THE UNIVERSITY OF MELBOURNE

# FOOD20006

# Food Microbiology & Safety



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# Foodborne diseases Infections

**Ray and Bhunia Ch** 26

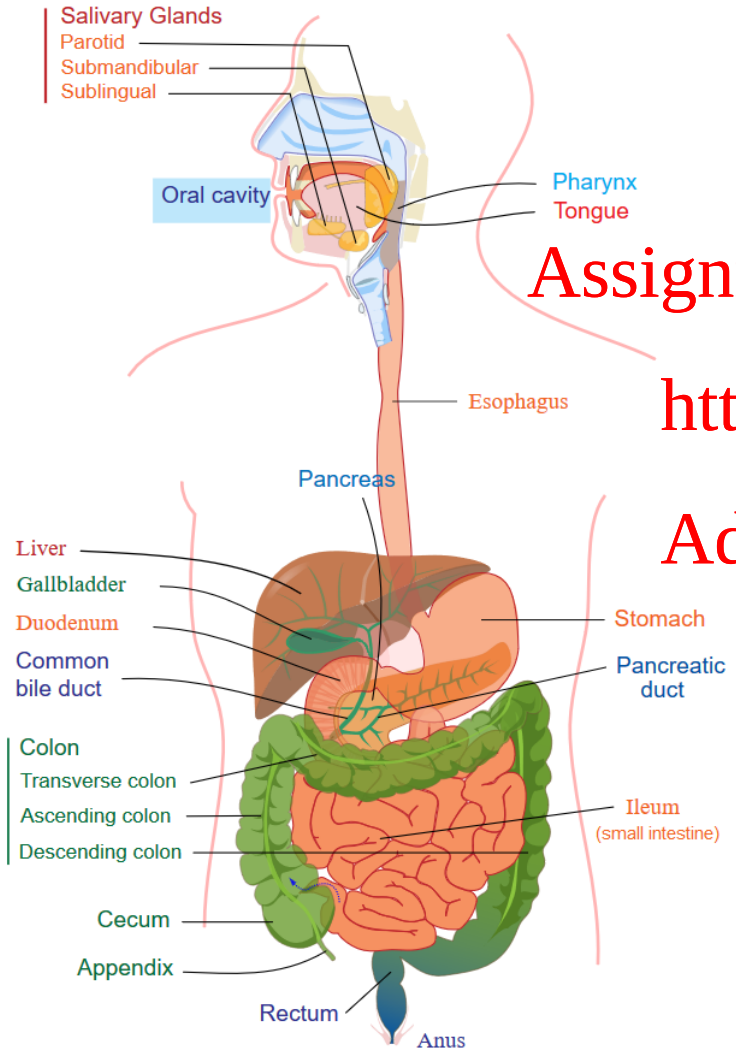
# Intended learning outcomes

Understand the difference between a foodborne illness caused by a preformed toxin (intoxication), and by a pathogenic microorganism (infection) and by a toxigenic pathogen (toxicoinfection)

Understand the biology and characteristics of ***Escherichia coli***, and why there are harmless and pathogenic types

Identify other bacteria and viruses that commonly cause foodborne infections

# Pathogenesis of foodborne bacterial infections




Foodborne **infection** occurs by a number of essential steps

- from ingestion of food containing pathogens, to the expression of symptoms of the disease.

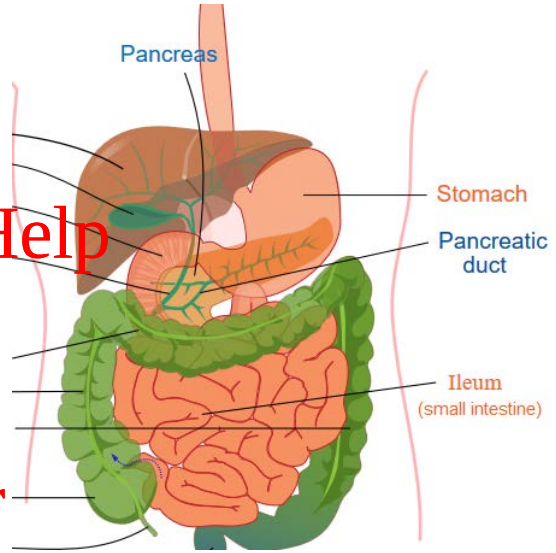
The diagram is useful to understand the steps involved

# Pathogenesis of foodborne bacterial infections

Steps required to cause bacterial disease by infection

1. Live bacteria ingested with food
2. survive passage through stomach (low pH)
3. penetrate mucous layer of intestinal tract
4. attach to epithelial cells of intestine
5. establish infection in/on epithelial cells
6. multiply, produce toxins or invade cells/body -> **symptoms**





# Toxico-infections vs infections

Generally, toxicoinfections involve

- either sporulation or
- lysis of bacterial cells and release toxins from lysed cells in gastrointestinal tract.



Examples of pathogens that casue food borne toxicoinfections are

*Aeromonas hydrophila*

*Bacillus cereus*

*Clostridium perfringens*

enterotoxigenic *Escherichia coli* (ETEC)

*Vibrio cholerae*.

