

# Emerging Food-Borne Bacterial Pathogens



**Prof. Rama Chaudhry**

**Department of Microbiology, All India Institute of Medical Sciences,  
Ansari Nagar, New Delhi, INDIA.**



- Food borne diseases result from ingestion of a wide variety of foods contaminated with pathogenic microorganisms, microbial toxins, or chemicals.

- Actual incidence unknown, estimated app./ year
  - 76 million cases,
  - 325,000 hospitalizations,
  - 5000 deaths.

(Mead et al, Food related illness and death in US, EID 1995;5:607-24)
- From 1993-97, 550 outbreaks, affected 17,000 persons in US.  
(MMWR 2000;49:1-51)
- In 2005 16,614 cases were reported, 205 outbreaks and 159 linked to restaurants.  
(MMWR, 2006,55:392)

# **What are emerging pathogens?**

- 1. Infectious diseases whose incidence has increased in the past 2 decades or threatens to increase in the near future**
- 2. New infections resulting from changes or evolution of existing organisms**
- 3. Known infections spreading to new geographic areas or populations**

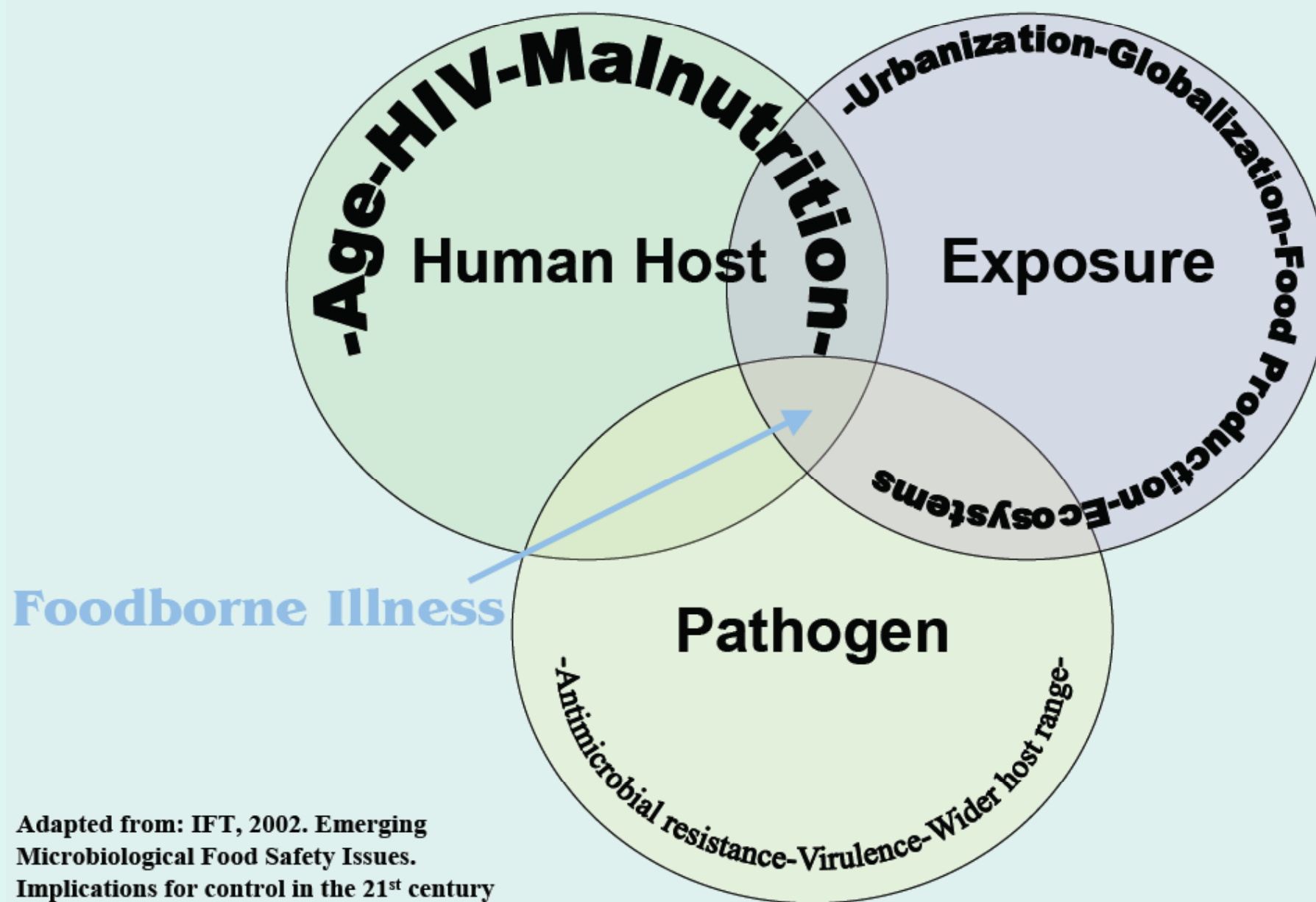
# **What are emerging pathogens?**

**4.Old infections reemerging as a result of their appearing in new vehicles**

**5.Previously unrecognized infections**

**6.Organisms on which to keep a watchful eye**

# Factors in the Emergence of Pathogens



Adapted from: IFT, 2002. Emerging  
Microbiological Food Safety Issues.  
Implications for control in the 21<sup>st</sup> century

