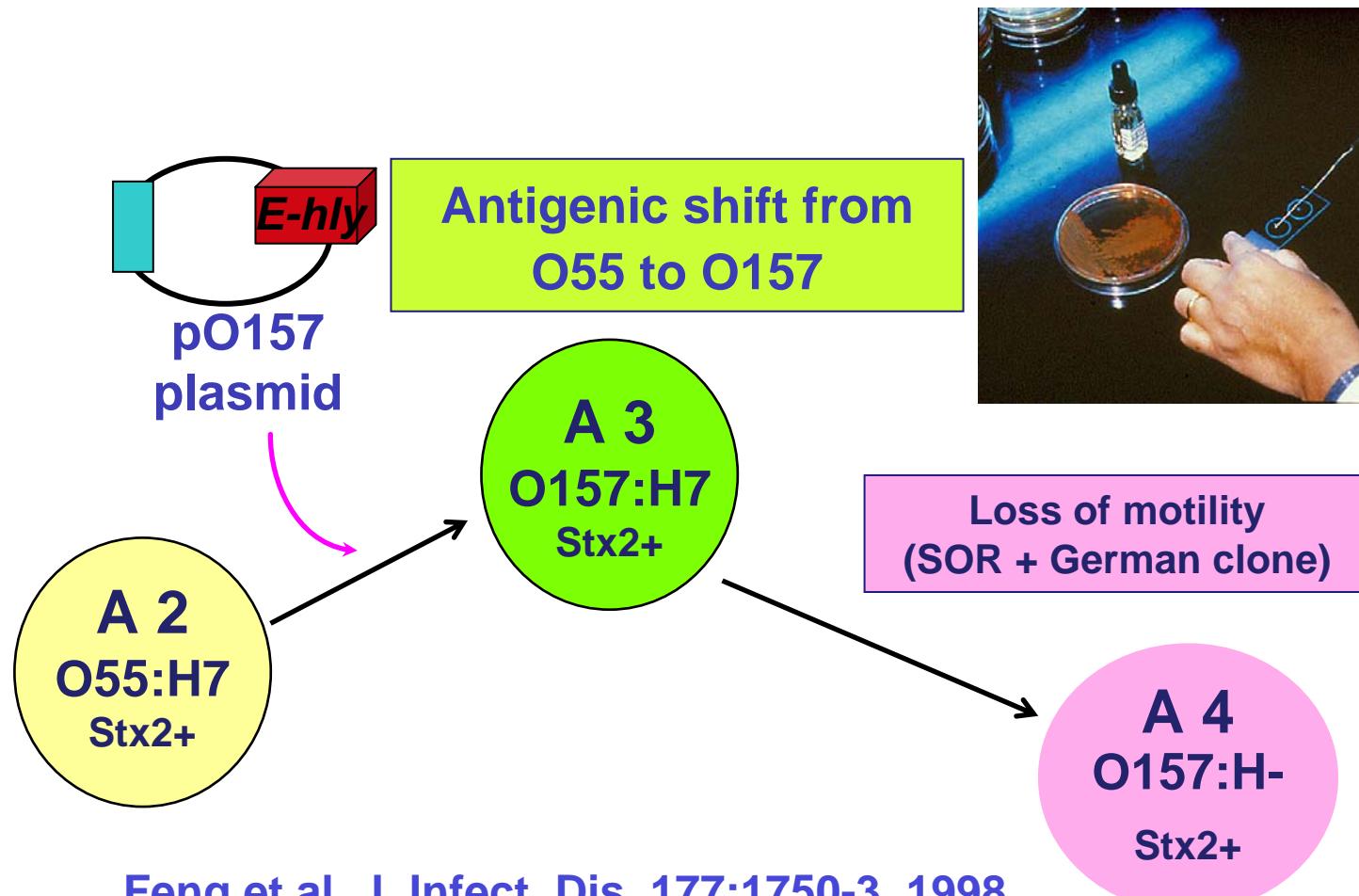
Stx2  
phage



Feng et al. J. Infect. Dis. 177:1750-3, 1998

Friedrich et al.. J. Clin. Microbiol. 42:4697-701, 2004;  
Wick et al. J. Bacteriol. 187:1783-91, 2005