# Toxico-infections vs infections

Foodborne infection occurs from the consumption of food (and water) contaminated with pathogenic enteric bacteria and viruses which invade the body via the GIT or colonise the gut epithelium

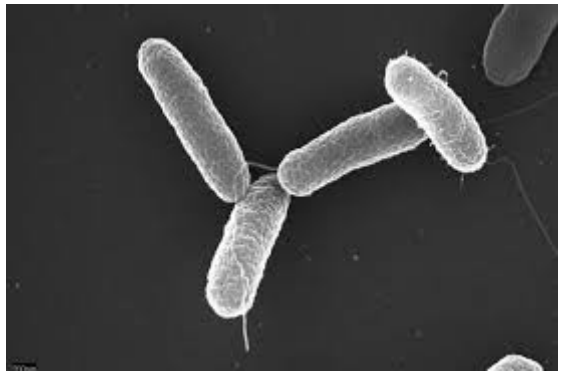
Examples of bacterial pathogens that cause food borne infections are

*E. coli*

*Salmonella*

*Campylobacter*

*Listeria*



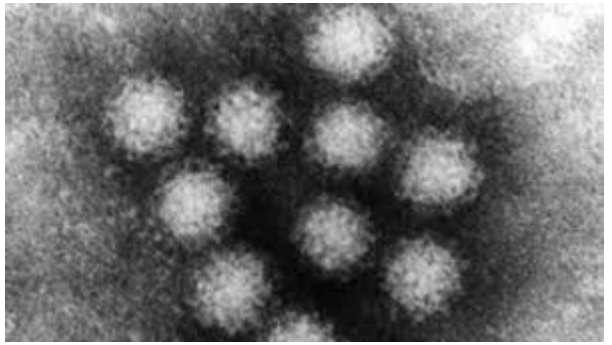
Examples of viral pathogens that cause food borne infections are

Norovirus

Hepatitis A virus

Hepatitis E virus

Human rotavirus



# Pathogenesis of foodborne bacterial infections

How many bacteria are needed to cause an infection?

- Can be measured by the '50% infectious dose', which is the number of infectious organisms that will cause disease in 50% of inoculated animals. Abbreviated as the ID50
- ID50 varies with species: 10 for EHEC, to $10^5$ for *Listeria*



How long from ingestion to symptoms (incubation period)?

- Generally after 24 hours, as all the steps (above) take time
- Compare this with the 30min–2hr needed for intoxications

Local symptoms (abdominal pain, diarrhoea, vomiting)

Systemic (non-enteric) symptoms, for those bacteria that can spread through the body to other target organs, e.g. *Listeria*

# Foodborne bacterial infections

**Chpt 26**

Where disease is caused by infection by a bacterial species



Main examples: *E.coli, Salmonella, Campylobacter, Listeria*

Read the sections about *E.coli, Salmonella, Campylobacter, Listeria* in chapter 26

# *Escherichia coli*

Gram negative rod, facultative anaerobe, ‘coliform’, family *Enterobacteriaceae*

Most strains are **harmless commensals**, commonly present in mammalian intestines. **Some** are pathogens

Survive well in water, hence is used as an indicator of faecal contamination e.g. by sewage or animal manure



No significant heat resistance (D60°C of 0.1min)

Can survive refrigerated or frozen temperatures for extended periods

Also survives when dried on the surfaces of seeds!! Then grows when seeds treated with water to germinate



Optimum growth temperature ~ 37°C (mesophiles)

Growth down to 4.4 pH is possible, neutral pH preferred

Minimum Aw of 0.95

# *E.coli* on MacConkey Agar

**Isolation:** easily done on selective/indicator media

e.g. MacConkey agar

Red colonies due to **fermentation of lactose (Lac+)**

Medium also has bile salts so both an indicator (differential) and selective medium.



# *Escherichia coli:* pathogenic types

Most, but not all, *E. coli* are harmless commensals.

- But some of the worst outbreaks of food-borne illness around the world have been due to **pathogenic strains** of *E.coli*.

Both the harmless, commensal *E.coli* and the pathogenic *E.coli* are members of the same species.

Pathogenic types differ by the **possession of virulence genes**, such as those specifying enterotoxins e.g. shiga toxin.

# *Escherichia coli:* pathogenic types

Pathogenic *E.coli* are divided into **4 main groups:**

Enterotoxigenic *E. coli* (ETEC)

Enteroinvasive *E. coli* (EIEC)

Enteropathogenic *E. coli* (EPEC)

Enterohaemorrhagic *E. coli* (EHEC/STEC) e.g. type O157:H7

* STEC = shiga toxin producing *E.coli*.

