Emerging Microbial Hazards

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Overview

- Microbial hazard
- What is a pathogen
- Foodborne disease
- Emerging pathogens
- Factors in the appearance of emerging pathogens
- Case studies:
  - *Cryptosporidium*
  - *E.coli* O157:H7
  - Noroviruses

Microbial hazard

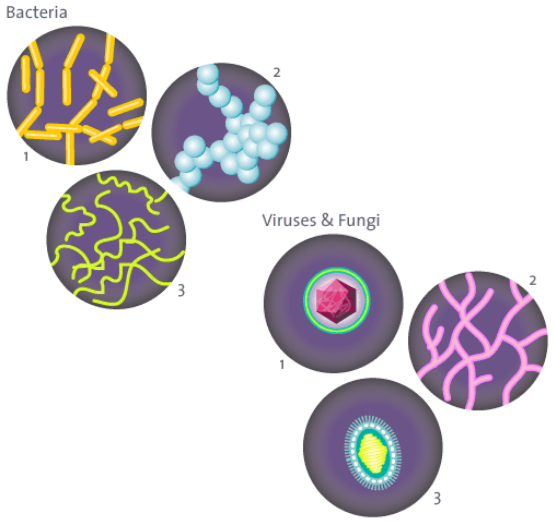
- Term generally used to refer to the micro-organisms: bacteria, fungi or viruses, that pose a risk of causing foodborne illness if they reach a sufficient number of cells on or in food contaminated with them.

- illnesses are caused by pathogenic bacteria

What is a pathogen?

PATHOGEN: “that with produces suffering”

A pathogen is a biological agent that causes disease in its host



Pathogens include:

- Bacteria (e.g. Strep throat)
- Viruses (e.g. hepatitis A)
- Fungi (e.g. athletes foot)

Foodborne pathogen: pathogen that has been acquired in food or the food chain

Foodborne disease

"a disease of an infectious or toxic nature caused by,
or thought to be caused by, the consumption of food or water",

Economic burden due to costs incurred from division
of resources to patient treatment

"Food and waterborne diarrhoeal diseases,
for example, are leading causes of illness
and death in less developed countries, killing
an estimated 2.2 million people annually,
most of whom are children"

WHO Global Strategy for Food Safety, 2002

Some statistics

Foodborne illness causes :

76 million illnesses

325,000 hospitalisations

5000 deaths

In the U.S., according to the CDC

The cost of five foodborne pathogens

-6.9 billion USD in 2000 USDA ERS estimates

Five foodborne outbreaks in 1996

- 300-700 million GBP in 1996 in England and Wales

Unknown pathogens

Known pathogens account for:

19% of total estimated number of cases

36% of deaths

Great majority of foodborne illness caused by unknown or emerging pathogens

Pathogens that have acquired antibiotic resistance

Emerging pathogens

- New, re-emerging or antimicrobial-resistant infections
- Incidence has increased in the last 20 years
- Incidence threatens to increase in the near future
- 13 out of 27 “top” US pathogens identified within last 25 years

Aetiology	Reservoirs	Mode of transmission	Distribution
Verocytotoxigenic *Escherichia coli*	Livestock and water	Meat, faeces, direct contact	Worldwide
Campylobacteriaceae - *Campylobacter* species - *Arcobacter species* - *Helicobacter species*	Poultry and pigs	Meat and faeces	Worldwide
Salmonella Typhimurium DT104	Livestock and wildlife	Faeces, eggs, meat and direct contact	Worldwide
Listeria monocytogenes	Livestock, wildlife, environment	Meat, milk, faeces and vegetation	Worldwide
Vibrio species - *V. cholerae* O1 - *V. parahaemolyticus* - *V. vulnificus*	Aquatic environment, fish and seafood	Fish, seafood, faeces	Worldwide
Listeria monocytogenes	Environment, livestock and humans	Milk and dairy products, fish, poultry, raw meat, ready-to-eat meat products and person-to-person transmission	Worldwide
Yersinia enterocolitica	Livestock, wildlife, fish and water	Meat, faeces and raw fish	Worldwide
Aeromonas species	Aquatic environments and livestock	Meat, milk, seafood and vegetables	Worldwide
Mycobacterium bovis	Cattle	Milk	Worldwide
Brucella melitensis	Sheep and goats	Milk	Worldwide
Enterobacter sakazakii	Not known	Powdered infant milk formula	Worldwide

Emerging pathogens

- Already present in the environment
- Change in conditions give a selective advantage
  - Infective agent
  - Host
  - Environment

Why do foodborne pathogens emerge?