# Stepwise evolution of *E.coli* O157

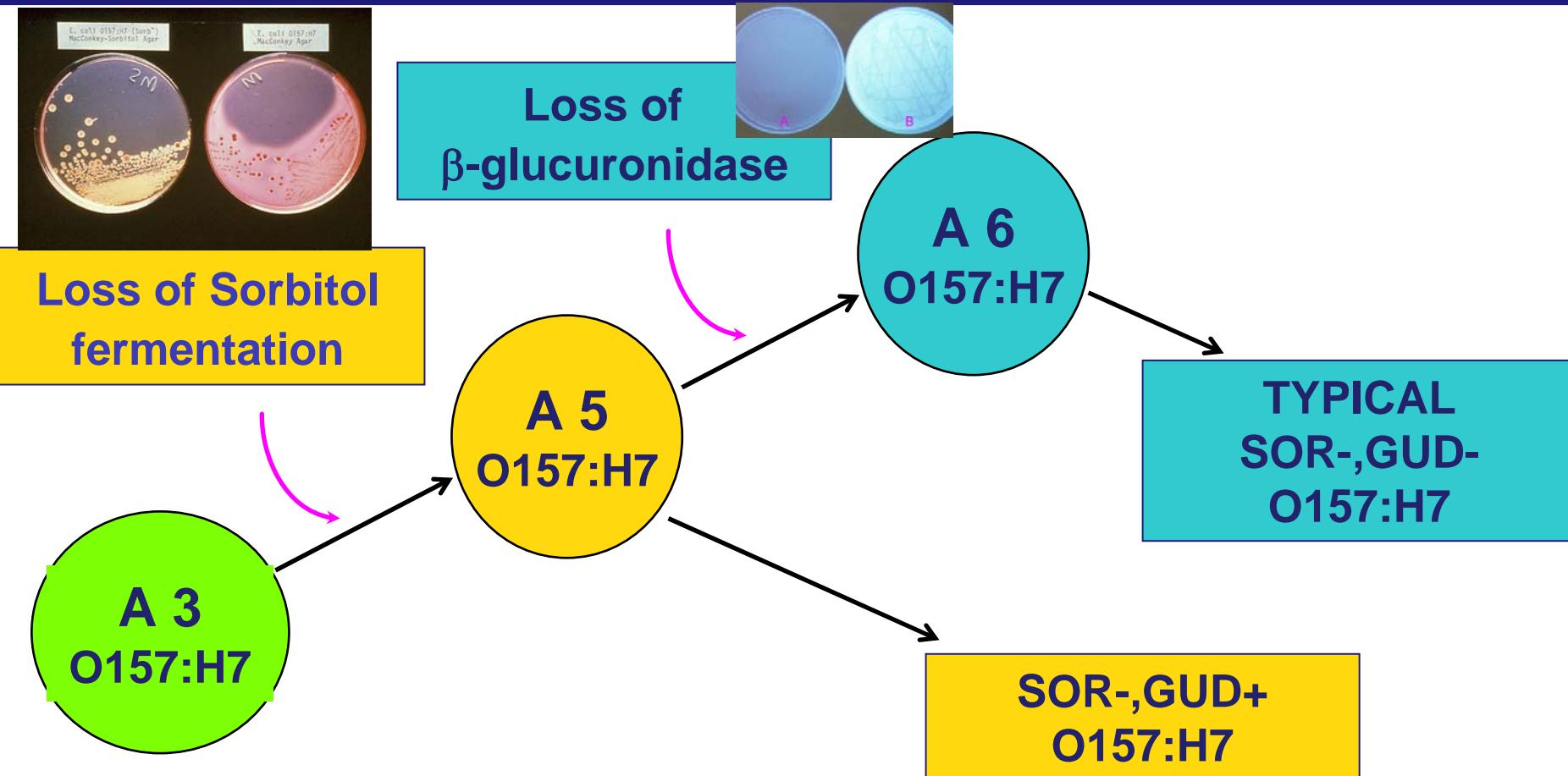


Feng et al. J. Infect. Dis. 177:1750-3, 1998