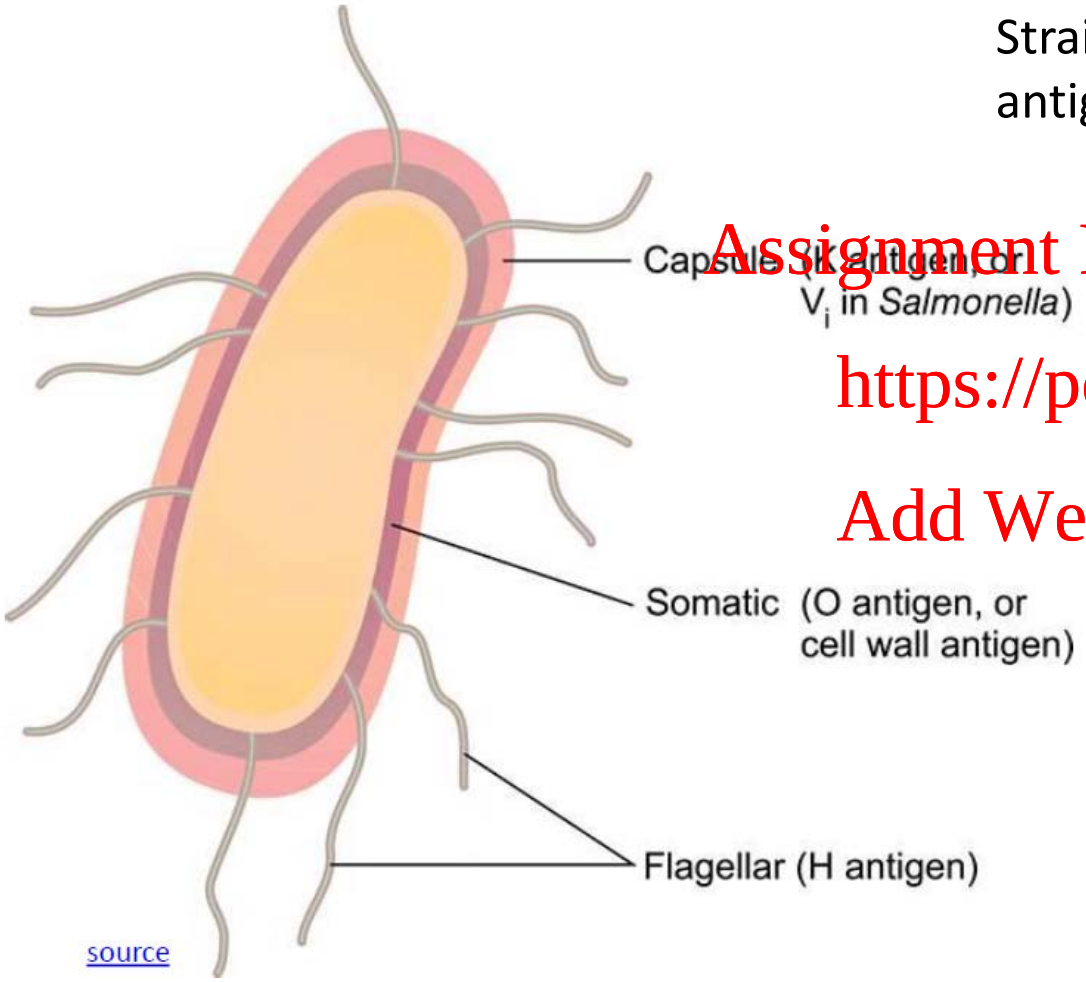


These differ from each other in the virulence genes carried the serotype numbers O157 and H7 refer to serological reactions to a panel of antibodies to cell surface antigens.

- O is for cell wall structures
- H is for flagella proteins.

https://en.wikipedia.org/wiki/Escherichia_coli_O157:H7

# *Escherichia coli:* pathogenic types




source

Strains are serotyped using panels of antibodies to O and H antigens

e.g. if the O antigen reacted with O antibody 157 = O157

if the flagella reacted with H antibody 7 = H7

-> the strain = O157:H7

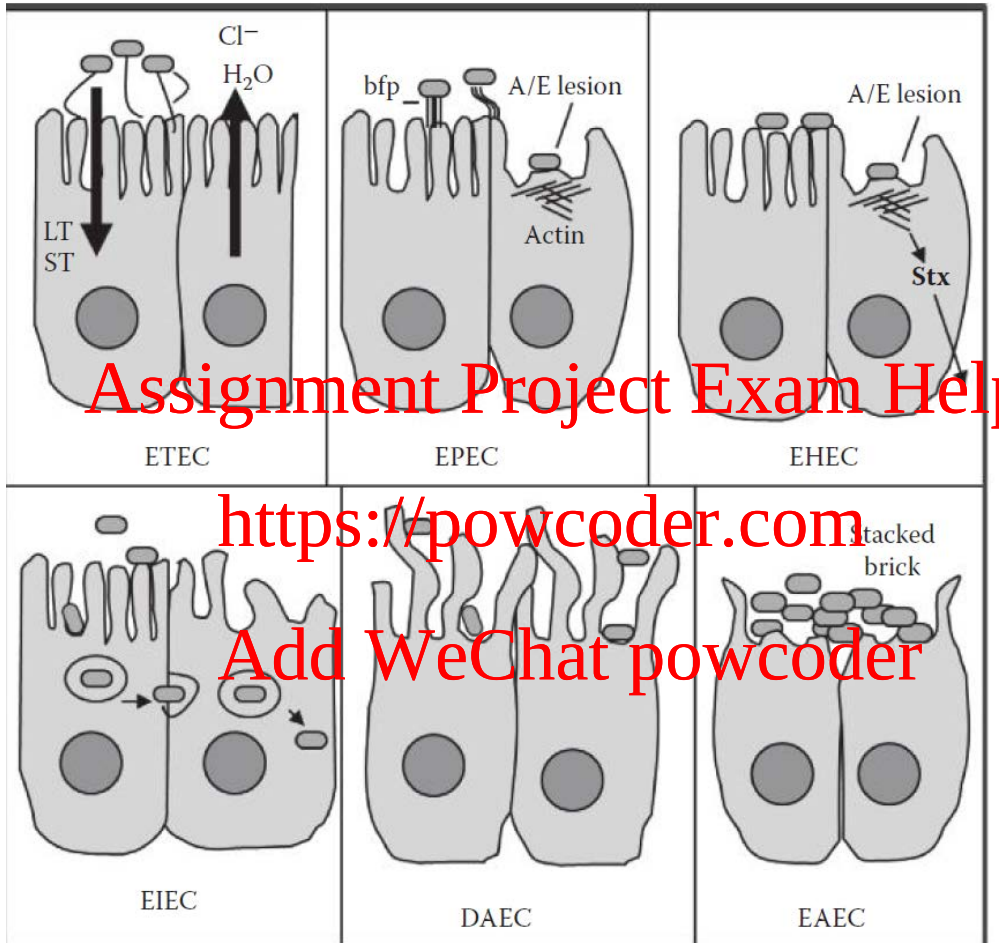





Figure 26.3 Mechanism of *Escherichia coli*–induced cell damage in intestinal villous epithelial cells by enterotoxigenic *Esc. coli* (ETEC), enteropathogenic *Esc. coli* (EPEC), enterohemorrhagic *Esc. coli* (EHEC), enteroinvasive *Esc. coli* (EIEC), diffusely adhering *Esc. coli* (DAEC), and entero-aggregative *Esc. coli* (EAEC). LT, heat-labile toxin; HT, heat-stable toxin; bfp, bundle-forming pili; A/E lesion, attachment/effacement lesion showing actin accumulation; Stx, Shiga-like toxin.