# Factors in the Emergence of Pathogens

- Microbial adaptation and change
- Human susceptibility to infection
- Climate and weather
- Changing ecosystems
- Human demographics and behaviour
- Economic development and land use
- International travel and commerce
- Technology and industry
- Breakdown of public health measures
- Poverty and social inequality
- War and famine
- Lack of political will
- Intent to Harm

# Bacterial agents

- *B.cereus*
- *Botulism, food borne*
- *Brucella spp.*
- *Campylobacter spp*
- *C.perfringens*
- *E.coli O157:H7*
- *E.coli, non – O157 STEC*
- *E.coli, enterotoxigenic*
- *E.coli, other diarrheogenic*
- *Listeria monocytogens*
- *S.typhi*
- *Salmonella, nontyphoidal*
- *Shigella spp*
- *Staphylococcal food poisoning*
- *Streptococcus, foodborne*
- *V.cholerae, toxigenic*
- *V.vulnificus*
- *Other Vibrios*
- *Y.enterocolitica*
- *Y.pseudotuberculosis O3*

(Red- emerging pathogens)



- Parasitic agents

- *C.parvum*
- *C. cayatanensis*
- *G.lambliia*
- *T.gonodii*
- *T.spiralis*
- *E.histolytica*

- Metazoan parasites

- Round worm
- Tapeworm
- Flukes

- Viral agents

- *Norwalk viruses*
- *Rotavirus*
- *Astrovirus*
- *Hepatitis A virus*
- *Hepatitis E virus*
- *Influenza*

- Fungi

- *Aspergillus*
- *Fusarium*
- *Penicillium*

# Pathogenic Mechanism

Preformed  
Toxin

Toxin production invivo

Tissue  
invasion

Toxin /  
Tissue Invasion

*S.aureus*

*C.perfringens*

*C.jejuni*

*V.parahemolyticus*

*B.cereus* (SI)

*B.cereus*(LI)

*Salmonella*

*Y.enterocolitica*

*C.botulinum*

*C. botulinum*  
(infant botulism)

*Shigella*

*Enterotoxigenic E.coli*

*Invasive E.coli*

*V.cholerae* O1 /O139

*V.cholerae* non-O1

*STEC*

# Nontyphoidal Salmonellosis

- **Source:**

Eggs, Poultry, Undercooked meat, Unpasteurized dairy products, sea food.

- **Incubation period:** 6-48 hours

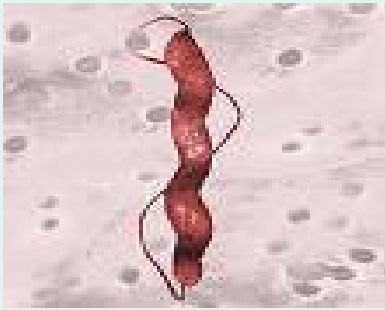
- **The serotypes are:**

Typhimurium	(19%)
Enteritidis	(18%)
Newport	(10%)
Heidelberg	(6%)



- **Antibiotic Resistance:**

*S. typhimurium* DT104- resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracyclines.



# *Campylobacter*

- **Incidence:**
  - 2.5 million cases per year (CDC estimate/NIAID)
- They are found in GIT of many animals used for food (poultry, cattle, sheep, and swine) and of many household pets (birds, dogs and cats).
- They do not cause illness in their animal hosts.
- **Source:** raw or undercooked food or direct contact with infected animals, unpasteurized milk.
- **Antibiotic resistance:** Resistance to ciprofloxacin, tetracycline, erythromycin have been reported.

# NEWER VEHICLE



- Outbreak of Salmonella Serotype Hartford Infections Associated With **Unpasteurized Orange Juice**.
- Acidic foods such as orange juice have been **thought to be unlikely vehicles** of food borne illness.
- All orange juice was recalled and the processing plant closed.
- **Pasteurization** or other equally effective risk-management strategies should be used in the production of all juices.

(Kim A. Cook et al, JAMA, November 4, 1998—Vol 280, No. 17 )

# Detection of Campylobacter and Salmonella in Turkey semen

- The contaminated pooled semen spread bacteria throughout entire flocks of hen **via artificial insemination**.
- Results demonstrate that **semen extenders tested did not eliminate** these bacteria from semen.
- Hence concluded that semen may be a **potential vehicle** for Salmonella and Campylobacter transfer to hens.

(A. M. Donoghue et al, Poultry Science 2004 ,83:1728–1733)

# An International Outbreak of Salmonellosis Associated with Raw Almonds



- During 2000-01, an outbreak due to *Salmonella enteritidis* (SE) phage type 30 (PT30), a rare strain, was detected in Canada.
- The case-control study was conducted to test the hypothesis of an association between raw whole almond consumption and infection.
- This was previously undocumented finding, leading to recall of this product and a review of current industry practices.