Friedrich et al. 2004. J. Clin. Microbiol.; 42

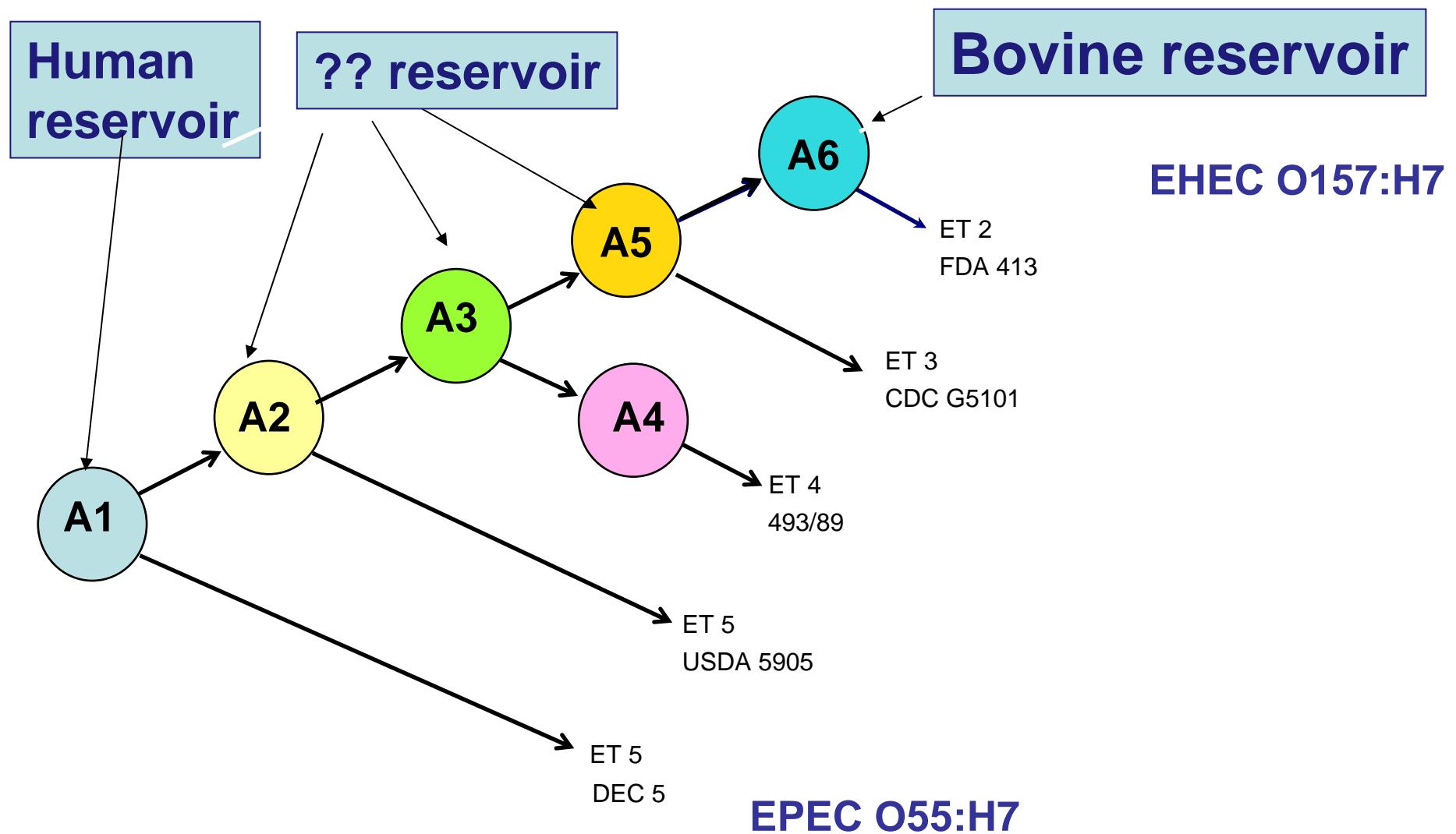
Wick et al. J. Bacteriol. 187:1783-91, 2005