- Globalisation of the food supply
- Changes in agricultural practices
- Inadvertent introduction of pathogens into new geographic areas
- Travellers, refugees, and immigrants exposed to unfamiliar foodborne pathogens while abroad
- Changes in microorganisms
- Change in the human population
- Changes in lifestyle

Globalisation of the food supply

- Improvement in international travel
  - From months to hours
- Dissemination of food animals and food products worldwide
- Availability of various produce year-round

Globalisation of the food supply

- Longer food chain-negative effect on food safety
  - Increased opportunity for contamination
  - Time/temperature abuse of products
  - Rapid spread of infectious agents over large distances
  - Emergence of particular pathogens in particular areas for the first time
  - Trend towards diffuse and widespread outbreaks

Globalisation of the food supply

- Difficult to trace source of outbreak
- Outbreak caused by a wide variety of foods
  - Traditionally meat, poultry, seafood, pasteurised milk
  - Now “low risk” foods e.g. apple juice, pepperoni, fresh fruit and vegetables

- *E. coli* O157:H7 outbreak associated with spinach in US (Sept 06)
  - 199 people infected across 26 states
  - 3 deaths
  - Contaminated irrigation water thought to be the cause

Changes in agricultural practices

- Intensive agriculture(high input)
  - High external input e.g. use of fertilisers and pesticides
  - More food produced per acre compared to other systems
  - Reduction of biodiversity
  - Irreversible soil erosion
  - Run-off to aquatic systems

Agricultural practices

- Extensive farming(low input)
  - Reduction of external inputs
    - Antibiotics, pesticides, herbicides, synthetic fertilisers
  - Diversification of crops and animals
  - Organic farming: a type of low input farming
    - Code of principles that are regulated internationally

Intensive farming

- May have lead to emergence and increased prevalence of *Salmonella*, *Campylobacter*, *E. coli* O157:H7
  - High stocking rates facilitates dissemination of pathogens
  - Increased prevalence of pathogens in animals going to slaughter
  - Spreading of animal wastes onto agricultural land
  - Feeding of grains

Intensive farming

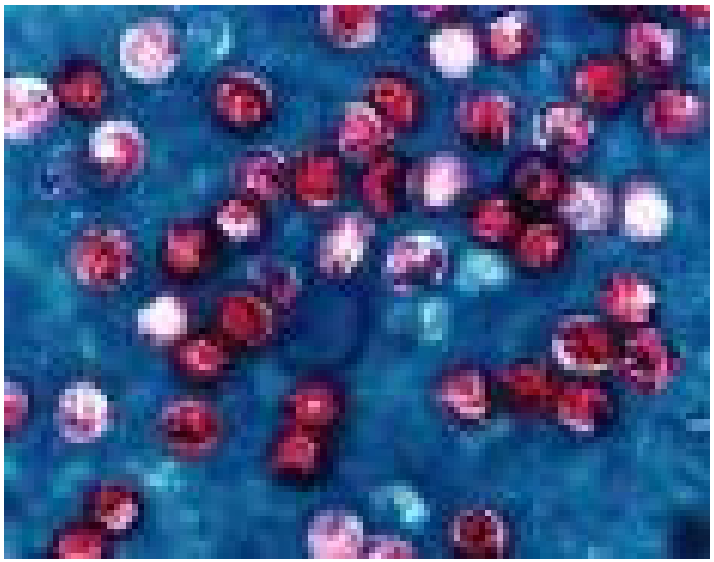
- Emergence of antibiotic resistant strains
  - Antibiotics are widely used in high input farming systems
    - To treat animal disease
    - To promote growth