# Enterohaemorrhagic *Escherichia coli* (EHEC/STEC)

Can cause major outbreaks, food-transmission, serious!

Illness ranges from mild disease to life-threatening!

i.e. from non-bloody diarrhoea to haemorrhagic colitis life threatening = **HUS** (**haemolytic uraemic syndrome**) = TTP (thrombocytopaenic purpura)



Adhere to intestinal epithelial cells and then damages underlying microvilli



Produces shiga-like toxins (stx) -> blocks protein synthesis -> causes cell damage to kidney endothelial cells, etc.



CDC link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4270005/

Serotype O157:H7 is common in Canada/USA

Serotype O111:H negative is more common in Australia

# *Escherichia coli:* pathogenic types

**Sources:**

**Often a zoonosis**

- **Faecal** contamination of water supplies and food handlers
- **Cattle** - good reservoirs for O157:H7 and similar EHEC strains
  - **Raw milk** (unpasteurised), undercooked meat (especially minced, insufficient cooking in general. Mincing distributes the bacteria throughout the meat
- **Salads/sprouts/fruit** – **contamination by sewage** or cattle dung. These food items are normally eaten raw, so always a danger....



Normal pasteurisation temperatures are sufficient to eliminate *E. coli* species

# German 2011 outbreak

Severe foodborne diarrhoea and kidney disease

~ 3800 cases

845 HUS

54 deaths




Caused by pathogenic *E.coli,* STEC

- **STEC** = shiga toxin producing *E.coli*
- **Very unusual serotype; unusually virulent**

**The bacterial culprit on a plate – Der Spiegel, May, 2011**

http://www.spiegel.de/international/germany/0,1518,765777,00.html

https://en.wikipedia.org/wiki/2011_Germany_E._coli_O104:H4_outbreak

# *E. coli* scare: cases '*likely to increase*'

BBC News 31st May 20011

**A deadly *E. coli* outbreak in Europe is expected to worsen in coming days, a German scientist has said.**



**Fourteen people have died** in Germany and one woman has now died in Sweden after a trip to Germany.



It is thought **cucumbers** from **Spain** caused the outbreak.

Germany bans the import of Spanish cucumbers.



Spanish exporters complain they are losing $200m (£120m) a week because of the bans and do not accept that their produce is the source of the outbreak

# Reaction to the cucumber crisis

**Germany:** Consumers told not to eat cucumbers, lettuces and raw tomatoes. 329 cases of *E.coli* confirmed; 14 deaths

**Spain:** The top European cucumber producer - threatens to seek compensation from the European Union for lost vegetables sales



**Russia:** Ban on all imports of cucumbers, tomatoes and fresh salad from Spain and Germany pending further notice



**Austria:** Ban on sale of cucumbers, tomatoes and aubergines imported via Germany



**Belgium:** Reported to have banned cucumber imports from Spain

**Netherlands:** Halted all cucumber shipments to Germany

**Ireland:** Spanish cucumbers removed from shelves

# Deadly germ comes from Spain!




# Shiga toxin producing *Escherichia coli*

Causing damaging gastrointestinal and kidney disease

Rare pathogenic *E. coli* bacterial strain involved, called serotype O104:H4 STEC

Strain is a genetic hybrid of different types of pathogen, a **mosaic** of different virulence traits

A decisive tool for identifying that there was a common source of infection among many different illnesses were modern molecular methods for DNA analysis and **strain DNA fingerprinting**

These gave rapid highly specific detection of the rare strain and were invented rapidly in the middle of the outbreak