# Stepwise evolution of *E.coli* O157



Feng et al. J. Infect. Dis. 177:1750-3, 1998  
Friedrich et al. 2004. J. Clin. Microbiol.; 42  
Wick et al. J. Bacteriol. 187:1783-91, 2005

# Stepwise evolution of *E.coli* O157



# *E.coli* O157 evolution: problems still open

**EPEC O55:H7 is a human pathogen: *EHEC* O157:H7 has its natural reservoir in ruminants .....**

**Why *E.coli* O157 appeared so abruptly in 1982 ??**

- *E.coli* O157 was rarely observed by Reference Laboratories of UK, Canada and USA between 1970 and 1982

**Why *E.coli* O157 is so widespread in cattle herds ??**

# *E.coli* O157: changing epidemiology

**1980's**

**Outbreaks in limited settings (restaurants,  
schools, day care centers)**

**1990's**

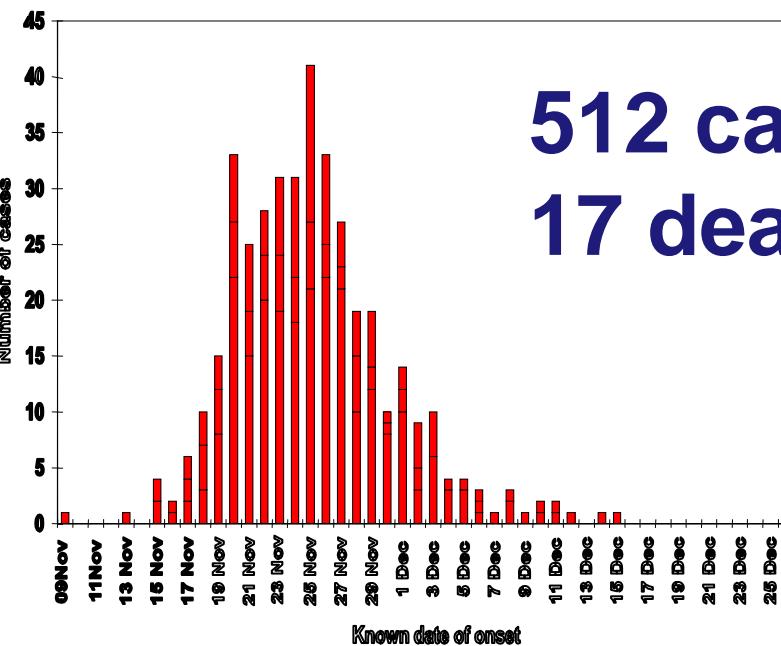
**Community-wide outbreaks, in large  
geographic areas and long period of times**

# USA, *E.coli* O157 multi-state outbreak, 1993

- Fast food chain, several States in the west coast
- Burgers (**not properly cooked**)
- 4 deaths
- 700 ill



# Central Scotland Outbreak of *E.coli* O157, 1996



512 cases  
17 deaths

- Contaminated “steak pie”
- Cross contamination with raw meat

**Il Messaggero**  
10/11

Allarme sanitario per un'epidemia  
Scozia, 6 morti intossicati  
da colibatteri nelle carni