Case Studies

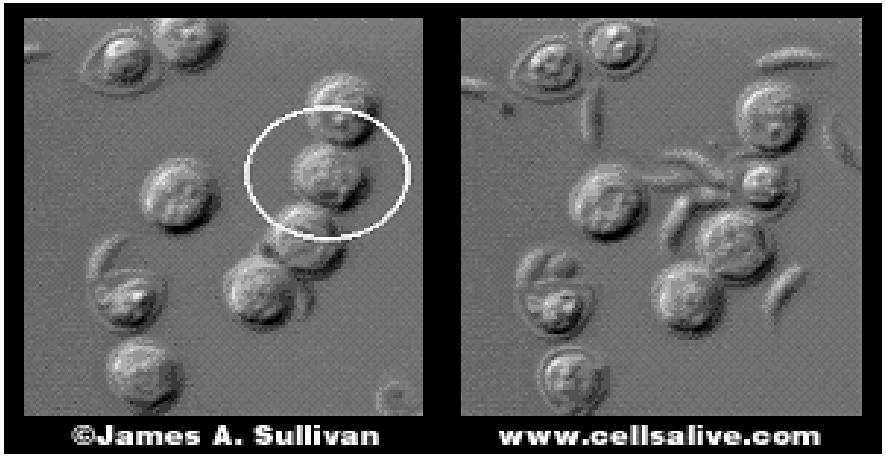
- Three case studies produced as part of Safe Foods project
  - *Cryptosporidium*
  - *Escherichia coli* O157:H7 in cattle
  - *Norovirus*

Case study*: Cryptosporidium*

Parasitic pathogen that causes a diarrhoeal illness called cryptosporidiosis



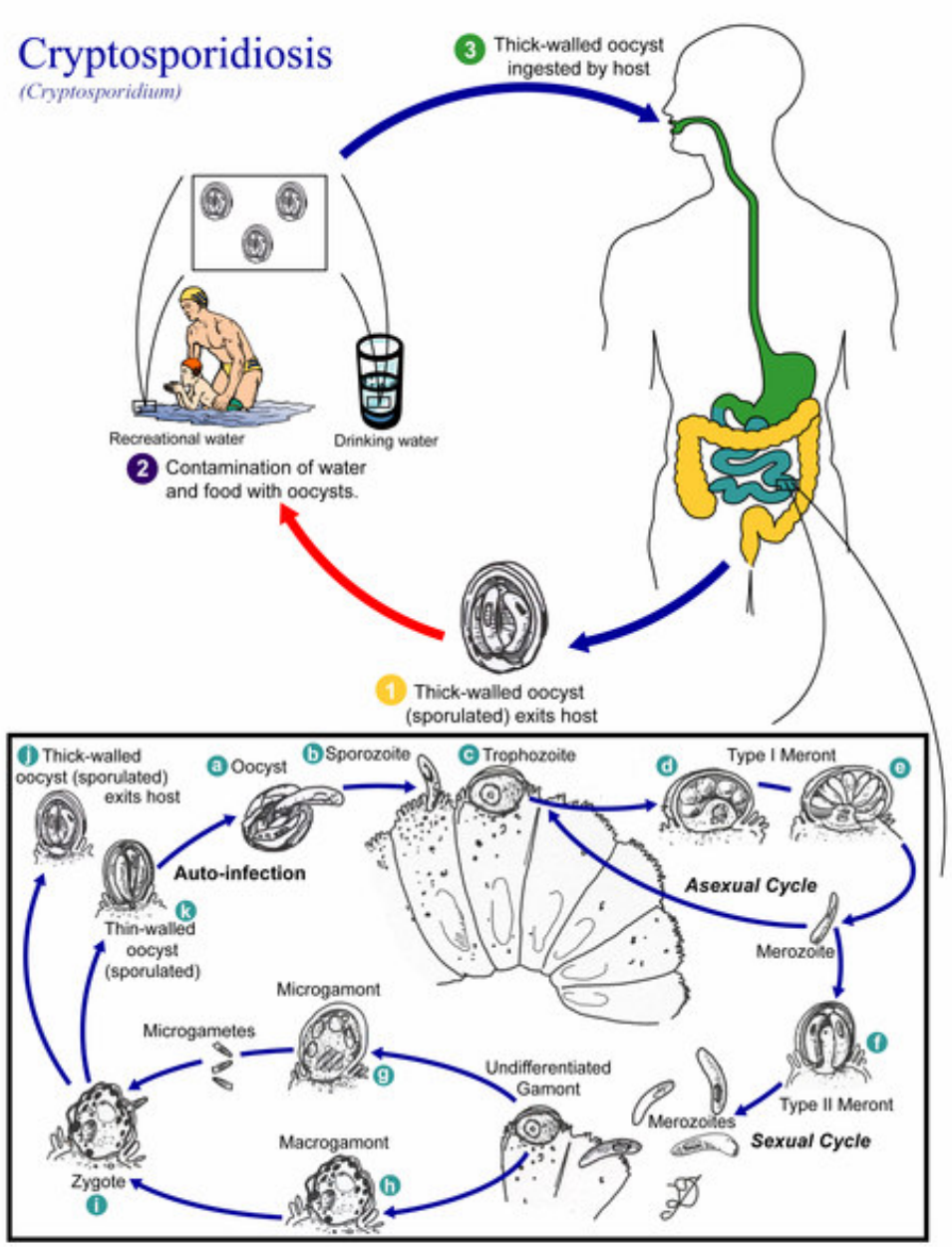
Cryptosporidium parvum in a fecal sample