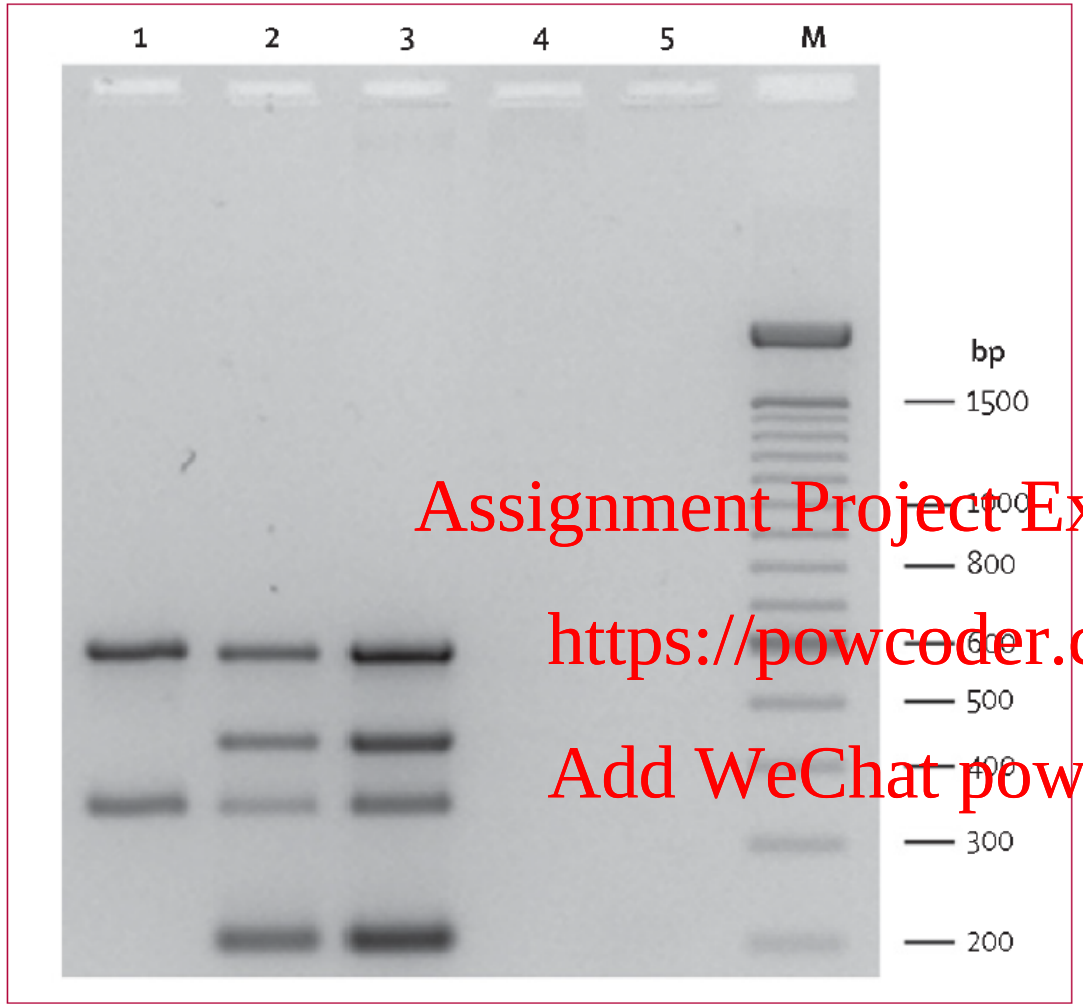





***Figure 1:* Detection of *Escherichia coli* O104:H4 outbreak strain**
The amplicons are $stx_2$ (584 bp), *terD* (434 bp), $rfb_{O104}$ (351 bp), $fliC_{H4}$ (201 bp). Lane 1: HUSEC037 (O104:H21; $stx_2$-positive, *terD*-negative, $rfb_{O104}$ -positive, $fliC_{H4}$-negative). Lanes 2 and 3: *E coli* O104:H4 outbreak isolates LB226692 and LB226541, respectively. Lane 4: *E coli* K12 negative control. Lane 5: reagent control. M: 100 kb molecular size marker.

Bielaszewska *et al.* 2011

# Results of genotyping

Comparison of the Spanish STEC isolate from cucumbers and the strain isolated from infected German patients showed that the two STEC strains were different!

**What does this mean?**

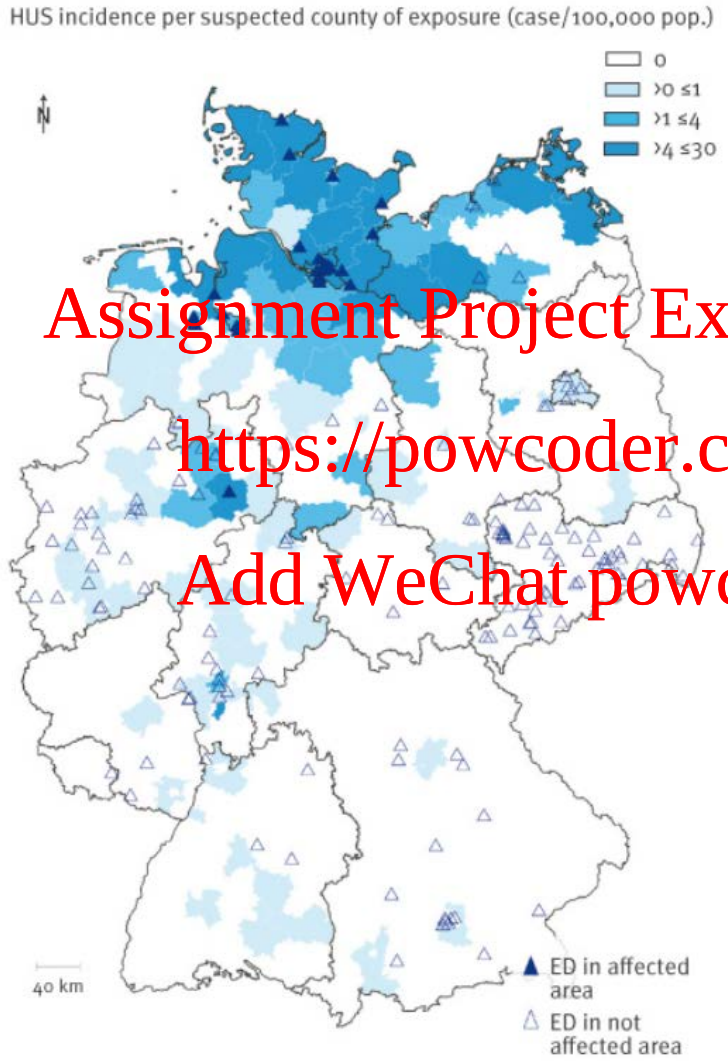


Epidemic profile of shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany - preliminary report. *New Eng. J Medicine* June 22, 2011 (10.1056/NEJMoa1106483)

http://www.nejm.org/doi/full/10.1056/NEJMoa1106483

**FIGURE 3**

Cumulative incidence of HUS cases per suspected county of exposure and emergency departments actively participating in the syndromic surveillance system, Germany, May–June 2011




ED: Emergency department; HUS: haemolytic uraemic syndrome; STEC: Shiga toxin-producing *Escherichia coli*.