LONDRA - E' salito ancora e rischia di aumentare, secondo le autorità sanitarie, il numero delle persone morte nella contea del Lanarkshire in Scozia dopo aver ingerito cibi prodotti con carni infette per la presenza del batterio E.Coli O-157. Sono sei i morti accertati e come nei precedenti casi si tratta di persone anziane. Un centinaio di persone sono rimaste intossicate, e una quarantina sono ancora ricoverate in ospedale, fra cui anche 17 bambini. «Speriamo di aver già toccato il culmine» ha affermato il responsabile della struttura sanitaria scozzese David Carter, affermando tuttavia che è ancora presto per dirlo con certezza e che comunque di fronte a sei decessi obbliga già parlare di epidemia. Le carni avariata, secondo la polizia, provengono da una macelleria della cittadina di Wishaw che fornisce parchi dettaglianti. Attraverso la radio e la tv le autorità, dopo aver istituito un numero verde d'emergenza, hanno invitato riveditori e cittadini a non vendere o consumare altre carni provenienti dalla macelleria in questione. Simili intossicazioni attribuite al batterio O-157 si sono verificate negli ultimi mesi anche in Giappone, con numerosi decessi e migliaia di intossicazioni.

**LA STAMPA** pag. 10

Le vittime sono tutte persone anziane: altri 100 in ospedale  
**Carni avariata, cinque morti**  
**Scozia, strage causata da un batterio**

**LONDRA**  
DAL NOSTRO CORRISPONDENTE

Dopo di emuccia pazzia, un'altra carne che uccide: quella infetta da E-coli O157, il bacillo che non perdona. In Scozia ha ucciso, nelle ultime ore, cinque persone anziane; una sesta, che per errore era stata data per morta ieri pomeriggio, è invece in condizioni disperate. L'epidemia ha colpito un centinaio di persone e circa 40 i casi più gravi - sono ancora ricoverate negli ospedali attorno a Glasgow. Il guaio è che E-coli O157, a differenza dei suoi confratelli, non conosce cure: o riesce l'organismo a debellarlo - e questo accade in genere nel 90 per cento dei casi - o si muore. Colpisce e blocca i reni; e infatti due bambini di 4 e 5 anni - più facilmente colpiti degli adulti - sono gravissimi e sono sottoposti da due giorni a dialisi continua nel tentativo di nere il «bug» in vita. Diarrea e vomito sono i primi sintomi, come per qualsiasi avvelenamento da ciabatta: ma poi arrivano le prime perdite di sangue dall'intestino, infine vengono colpiti i reni.

L'epidemia scozzese, si è scoperto, ha avuto origine da una partita di carne precotta - roastbeef, arrosti di maiale, prosciutti cotti - distribuita in numerosi negozi e supermercati attorno a Glasgow. Tutta carne fornita da una macelleria di Wishaw: la premiata ditta John Barr & Son, ora chiusa dalle autorità sanitarie che stanno cercando di scoprire il perché. E' una macelleria, per ironia della sorte, insospettabile: detentrice del titolo di «macelleria scozzese dell'anno». La migliore, insomma, nella Scoria delle carni sopravvive. Ma per la carne, a cominciare da emuccia pazzia, il 1996 non sarà certamente ricordato come anno doc in queste isole.

La prima vittima - martedì sera - è stato Harry

# Outbreak of *E.coli* O157, Japan 1996



WORLD  
NEWS  
STORY PAGE



Health officials search  
for the source of the  
outbreak (CNN)

## Radish sprouts in school meal

- 10,000 school children ill
- 8 deaths

Giappone:  
ancora una vittima  
del colibatterio  
«O-157»

L'epidemia ha già contagiato 5 mila persone e 3 bambini sono morti

Un batterio-killer  
uccide i giapponesi

In corso due bimbi colpiti  
dal virus-killer «O-157»