Electron micrograph of *Cryptosporidium parvum*

Life cycle of Cryptosporidium

- Obligate intracellular protozoan parasites
- Infective stage: highly resistant oocysts
- Low infective dose



Cryptosporidium

- Identified as human pathogen in 1976
- 1993 Milwaukee outbreak
- Implications for
  - Immunocompromised individuals
  - Farm animals
- Consists of 13 recognised species
  - *C. parvum*, *C. hominis*, (human)
  - *C. felis* (cats), *C. canis* (dogs)

Cryptosporidium

- Most significant microbial pathogens to emerge
- Concerns because:
  - Cryptosporidium can be transmitted through water and food
  - Capable of causing a high degree of morbidity
  - No effective anti-parasitic treatment to eradicate from the GI tract in symptomatic individuals

Cryptosporidiosis

- *C. hominis* responsible for most outbreaks of human cryptosporidiosis in many regions
- Europe: *C. parvum* dominant
  - Intensive animal husbandry
  - *C. meleagridis* emerging as problem
  - Other species found have broad host range
- Humans major source of infections

Food borne Cryptosporidiosis

- In 1999, 10% cases via food in U.S.
- The rest contaminated water or person to person
- Raw fruits and veg, raw milk, meat and meat products, apple cider
- Hard to incriminate as *Cryptosporidium* hard to detect- underestimation of cases

Exposure assessment of *Cryptosporidium*

- Risk factors depend on
  - Quality of raw materials
  - Process steps and process environment
  - Product composition, packaging, storage conditions
  - Growth not important as Cryptosporidium cannot grow outside host, but viability reduction is important

Risk factors

- Water-raw sewage, treatment process, amount of water consumed
- Raw fruit and veg
  - Cultivation
  - Harvesting
  - Transport and storage
  - Industrial processing
  - Food preparation by consumer

Risk factors

- Meat products
  - Slaughterhouse
    - contact with contaminated water, contaminated environment, contamination of feed, cross contamination between animals, age influence of animals, influence of season
  - Storage of meat
  - Processing options