(S. Isaacs et al, Journal of food protection 2005, 68, 191-98)

# Insight of Resistance



- *S. typhimurium* phage type **DT104** is the most prevalent multiresistant / hypervirulent strain.
- Multiresistance in DT104 is conferred by an integron structure, **Salmonella genomic island 1 (SGI1)**.

(Steve A. Carlson et al , INFECTION AND IMMUNITY, Feb. 2007, p. 792–800)

- The detection of **DT104 and DT193** in **shellfish** emphasizes monitoring the presence of Salmonella in routine surveillance of live bivalve molluscs.
- Two DT104 isolates and a PT 193 were resistant to multiple antibiotics.

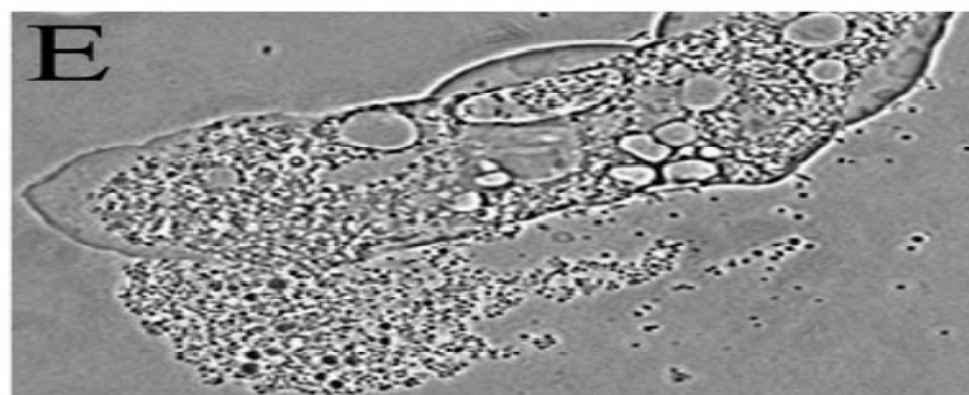
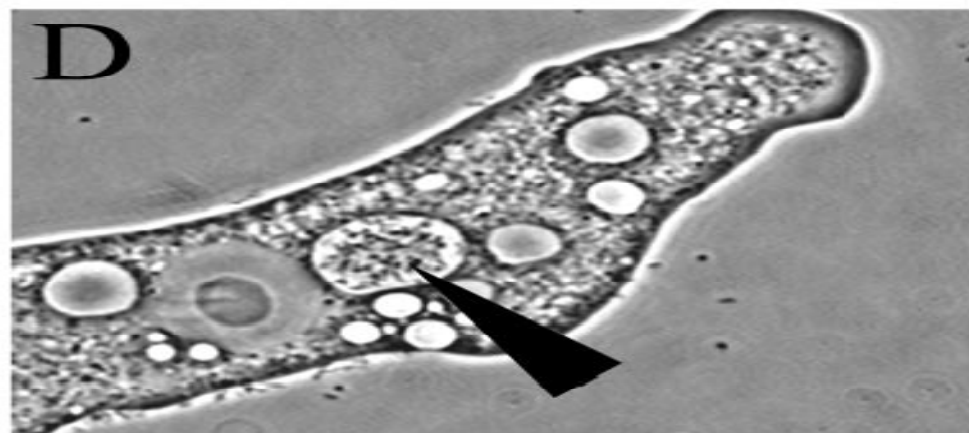
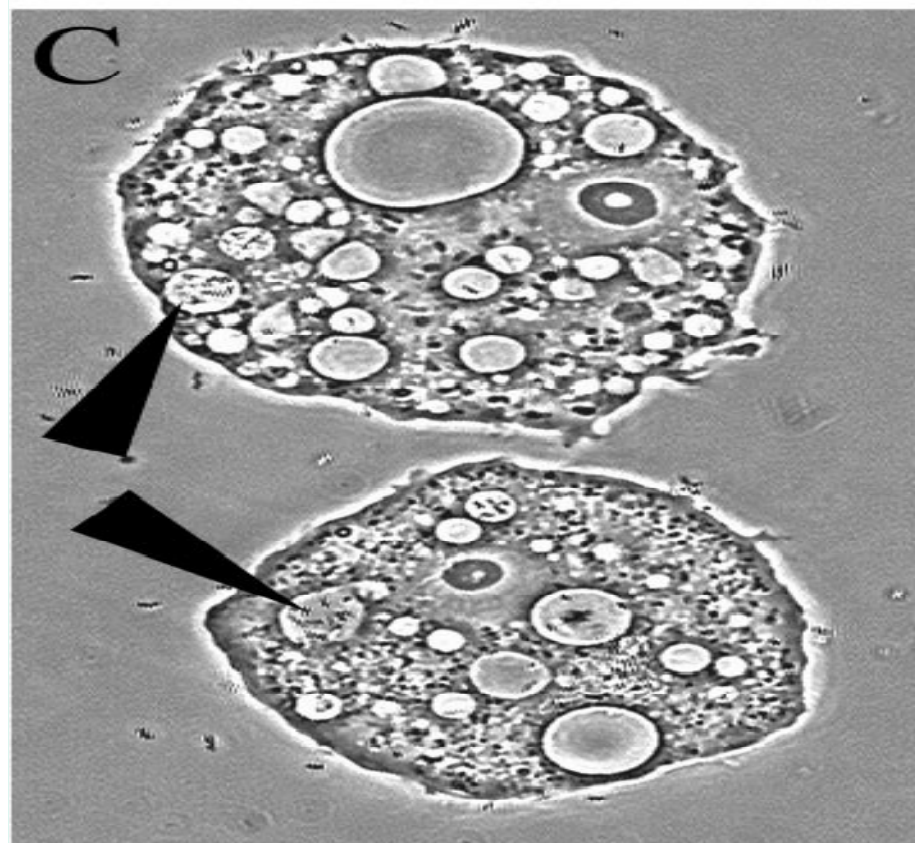
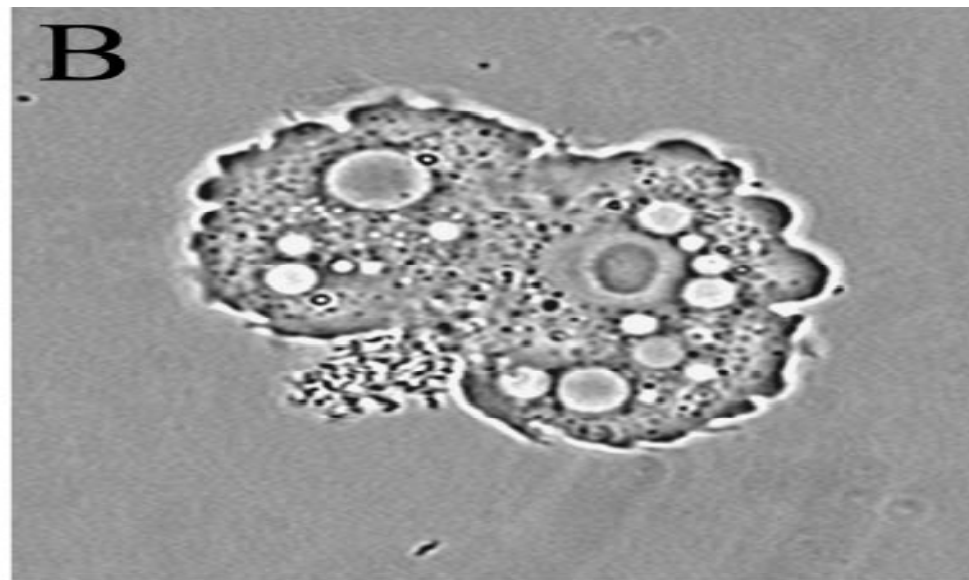
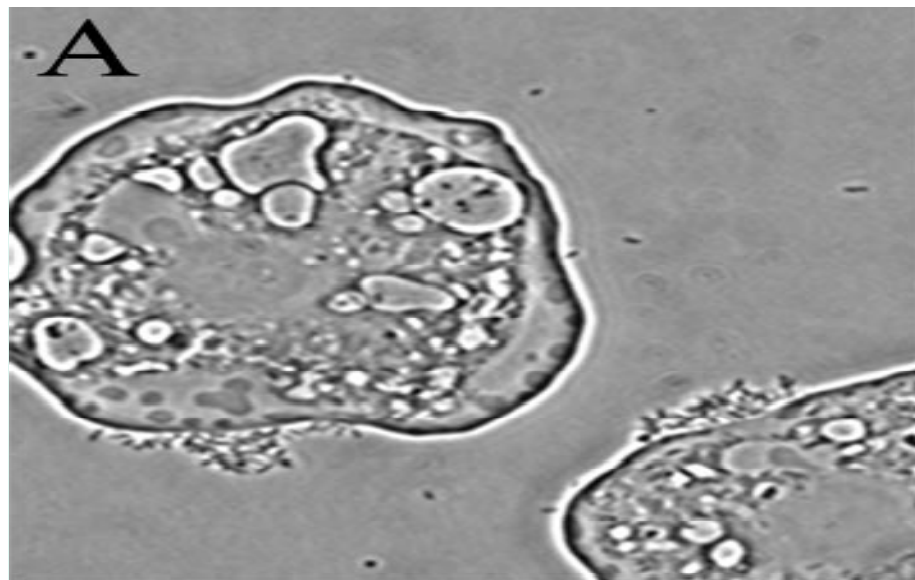
(Jaime Martinez-Urtaza et al, APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 2004, p. 4030–4034)