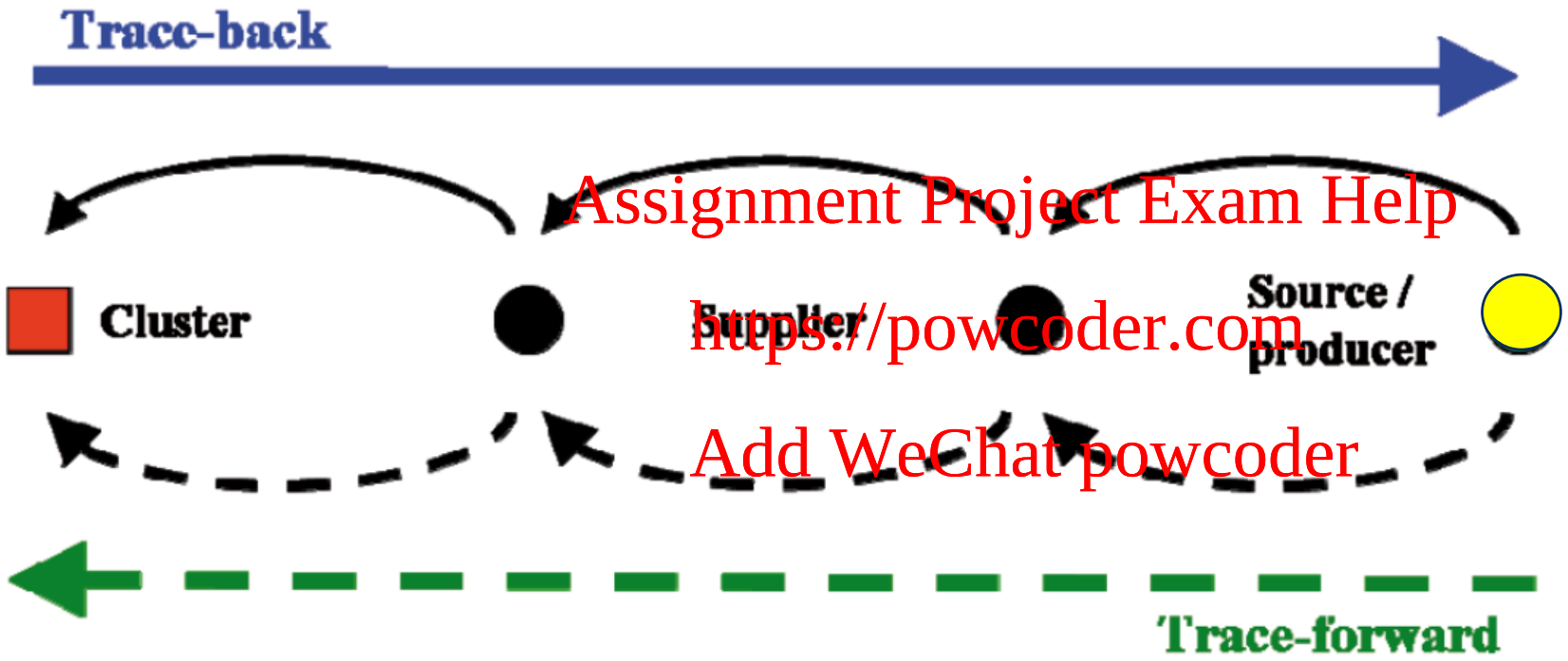





**Fig. 3: Schematic presentation of tracing strategies**
(1) Identification of "infection clusters" (RKI), (2) Backward Tracing: cluster → common producer/source (blue), (3) Forward Tracing: producer/source → other clusters (green)

# Chronology of the outbreak



Fig. 2: Top: BfR personnel sampling sprouts and seeds. Bottom left: Preparing samples at the NRL *E. coli*. Bottom right: Fenugreek seeds are the source of the disease outbreaks

# EFSA technical trace-back summary report

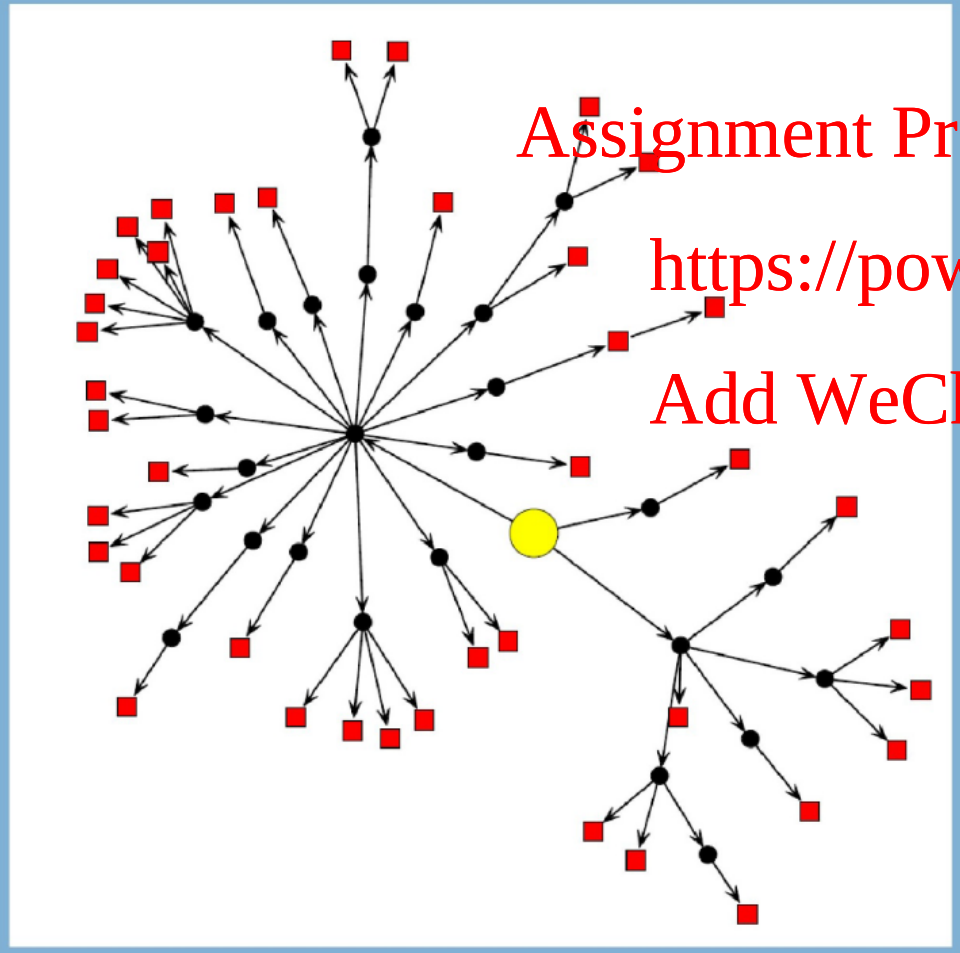
The epidemiological information currently available on this STEC outbreak in Germany suggests that STEC **O104-contaminated sprouts** are the vehicle of infection. This link is based on the results of a cohort study which is corroborated by analysis of trace-back and trace-forward studies carried out by the German EHEC Task Force and the authorities of Lower Saxony.