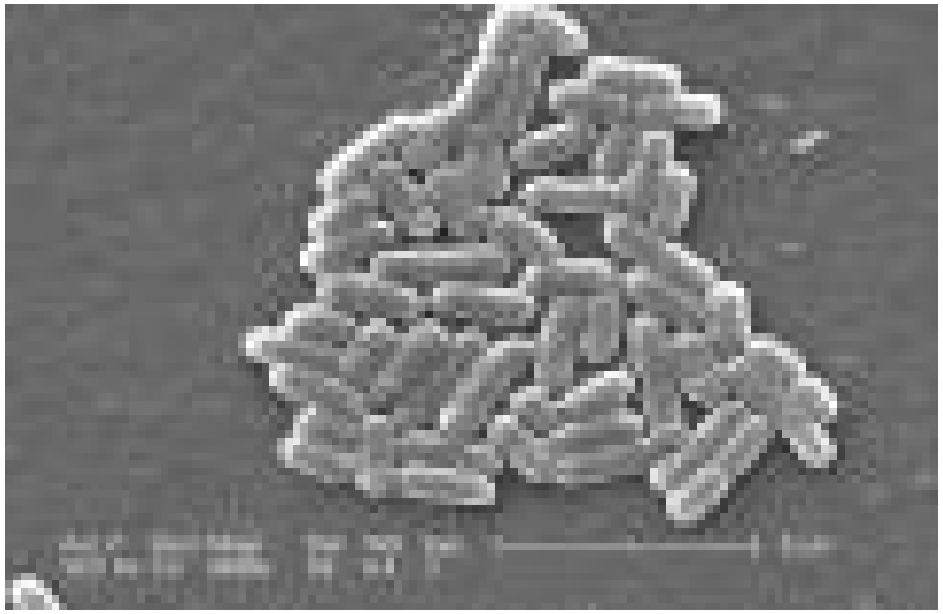
Cryptosporidium detection methods

- Direct examination of stool preparations
- Epifluorescence microscopy for food, water and environmental samples
- Monoclonal antibodies conjugated to FITC
- PCR

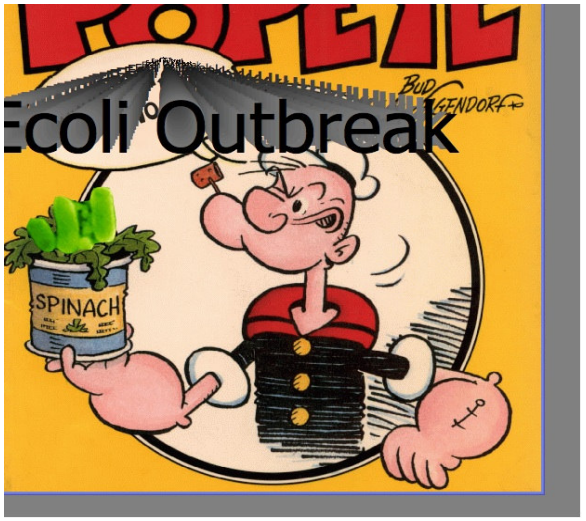
Cryptosporidium: Recommendations

- Difficult to prevent in food manufacturing
- Changes in animal husbandry and agricultural practices
- Broad host range of some strains
- Primary hazard in water supply, potential problem for fresh produce
- Not a significant problem in foods sufficiently heat treated
- More research into surveillance, epidemiology and detection methods needed
- Increased globalization of food chain increases risk
- Number of immunocompromised individuals increasing, making *Cryptosporidium* a greater hazard

Case study: *Escherichia coli* O157:H7 in cattle



Electron micrograph of *E. coli*



With reference to the recent outbreak of *E. coli* O157:H7in the U.S. associated with contaminated Spinach

E. coli O157:H7

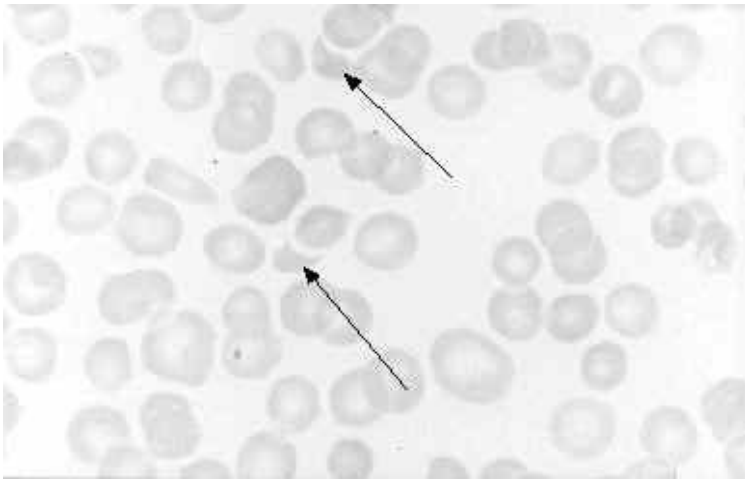
- Produce toxins:
  - Toxic to Vero cell cultures (vt1/vt2)
  - Similar to shiga toxins produced by *Shigella* (shiga-like toxin I or II)
- Verotoxin-producing *E. coli* (VTEC) or shiga-like toxin-producing *E. coli* (STEC)
- Survive in many environments
- Low infective dose
- Vulnerability of susceptible populations

E. coli O157:H7

- Since 1981 number of reported cases has increased
  - Improved surveillance programmes
  - Better detection methods
- Facultative anaerobic Gram-negative bacterium
- Found in GI tracts of mammals
- Somatic O157 antigen, flagellar H7 antigen

E. coli O157:H7, Complications

- Causes Haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) in humans
- Sometimes causes Trombotic trombocytopenic purpura (TTP)



A blood film from a patient with TTP showing relatively minor changes. Platelets are severely reduced and there are occasional red cell fragments (arrowed).