# Protozoan *Acanthamoeba polyphaga* as Potential Reservoir for *Campylobacter jejuni*

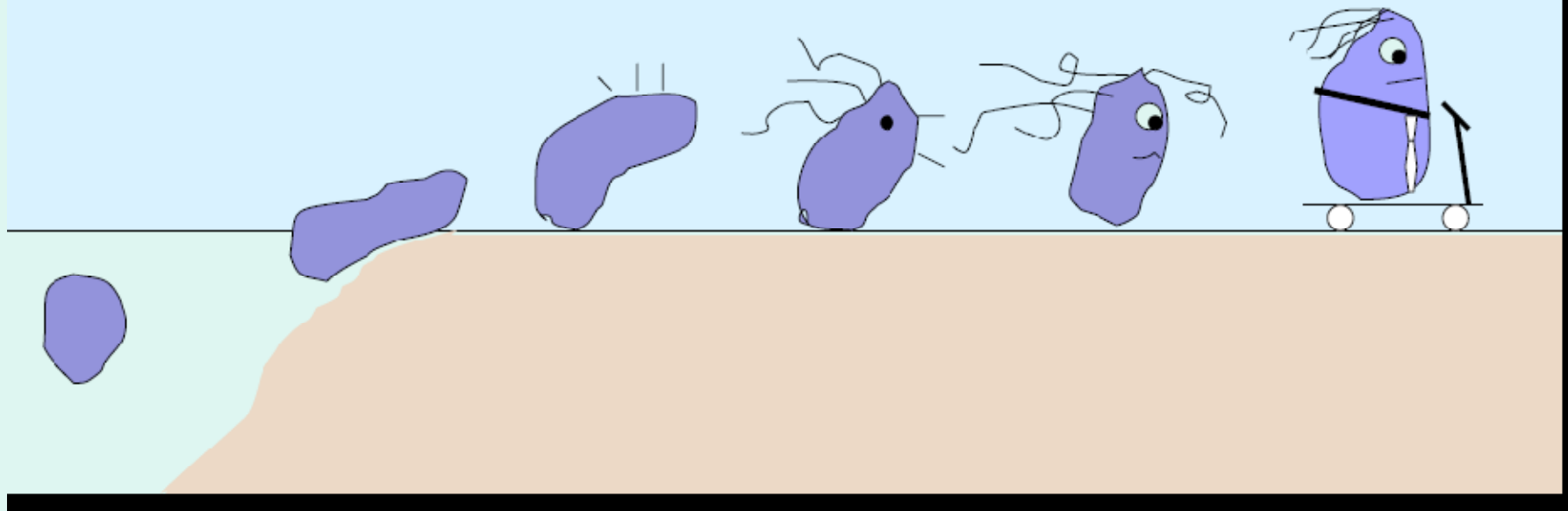
- The four different *Campylobacter jejuni* strains infect the protozoan *Acanthamoeba polyphaga*.
- *C. jejuni* cells survived for longer periods when cocultured with amoebae.
- A resuscitation of bacterial cultures was observed after reinoculation into fresh amoeba cultures.
- Amoebae may serve as a nonvertebrate reservoir for *C.jejuni* in the environment.

(Diana Axelsson-Olsson et al, APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 2005, p. 987–992 )



# Emerging Pathogens

2. New infections resulting from changes or evolution of existing organisms



# *Escherichia coli*

- Shiga-toxin producing *E.coli* (STEC)
- **Source:** undercooked ground beef, manure from cattle or other animals can contaminate produce (potatoes, lettuce, sprouts, fallen apples).
- **Incubation period** : 3-4 days.
- hemorrhagic colitis and the hemolytic uremic syndrome (HUS)
- **O 157:H7** is the most prominent serotype.
- Other pathotypes of *E.coli* are acquired predominantly through fecal-oral route.

# Isolation of shiga toxin producing *E.coli* from dairy cattle and beef , Calcutta

- The study provided the first evidence that dairy cattle in Calcutta is the potential reservoir for virulent O157:H7 strain.
- Also identified that beef marketed in Calcutta , as a source for virulent O157:H7 strain.
- The isolation of eaeA and hyl A positive O157:H7 strains from meat reflected it as pathogenic strain.
- No STEC was isolated from diarrhoea cases.
- The low prevalence of STEC associated diarrhoea in India may be due to cooking practices and small proportion of population that consume beef.

(Shanta dutta et al, J. Med. Micro 2004, 29 765-767)

# Non- O 157 *E.coli* Outbreaks

- **O111**- 1994-2000 - USA, Canada  
- Ground beef, unpasteurized apple, salad.
- **O129**- 1999-2006- USA, Canada  
- lettuce, lake water.
- Other emerging are **O26**, **O103**, **O45**.



# Use of **Copper Cast** Alloys To Control *E.coli* O157 Cross-Contamination during Food Processing



- The results demonstrated the antimicrobial properties of cast copper alloys with regard to *E. coli* O157, and consequently these alloys have the potential to aid in food safety.
- With beef juice, only one alloy (95% Cu) completely killed the inoculum at 22°C.
- For stainless steel, no significant reduction in cell numbers occurred.

(J. O. Noyce et al, APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 2006, p. 4239–44)





# *Clostridium perfringens*

- **Type A** is the most common cause of food poisoning.
- The cytotoxin is produced by >75% of strains isolated from cases of food borne disease.
- **Source**: meat, meat products and poultry.
- **Outbreaks**: due to problem in cooling and storage of food cooked in bulk.
- During reheating , they sporulate and germinate.
- **Incubation period**: 8- 24 hours.