Giappone  
si aggrava  
l'epidemia

TOKYO — Si allarga in Giappone l'epidemia da vi-

Unità pagina 15

Il batterio piega il Giappone  
8mila contagiati tra bimbi e anziani. 4 morti

LA PIÙ GRAVE EPIDEMIA REGISTRATA TRA I MINORI

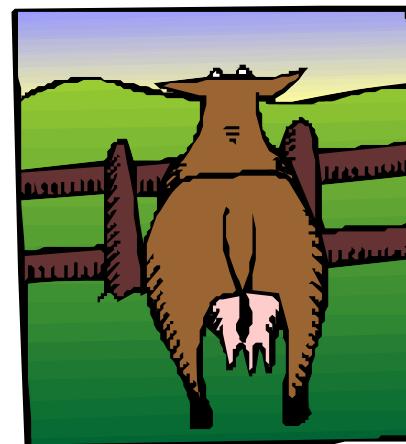
Giappone, settemila bambini intossicati

Chiuse scuole e piscine, ma il contagio non si ferma

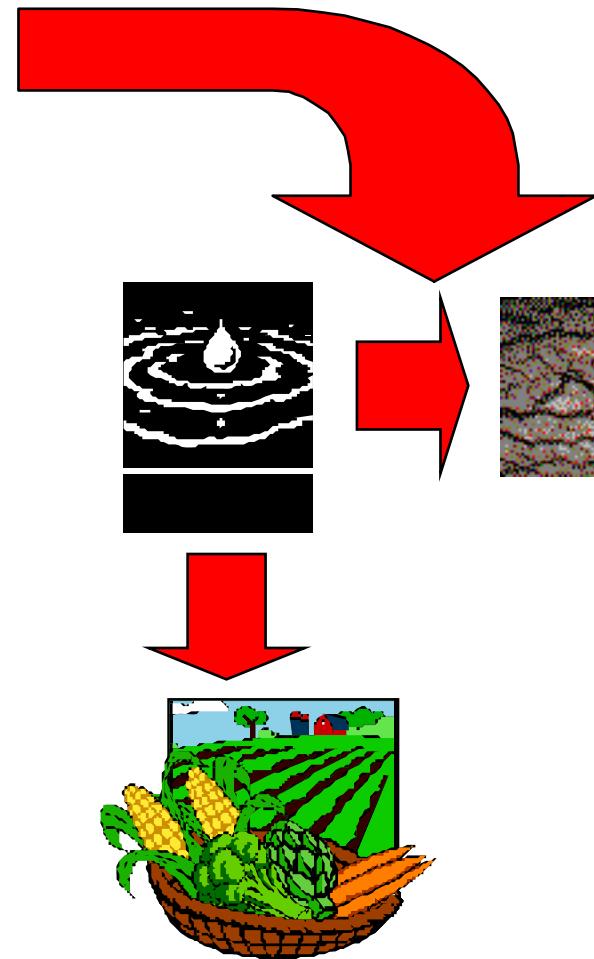
bilancio diffuso dalla autorità per la di 6.784 — ne che ha un'incubazione di circa due sei

# *E.coli* O157 modes of transmission

Foodborne



Environmental contamination



# Outbreak of *E.coli* O157 associated with vegetables

● USA, 1996	54 cases,	lettuce
● USA 1996	14 cases,	apple cider
● USA/Canada 1996	70 cases,	apple cider
● Japan, 1997	96 cases,	sprouts
● Michigan, 1997	108 cases,	sprouts
● Indiana, 1998	27 cases,	cabbage
● Ohio, 1999	30 cases,	cabbage
● Oklahoma, 1999	7 cases,	apple cider

# *E.coli* O157 - USA - September 2006

## Outbreak associated with fresh spinach

- 192 confirmed cases
- 30 cases of HUS
- 2 deaths



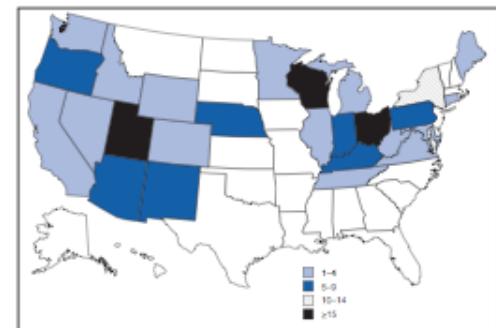
*MMWR Dispatch*  
Vol. 55 / September 26, 2006



### Ongoing Multistate Outbreak of *Escherichia coli* serotype O157:H7 Infections Associated with Consumption of Fresh Spinach — United States, September 2006