E. coli O157:H7

- 1982, recognised as a human pathogen
- Three hypothesis for emergence
  - Recently emerged but conditions for spread always present
  - Slaughter and meat processing practices modified promoting contamination
  - Existence in meat supply, but consumer practices changed
- Changes at several levels of food chain

Virulence factors

- Most important
  - Shiga toxins I and II (encoded by *stx*1 and *stx*2); cytotoxic
  - Intimin protein (encoded by *eae*)
- Others include
  - EAST1 toxin (encoded by *ast*A)
  - toxB protein
  - Associated attaching/effacing components (type III secretion system, Esp proteins, etc.)

Reservoirs/modes of transmission of *E. coli* O157: H7

- Isolated from many species
  - Sheep, goats, deer, pigs, cats, horses, gulls
- Cattle are major reservoir for *E. coli* O157:H7 and other VTEC
- Routes of transmission
  - Contaminated bovine products
  - Direct contact with infected animals
  - Consumption of contaminated foods
  - Person to person
  - Swimming/drinking contaminated water

E. coli O157:H7

- Seasonal variation in shedding patterns
  - Increases during summer and early fall
- Young animals have higher prevalence
- *E. coli* O157:H7 can be isolated from healthy and ill cattle faeces
- Widespread in beef and dairy herds, variable within animal and herd
- No gender significance
- Calves on pasture may be less exposed to bacteria
- Prevalence higher in grain fed cattle; but not sure of influence on shedding of organism

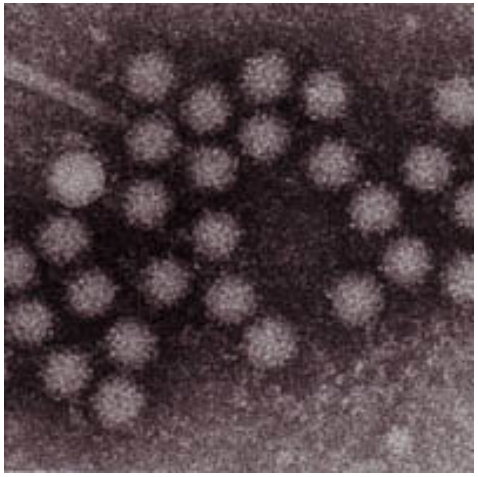
E. coli O157:H7

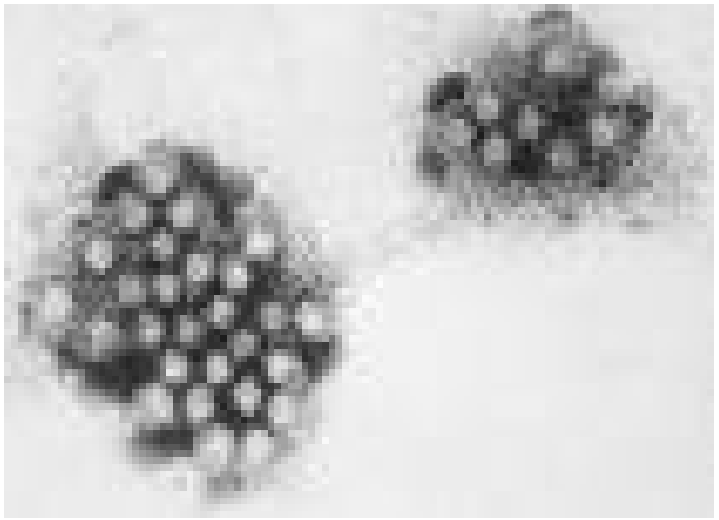
- Bedding material influences prevalence
- Faeces are vehicle for distribution of *E. coli* O157:H7
- Insects may play a role in hosts/vectors in dissemination
- Antimicrobial resistance occurs less in organic farming systems
- Example of “first world” emerging pathogen