# *Clostridium botulinum*



- **Food borne botulism**: from ingestion of preformed toxin in food contaminated with *C.botulinum*.
- **Intestinal botulism**: from ingestion of spores and production of toxin in intestine of infants (infant botulism) or adults.
- Toxin A,B,E and rarely F cause human disease.
- **Source**: home canned food- vegetables, fruit, condiments, meat and fish.
- **Incubation period**: 18-36 hours.

# Botulism Associated With Commercially Canned Chili Sauce-Texas and Indiana, July 2007

- On July 7 and 11, 2007, public health officials in Texas and Indiana, reported to CDC four suspected cases of food borne botulism, two in each state.
- Investigations conducted revealed that all four patients had eaten brands of Castleberry's hot dog chili sauce before illness began.
- **Toxin type A** was detected in the serum of one Indiana patient and in a leftover chili mixture obtained from his home.
- The manufacturer, recalled the implicated brand.

(*JAMA*. 2007;298:1154-1156)

# *Clostridium difficile* in Retail Ground Meat, Canada

- **Abstract**

*Clostridium difficile* was isolated from 12 (20%) of 60 retail ground meat samples purchased over a 10-month period in 2005 in Canada. Eleven isolates were toxigenic, and 8 (67%) were classified as toxinotype III. The human health implications of this finding are unclear, but with the virulence of toxinotype III strains further studies are required.

(Alexander Rodriguez-Palacios et al, EID Volume 13, Number 3–March 2007 )

# **Methicillin (Oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans**

- From May 2001 to April 2003, various types of specimens from **cattle**, **pigs**, and **chickens** were collected and examined for the presence of MRSA.
- The data suggested that the genomes of the six animal MRSA isolates were very closely related to those of some human MRSA isolates and were a **possible source of human infections** caused by **consuming contaminated food products** made from these animals.

( Lee JH et al, Appl Environ Microbiol 2003 Nov;69(11):6489-94)

# An Outbreak of *Yersinia pseudotuberculosis* O3 Infections Associated with Consumption of Iceberg Lettuce in Finland

- The source for *Y. pseudotuberculosis* (YP) infections has remained unknown in previous outbreaks.
- In [October 1998](#), reported YP, serotype O3 (YP-O3) infections increased markedly.
- Case-patients were more likely than controls to report having [eaten iceberg lettuce in the 2 weeks before illness onset](#) (71% vs. 42%; matched OR, 3.9; 95% CI, 1.3-9.6).
- No other fresh produce, or meat product was associated with illness.

(NUORTI JP et al, *Antimicrob Agents Chemother.* 1999 Sep 26-29; 39: 693 )

# *Vibrio parahaemolyticus*



- Infections with this organism have been associated with the consumption of raw, improperly cooked, or cooked, recontaminated fish and shellfish.
- Outbreak of *V. parahaemolyticus* Infections Associated with Eating Raw Oysters -- Pacific Northwest, 1997.
- Outbreak of *V. parahaemolyticus* Infection Associated with Eating Raw Oysters and Clams Harvested from Long Island Sound -- Connecticut, New Jersey, and New York, 1998.

(MMWR :47,1997)

( MMWR: 48,1998)

- This highlights the need for enhanced surveillance for human infections.
- PCR approach used to monitor the occurrence and distribution of this newly emerged pathogenic *V. parahaemolyticus* O3:K6 strain in coastal, marine, and ship ballast waters.

(Michael L. Myers et al, APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 2003, p. 2194–2200)

# Viable but non cultivable Vibrios

- Human volunteer studies suggested that VBNC *V. cholerae* maintains pathogenic potential in the environment with human passage effectively triggering an outgrowth of the cells to the viable and cultivable state

Ecological study of *Vibrio cholerae* in Vellore Epidemiol. Infect. (2000), 124, 201±206.

- In such scenario we need to have Molecular tools which can explore this state of organisms



# Food Net

- The **Food borne Diseases Active Surveillance Network** (FoodNet) of CDC's Emerging Infections Program collects data from **10 U.S. states** regarding diseases caused by enteric pathogens transmitted commonly through food
- Food-Net quantifies and monitors the incidence of these infections by conducting **active, population-based surveillance for laboratory-confirmed illness**

## In 1996

- Active surveillance began for laboratory-confirmed cases of  
*Campylobacter*  
*Escherichia coli* O157  
*Listeria monocytogenes*  
*Salmonella*  
*Shigella*  
*Vibrio*  
*Yersinia enterocolitica* infections

## In 1997

- Laboratory-confirmed cases of *Cryptosporidium* spp. and *Cyclospora cayetanensis* infections was added

## In 2000,

- Data on *STEC* -O157 and comprehensive information on hemolytic uremic syndrome (HUS) was added.

## The data indicate

- The relative frequency of diagnosed infections.
- Demonstrate substantial regional variation.
- Suggest trends in incidence.