On September 13, 2006, CDC officials were alerted by epidemiologists in Wisconsin and Oregon that fresh spinach was the suspected source of small clusters of *Escherichia coli* serotype O157:H7 infections in those states. On the same day, New Mexico epidemiologists contacted Wisconsin and Oregon epidemiologists about a cluster of *E. coli* O157:H7 infections in New Mexico associated with fresh spinach consumption. Wisconsin public health officials had first reported a cluster of *E. coli* O157:H7 infections to CDC on September 8. On September 12, CDC PulseNet had confirmed that the *E. coli* O157:H7 strains from infected patients in Wisconsin had matching pulsed-field gel electrophoresis (PFGE) patterns and identified the same pattern in patient isolates from other states. This report describes the joint investigation and outbreak-control measures undertaken by state public health officials, CDC, and the Food and Drug Administration (FDA). This investigation and additional case finding

FIGURE 1. Number of confirmed cases (N = 183)\* of *Escherichia coli* serotype O157:H7 infection, by state — United States, September 2006



\* Confirmed cases reported as of 1:00 p.m. EDT on September 26, 2006.

# Waterborne outbreak of *E.coli* O157, Walkerton Canada, 2000

Walkerton

## **E. coli TRAGEDY**

### Contamination of municipal drinking water

- Population 5,000
- Ill 2,300
- Deaths 7



# *E.coli* O157 environmental contamination

## Children visiting

- Farms
- Petting zoos
- Fairs
- Camping



# *E.coli* O157 by direct and indirect animal contact

## The New England Journal of Medicine

---

Copyright © 2002 by the Massachusetts Medical Society

---

VOLUME 347

AUGUST 22, 2002

NUMBER 8



### AN OUTBREAK OF *ESCHERICHIA COLI* O157:H7 INFECTIONS AMONG VISITORS TO A DAIRY FARM

JOHN A. CRUMP, M.B., CH.B., D.T.M.&H., ALANA C. SULKA, M.P.H., ADAM J. LANGER, D.V.M., CHAD SCHABEN, M.P.H.,  
ANITA S. CRIELLY, M.S., ROBERT GAGE, M.S.P.H., MICHAEL BAYSINGER, B.S., MARIA MOLL, M.D., GISELA WITHERS, M.S.,  
DENISE M. TONEY, PH.D., SUSAN B. HUNTER, M.S., R. MICHAEL HOEKSTRA, PH.D., STEPHANIE K. WONG, D.V.M., M.P.H.,  
PATRICIA M. GRIFFIN, M.D., AND THOMAS J. VAN GILDER, M.D., M.P.H.

**“Contact with calves and their environment, hand  
washing protective”**

---

Istituto Superiore di Sanità - Dipartimento Sanità Alimentare e Animale  
Community Reference Laboratory for *E.coli*

# Prevention of *E.coli* O157 infection

## School visits to farms

### **E. coli O157** Schools Information Pack



### Health Tips for Teachers Leading School Visits to Farms



1934/3 7/2007

#### Before the visit

- 1 Check that the farm is authorised for school visits and has suitable washing facilities – soap, very warm water and clean towels (preferably disposable) or blow-driers.
- 2 Ensure there is an adequate number of supervising adults, taking into account the age and stage of development of the pupils.
- 3 Explain to the pupils that they cannot be allowed to eat or drink anything, including crisps, sweets etc. while touring the farm, because of the risk of infection.