E. coli O157:H7 Recommendations

- Not enough information to be sure *E. coli* O157:H7 emerged
- Further research needed to elucidate mechanisms
  - Influence of bedding material, diet and starvation, milk based diets, survival in manure, seasonal variations, role of farm types, occurrence of antibiotic resistance, role of flies or other animals
- From this information
  - Modifications to control *E. coli* O157:H7

Norovirus/Norwalk like viruses





Negative-stain Transmission Electron Microscopy

Noroviruses (NoV)

- Formerly known as Norwalk-like viruses or small round structured viruses
- Group of small nonenveloped RNA viruses that belong to the *Caliciviridae* family
- Lack of envelope-resistant to disinfectant
- Over 15 genotypes based on capsid gene

NoV

- Thought to be most common cause of viral gastroenteritis worldwide
- Short incubation period (12-48 h)
- Symptoms
  - Diarrhoea, vomiting, fever, nausea, chills, weakness, “gastric/stomach” ‘flu
- NoV outbreaks common in closed environments like day care centres, nursing homes, cruise ships

NoV detection

- In stool samples
  - Electron microscopy
    - need 10^5-10^6 samples/g
    - Specialised equipment
    - Highly skilled personnel
  - Enzyme immunoasays
    - Simple
    - Fast screening
  - Molecular biology
    - Detect viral genome (RT-PCR)
    - Very sensitive, allows for strain typing

NoV epidemiology

- Transmitted via faecal-oral route
- Minimal infective dose 1-10 particles
- Asymptomatic carriers shed virus
- Incidence
  - 190 per 1000 persons in UK (1999)
  - 283 per 1000 persons in The Netherlands (2001)

NoV outbreaks

- “winter vomiting disease”
- Due to transmission from
  - Person to person
  - Food
  - Water
  - Environmental
    - Carpets
    - Fomites
- Commonly combination of many routes

Food borne outbreaks of NoV

- Can be contaminated with NoV anywhere from farm to fork
- NoVs are highly stable-survive many food processes
- Sweden, March 1999
  - 30 day care centres
  - 1500 ill
  - 12 day outbreak
  - Asymptomatic or food handler source of infection

Food borne outbreaks of NoV

- The Netherlands, 2001
  - New Year's reception buffet
  - 231 people ill
  - Symptomatic baker source of infection
- Budapest, Hungary, 2001
  - 13 nurseries, 3 primary schools
  - 1036 ill
  - Source of infection undetermined

Food borne outbreaks of NoV

- South Hungary, 2003
  - 1109 ill
  - Cottage cheese culprit
  - One person found to be shedding virus

Risk foods for NoV

- Filter feeding shellfish
- Ice
- Raspberries
- Salad vegetables
- Poultry
- Red meat
- Fruit
- Soups
- Desserts
- Savoury snacks
- Sandwiches

Raw/untreated food is greatest risk

Prevention and control of NoV

- Good agricultural practice
- During food preparation:
  - Good manufacturing practice (GMP)
  - Hazard Analysis and Critical Control Point (HACCP)
- Virus tracking
  - Foodborne viruses in europe network (www.eufoodborneviruses.net)
  - Epidemiology of outbreaks in 13 EU countries

NoV

- NoV can recombine when two strains infecting one cell
- New strains evolve rapidly
- Explaining reason behind emergence of this RNA virus