## FoodNet provides data for

- monitoring food borne illnesses.
- Interventions designed to reduce them.

## In 5 years(1996-2000) of FoodNet data collection,

- *Campylobacter* was the most frequently diagnosed pathogen.
- Followed by *Salmonella*, *Shigella*, and *E. coli* O157.
- However, substantial regional and year to year variation occurred.

Organisms	1996	2000	2005	2010 (GOAL)
<i>Campylobacter</i>	23.5	22.4	12.72	12.3
<i>Salmonella</i>	14.5	13.45	14.55↑	6.8
<i>Shigella</i>	8.9	9.95	4.61	
<i>E.Coli</i> O:157	2.7	2.55	1.06	1.0
<i>Yersinia</i>	1.0	0.55	0.36	
<i>Listeria</i>	0.5	0.3	0.3	0.25
<i>Vibrio</i>	0.1	0.45	0.27	

MMWR

# Comparing incidence of two Salmonella

Organisms	1996	2000
<i>Salmonella typhi</i>	3.9%	2.7%
<i>Salmonella enteritidis</i>	2.5%	1.8%

# Noroviruses

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 11, No. 5, May 2005

## Are Noroviruses Emerging?

Marc-Alain Widdowson,\* Stephan S. Monroe,\* and Roger I. Glass\*

[:: Home](#) > [Archives : Eurosurveillance weekly releases 2006](#) > [Volume 11 / Issue 9](#)

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Surveillance Report

volume	11
issue	9
date	7 September 2006

**Four outbreaks of norovirus gastroenteritis after consuming raspberries, Sweden, June-August 2006**

# Rotaviruses

- Acute gastroenteritis in children/infants
- Faeco-oral transmission, highly infective
- Global burden estimate: 138 million infections, over 600,000 deaths each year
- Severe disease preventable by live attenuated oral vaccines
- Vaccination programs being evaluated in Europe
- Found in raw retail meats

# Emerging Parasites

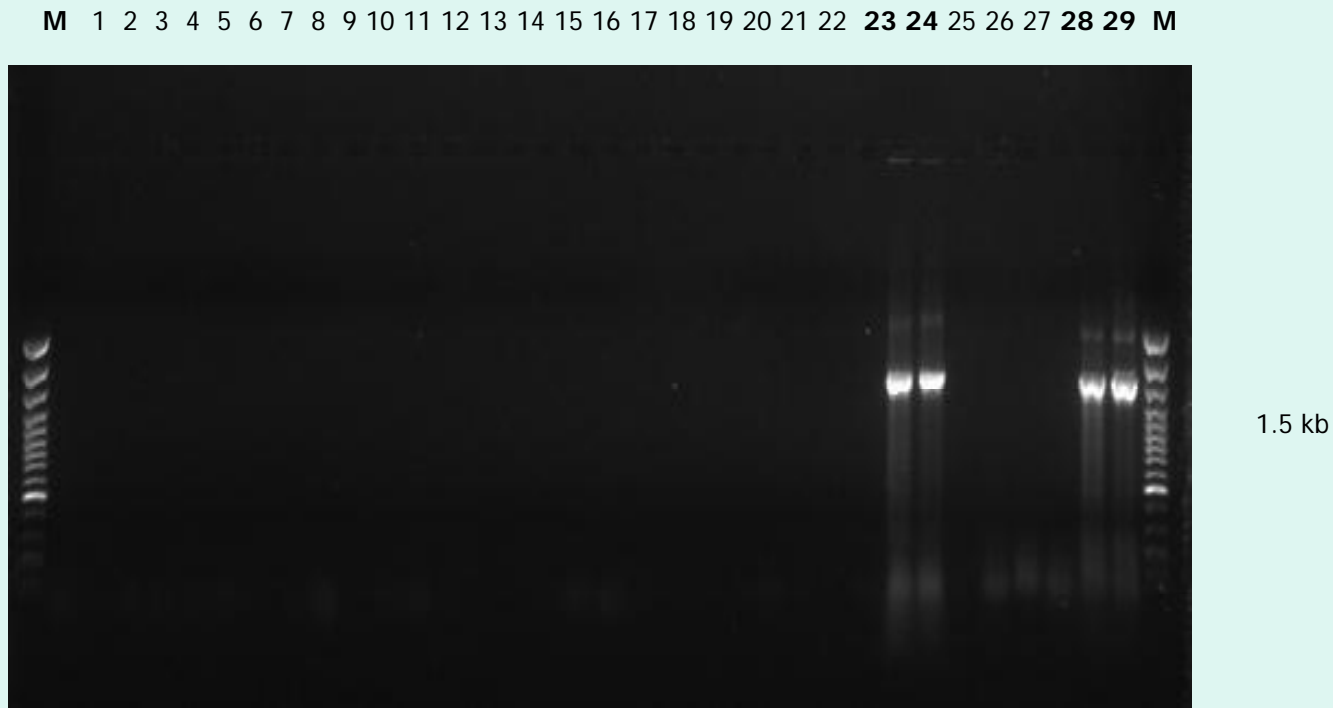
- *Cryptosporidium* spp. 2.95/100000 population reported in 2005.
  - Large outbreaks associated with visit to recreational water park in New York, 2004-05.
- *Cyclospora*
  - Atlanta GA, May 2000, 21 cases
    - raspberries (Guatemala)
  - Florida, Mar-Apr 2005, 293 cases
    - Basil (Peru)
  - Vancouver BC, Jun-Jul 2006, 14 cases
    - basil / garlic?