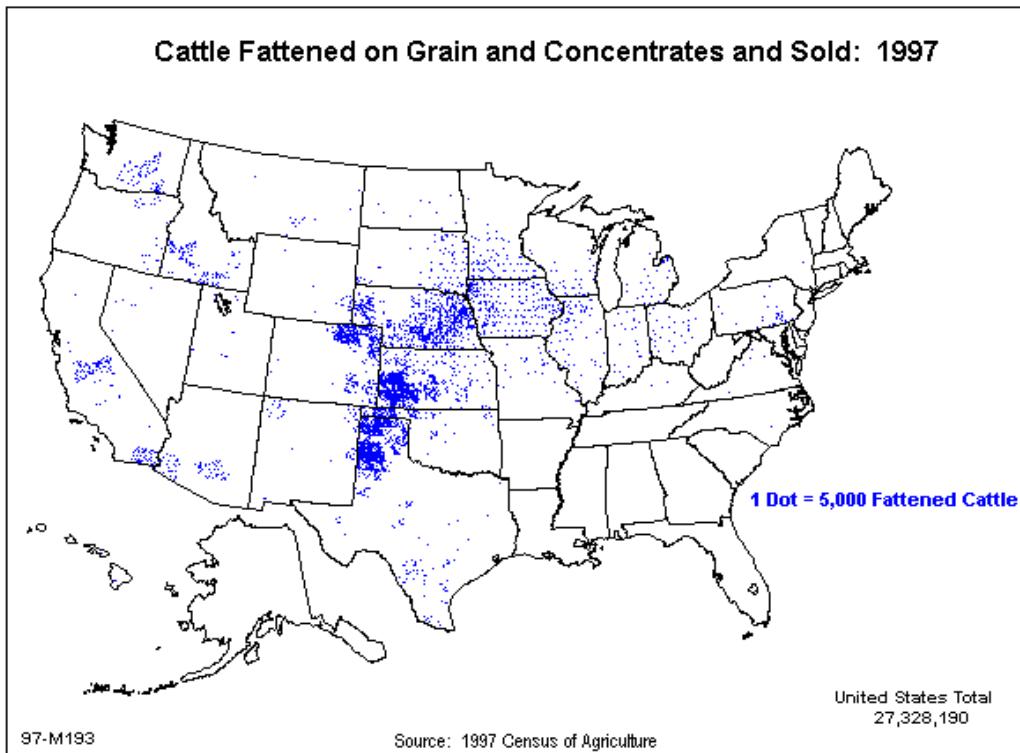
#### During the visit

- 4 If pupils are in contact with, or feeding, farm animals, warn them not to place their faces against the animals or put their hands in their own mouths afterwards.
- 5 Keep pupils away from sick animals.
- 6 After contact with animals and particularly before eating and drinking, ensure all pupils wash and dry their hands thoroughly. If young children are in the group, hand washing will require to be supervised.
- 7 Meal breaks or snacks should be taken well away from areas where animals are kept and pupils warned not to eat anything which has

# Animal Waste Pollution in America, an Emerging National Problem

## Democratic Staff Report, US Senate Agriculture Committee (1998) US Manure Estimates, 1997

- **5 tons of animal manure / person / year**
- **130 times greater than amount of human waste**



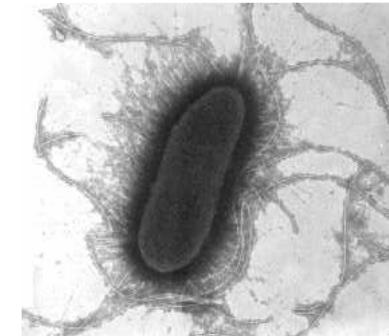
# Prevention *E.coli* O157 infections

- Farm level
- Slaughter house level
- Retail/catering level

# *E.coli* O157 prevention: farm level

**Cattle colonization cannot  
be avoided at present**

- Good husbandry practices
- Correct disposal of manure
- Attention to farm visits by school children



# *E.coli* O157 prevention: abattoir

- **Changing diet or starvation before slaughtering (?)**
- **Visual control of hide fecal contamination**
- **Hygienic dehiding**
- **Cleaning of the tools**
- **Correct removal of the gastrointestinal tract**
- **Carcass separation**
- **End process treatment (?)**

# *E.coli* O157 prevention: retail/catering

- Segregation of raw - cooked
- Hygienic packaging
- Training and licensing of personnel
- Consumer education (warning in labels, education by media)

# Thank you for attention !



Istituto Superiore di Sanità - Dipartimento Sanità Alimentare e Animale  
Community Reference Laboratory for *E.coli*