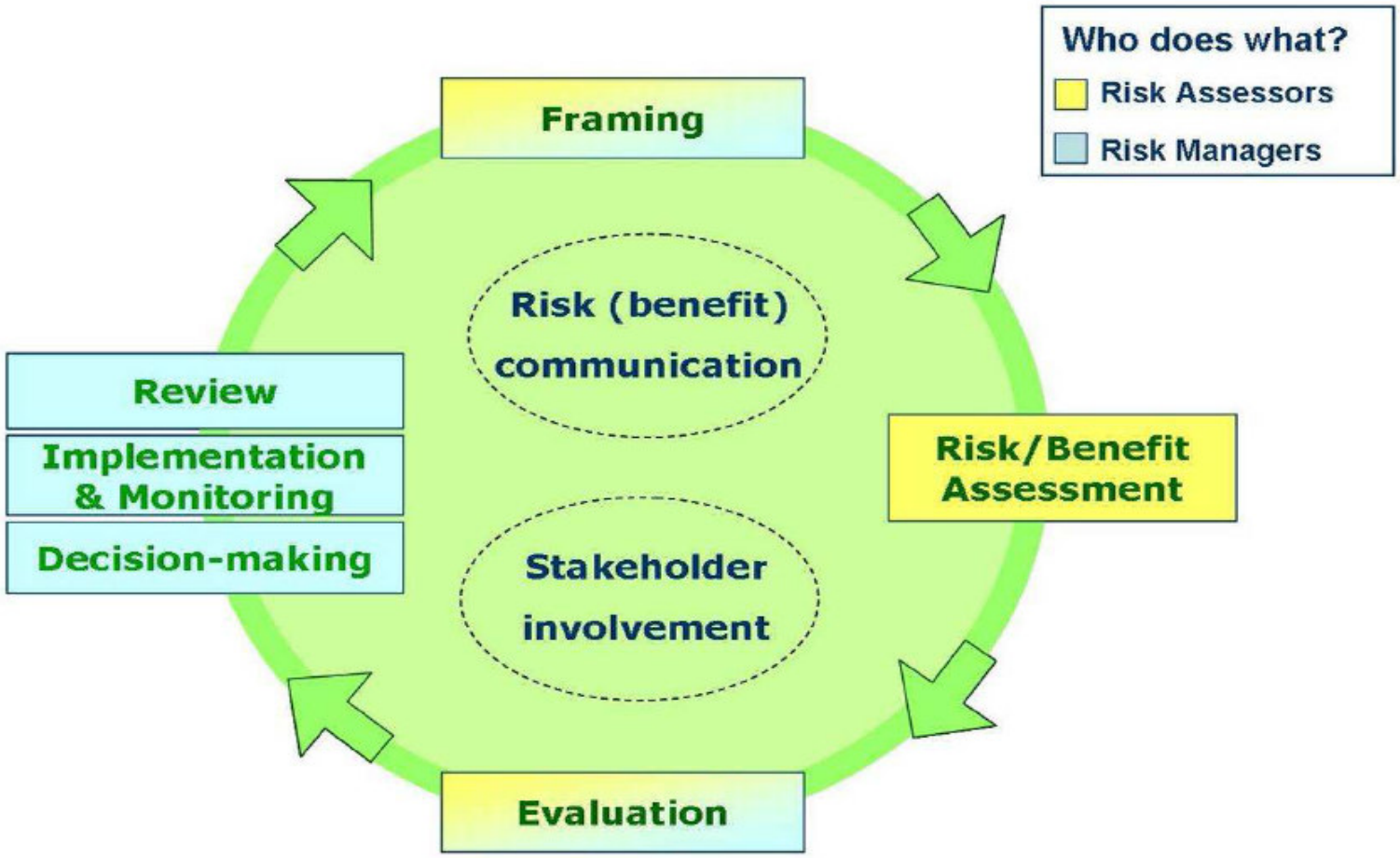
Summary

- Wide variety of factors involved in emergence
- Understanding of ecology of bacteria will help prediction
- To characterise emerging agents
  - Targeted surveillance systems
  - Outbreak investigations and research
  - Review pathogens that emerged in the past
  - Multidisciplinary teams

Summary

- Data used for
  - Risk analysis
  - Risk assessment
- Aid policy makers design most appropriate prevention strategies
- Safe foods overall aim
  - Assist in preparation of a new risk analysis approach for foods

Safe foods cycle: risk analysis framework



Based on the reports written for Safe Foods

To be published in Food and Chemical Toxicology

- **"Early Detection of Emerging Risks Associated with Food and Feed Production", Marvin, H.J.P., Kleter, G.A, Kelly, B. G., Ossendorp, B., Vespermann , A., Beczner, J. and Prandini, A.**
- **"Emerging microbial hazards in food and feeds and factors that influence their emergence", Byrne, C. M., Bolton, D. J., Howlett, B., Kelly, B. G., Orlova, O., Ossendorp, B. and Vespermann, A.**
- **"Case Study: *Cryptosporidium spp.*", *Orlova*, O., Kelly, B. G. and Santare, D.**
- **"Case Study: *Escherichia coli* O157:H7 in cattle", Vespermann, A., Howlett, B., Bräunig, J., Käsbohrer and Bolton, D. J.**
- **"Case Study: Norovirus", Koopmans, M., Duizer, E., Reuter, G. and Beczner, J.**


Thanks!
Any questions?