## Molecular methods

PCR for the early & correct detection of *Salmonella* serotypes in food/feces & blood

### Salmonella – PCR [ Genus specific ]



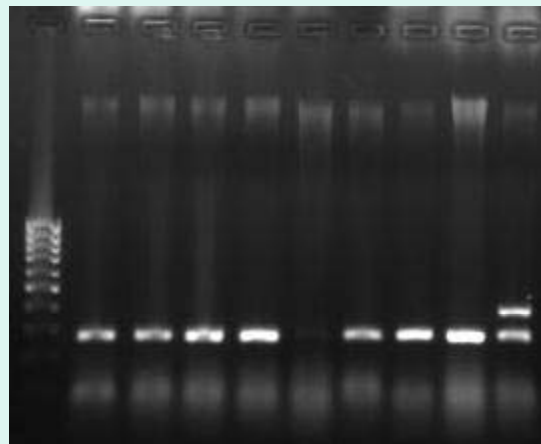
(R.Chaudhry et al, Medical Journal of Indonesia, suppl-1998)

# *Clostridium perfringens*

- Identification of *C. perfringens* using phospholipase C gene and enterotoxin gene by PCR

## Advantages:

- Invitro sporulation is difficult - enterotoxin detection Molecular level identification better



M - marker (100 Bprs)

Lane 1 to 8- Non enterotoxigenic *C. perfringens*

Lane 9 - Enterotoxigenic *C. perfringens*

# *Clostridium botulinum* neurotoxin gene

Lane M : Marker (100-1000 bp)

Lane 1: *C. botulinum* type A (782 bp)

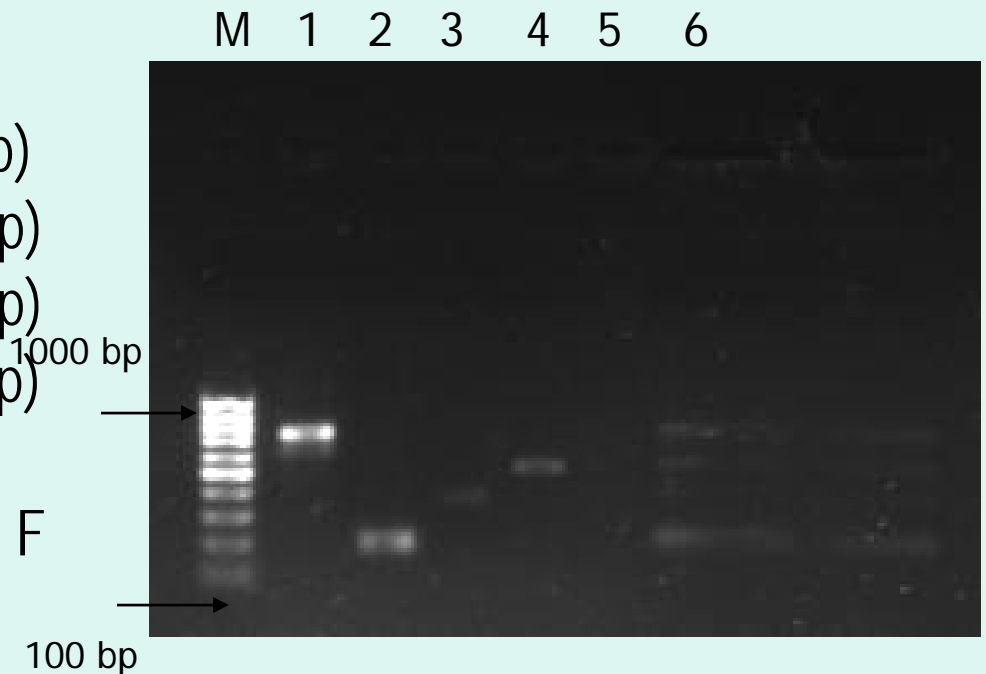
Lane 2 : *C. botulinum* type B (205 bp)

Lane 3 : *C. botulinum* type E (389 bp)

Lane 4 : *C. botulinum* type F (543 bp)

Lane 5 : Negative control

Lane 6 : *C. botulinum* types A,B,E & F



- Such active surveillance network needs to be established in each country to have true global burden of food borne pathogens so as appropriate interventions can be implemented at an early phase
- The formulation of internationally acceptable standards for food premises and of public health infrastructure would be of great value.

Evolving and adapting foodborne pathogens  
in a backdrop of environmental and behavioural  
changes provide new ecological niches into  
which evolving microbes can easily fit and  
prosper



leading to emerging and reemerging  
food borne pathogens



# THANK YOU

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