**A cohort study carried out by the Robert Koch Institute (RKI 2011b) involved guests of a restaurant which had been established as the place of exposure to the hazard for several cases. The food consumption of restaurant customers during the period of concern was ascertained not only through interviews, but also by checking food delivery lists and receipts. It established a high relative risk to develop bloody diarrhoea related to consumption of sprouts**.

# Results of the trace back and forward approach

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit



- Sprout Producer
- Distributor
- Outbreak clusters at the end of supply Chains (restaurants, canteens, supermarkets, groceries)

**41** well described outbreak clusters have been delivered with sprouts from the FBO in Lower Saxony

# Pinpointed a sprout farm

The German EHEC Task Force extensively investigated the possible source of infection in “Establishment A”, in particular water, personnel, and seeds.

- They showed that personnel were infected.



Whereas this may have contributed to the spread of the contamination, there was no indication that personnel had introduced the outbreak strain of *E. coli*.



- **Analyses of water and seeds have all proved negative to date**.



BERLIN, June 11 (Reuters) – No legal action against organic farm in northern Germany “Everything we have looked into until now shows the farm was flawless,” said Gert Hahne, spokesman for the consumer protection office of Lower Saxony state.

“It is hygienic and followed all the regulations. No matter how you look at it we don’t see any fault with the farmer, or any legal ground to hold them accountable”

**”You cannot punish someone for having bad luck.”**

# French outbreak – June 2011

**24th June**: France reported a cluster of patients with bloody diarrhoea, after having participated in an event in a town near Bordeaux on the 8th of June.

Adults (31-64 yr old), many with HUS. Many reported having eaten sprouts.

Infection with ***E. coli* O104:H4** was confirmed, and was **the same strain** as the German outbreak!

*Sprouts were not directly connected to Germany*



**What question would you ask at this point?**

# French outbreak – June 2011

*Sprouts were not directly connected to Germany*.

1. Suspected sprouts of fenugreek, rocket and mustard had been privately produced in small quantities by the organiser of the event

2. Seeds supplied by British company Thompson & Morgan, UK



**What question would you ask now?**

# Tracing the seeds…




TECHNICAL REPORT OF EFSA

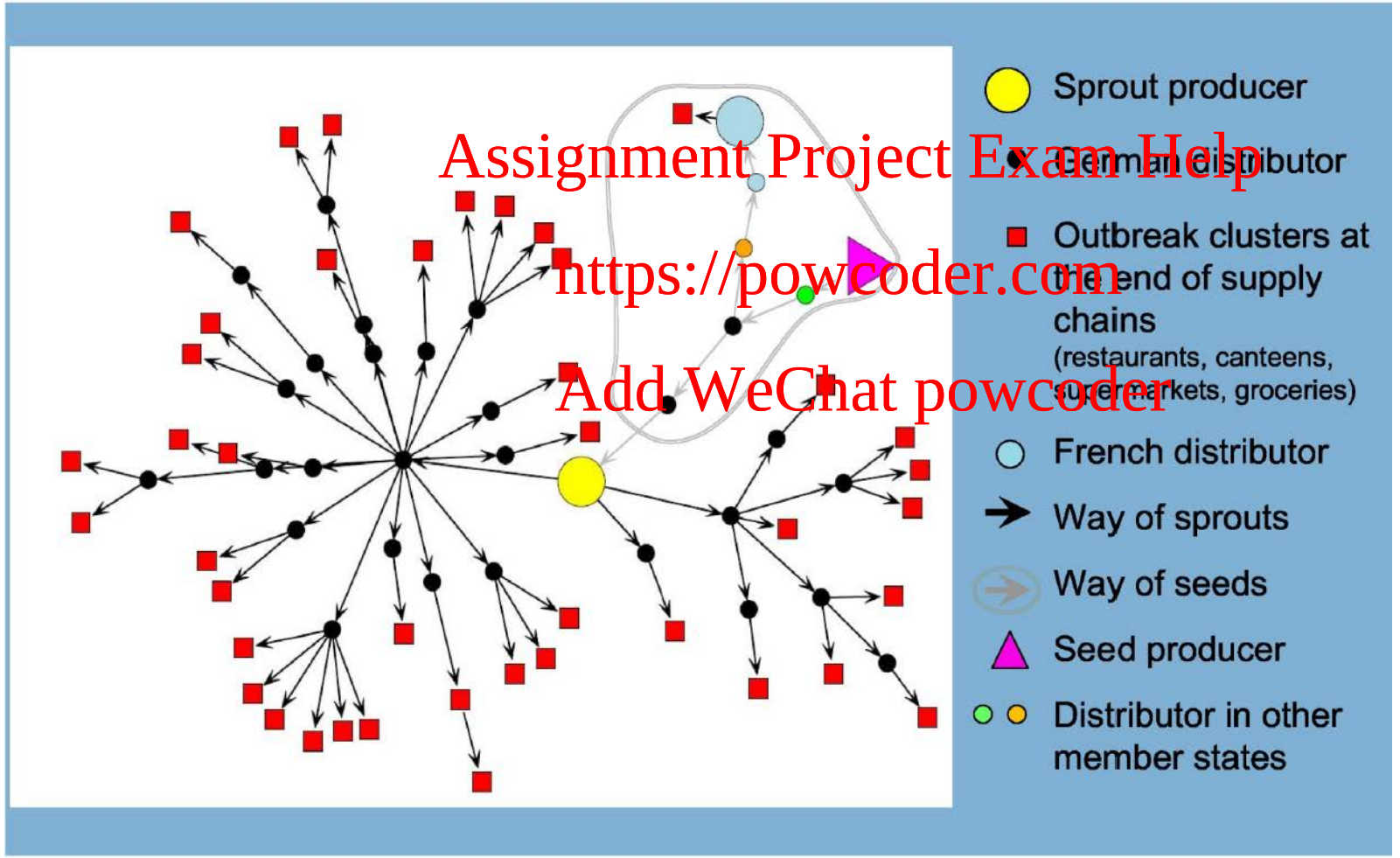
**Tracing seeds, in particular fenugreek *(Trigonella foenum-graecum)* seeds, in relation to the Shiga toxin-producing *E. coli* (STEC) O104:H4 2011 Outbreaks in Germany and France**[1]

**European Food Safety Authority**[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

# Link between the French and German outbreaks

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit



**B.** FENUGREEK IMPORTS FROM EGYPT TO THE GERMAN IMPORTER 2008-2011

| *Product name* | *Import date* | *Expiry date* | *Egyptian lot number* | *Importer lot number* | *Importer lot number* | *Amount (kg)* |
|---|---|---|---|---|---|---|
| Organic fenugreek "Bockshornklee" | 2008 | | | 542/08 | 5161 | |
| Organic fenugreek "Bockshornklee" | 15.12.2009 | 11/2011 | 48088 | 512/09 | 6832 | 15,000 |
| Organic fenugreek "Bockshornklee" | 28.10.2010 | 09/2012 | 8266 | 345/10 | 8223 | 10,000 |
| Organic fenugreek "Bockshornklee" | 14.02.2011 | 12/2014 | 2660002 | 51/11A | 8710 | 12,000 |

# Foodborne pathogens can live in plant tissues

*Amanda Deering and Robert Pruitt found that foodborne pathogens can live inside plant tissues*

http://www.sciencedaily.com/releases/2011/08/110815152049.htm



*E. coli* 0157:H7 was present in tissues of mung bean sprouts and *Salmonella* in peanut seedlings after the plants' seeds were contaminated with the pathogens prior to planting.



Amanda Deering, a postdoctoral researcher in food science, said seeds could be contaminated in such a manner before or after planting through tainted soil or water. "The pathogens were in every major tissue, including the tissue that transports nutrients in plants," said Deering, whose results were published in separate papers in the *Journal of Food Protection* and *Food Research International*.



# The sprouting process –the risk factors

Pathogenic bacteria can survive in/on seeds for hundreds of days

Impossible to absolutely sanitise seeds prior to sprouting (without killing seeds)

Germination provides ideal condition for bacterial growth (humidity, nutrients, warmth) for several days



- **Inherent risk if you eat raw sprouts**



Extra risk factors from **organic** certified sprouts:



- Seeds are at greater risk of faecal contamination from manure-compost
- Good seed sanitisation methods (hypochlorite) for seeds ruled out for ideological reasons
- Use of hot water for seed sanitisation is unsafe

# The disease track record

Over 40 outbreaks associated with sprouts

| | Pathogen | Culture confirmed cases | Location | Sprout type |
|---|---|---|---|---|
| 1988 | *Salmonella* Saintpaul | 143 | UK | Mung |
| 1994 | *Salmonella* Bovismorbificans | 595 | Sweden, Finland | Alfalfa |
| 1995 | *Salmonella* Stanley | 242 | USA, Finland | Alfalfa |
| 1996 | *Salmonella* Montevideo and Meleagridis | 508 | USA | Alfalfa |
| 2010 | *Salmonella* Bareilly | 231 | UK | Mung |
| 1996 | *Escherichia coli* O157:H7 | 2764 | Sakai City, Japan | Radish |
| 1997 | *E coli* O157:H7 | 126 | Japan | Radish |
| 1997 | *E coli* O157:H7 | 85 | USA | Alfalfa |
| 1998 | *E coli* O157 | 9 | USA | Alfalfa, clover |

***Table:*** **Notable outbreaks of food borne illness linked to seed sprouts**[10,11]



Hugh Pennington, Lancet Infectious Diseases 2011

## ABSTRACT

Sprouted seeds are young seedlings obtained from the germination of seeds. They are ready-to-eat foods which have caused large outbreaks. The bacterial pathogens most frequently associated with illness due to contaminated sprouted seeds are *Salmonella* and to a lesser extent STEC. *Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus* and *Yersinia enterocolitica* have also been transmitted by sprouted seeds, albeit very rarely. Dry seed contaminated with bacterial pathogens has been identified as the most likely initial source of sprout-associated outbreaks; although other routes of contamination (e.g. during production due to poor practices) may also occur. In some outbreaks, contamination of seeds with as low as 4 *Salmonella* per kg was sufficient for the sprouts to cause disease. Seeds purchased by sprouts producers are usually not grown specifically for this purpose. They may be contaminated during production, harvest, storage and transport, and there may be difficulties in traceability of seeds from production to sprouting. Bacterial pathogens on seeds may survive for long periods during seed storage. There is so far no guarantee of a bactericidal step which is able to control contamination of seeds with bacterial foodborne pathogens acquired prior to germination. Due to the high humidity and the favourable temperature during sprouting, bacterial pathogens present on dry seeds can multiply on the sprouts. Contamination with pathogenic bacteria must be minimized by identification of seed crops intended for sprouted seeds production before planting, and application of GAP, GHP, GMP, HACCP principles at all steps of the production chain. The relevance of decontamination treatments of seeds and of microbiological criteria is also discussed.