Pathogens in the Environment

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Indiana Water Resources Research Center

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Outline for Today

- Pathogens
- The intestinal zone
- Coliforms
- Indicators
- Fecal-oral transfer route
- What is fecal material
- Sources of fecal material in water

What are Pathogens?

- **Pathogens:** are biological agents that cause a disease (i.e., illness) in a host.
- **Environmental Pathogens:** Microorganisms that normally spend a substantial part of their lifecycle outside human hosts, but when introduced to humans cause disease with measurable frequency. . . (ASM)

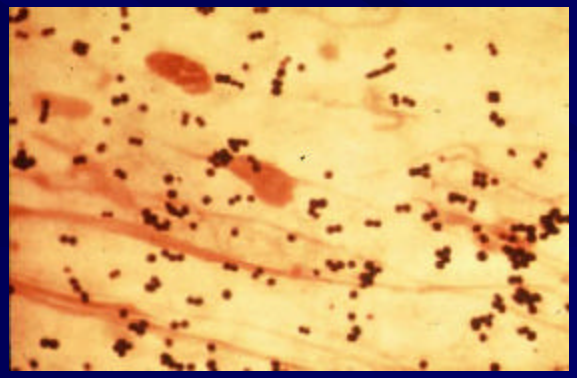
Pathogen is derived from the Greek "birth of pain"

Environmental Pathogens

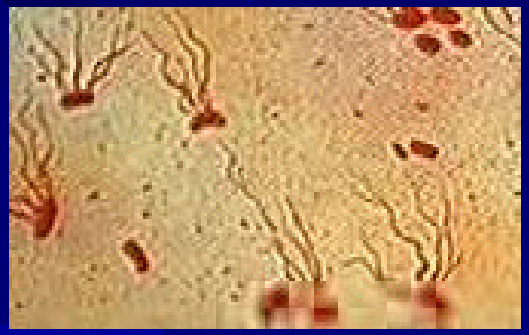
- Include:
  - **PATHOGENIC BACTERIA**
  - **INTEROVIRULENT *E. COLI* GROUP**
  - **PARASITIC PROTOZOA and WORMS**
  - **VIRUSES**
  - **PRIONS**

A Few Pathogenic Bacteria

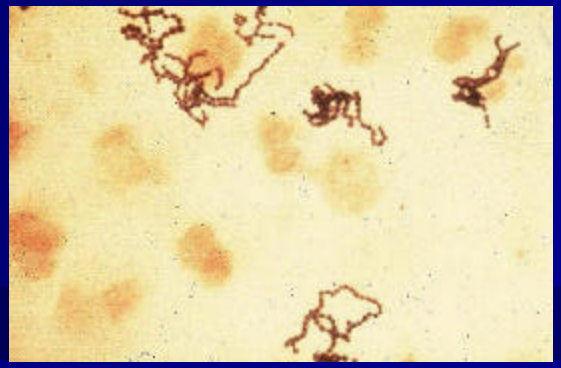
- *Salmonella spp.*
- *Clostridium botulinum*
- *Staphylococcus aureus*
- *Campylobacter jejuni*
- *Yersinia enterocolitica & Yersinia pseudotuberculosis*
- *Listeria monocytogenes*
- *Vibrio cholerae O1*
- *Vibrio cholerae non-O1*
- *Streptococcus*
- *Shigella spp.*
- *Vibrio parahaemolyticus and other vibrios*
- *Vibrio vulnificus*
- *Clostridium perfringens*
- *Bacillus cereus*
- *Aeromonas hydrophila and other spp.*
- *Plesiomonas shigelloides*
- *Miscellaneous enterics*
  - *Klebsiella, Enterobacter, Proteus, Citrobacter, Aerobacter, Providencia, Serratia*



Staphylococcus aureus



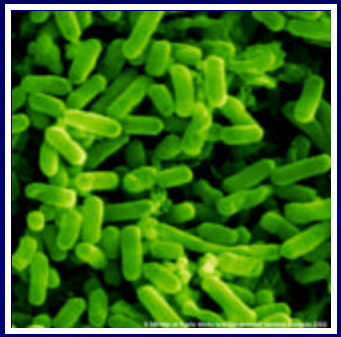
Salmonella typhi



Streptococcus pyogenes

http://www.geocities.com/capecanaveral/3504/gallery.htm

Pathogenic *E. coli*



- *Escherichia coli* - enterotoxigenic (ETEC)
- *Escherichia coli* - enteropathogenic (EPEC)
- *E. coli* O157:H7 enterohemorrhagic (EHEC)
- *Escherichia coli* - enteroinvasive (EIEC)

Capacity of Enteric Pathogens to Infect in Humans and Animals

Organism	Human	Cattle	Pigs	Poultry
Salmonella spp	1	0-13	0-38	10-100
E. coli O157:H7	1	16	0.4	1.3
Campylobacter jejuni	1	1	2	100
Yersinia enterocolitica	0.002	< 1	18	0
Giardia lamblia	1-5	10-100	1-20	0
Cryptosporidium spp	1	1-100	0-10	0

Human and Animal Pathogens in Manure, Olson, M. E.

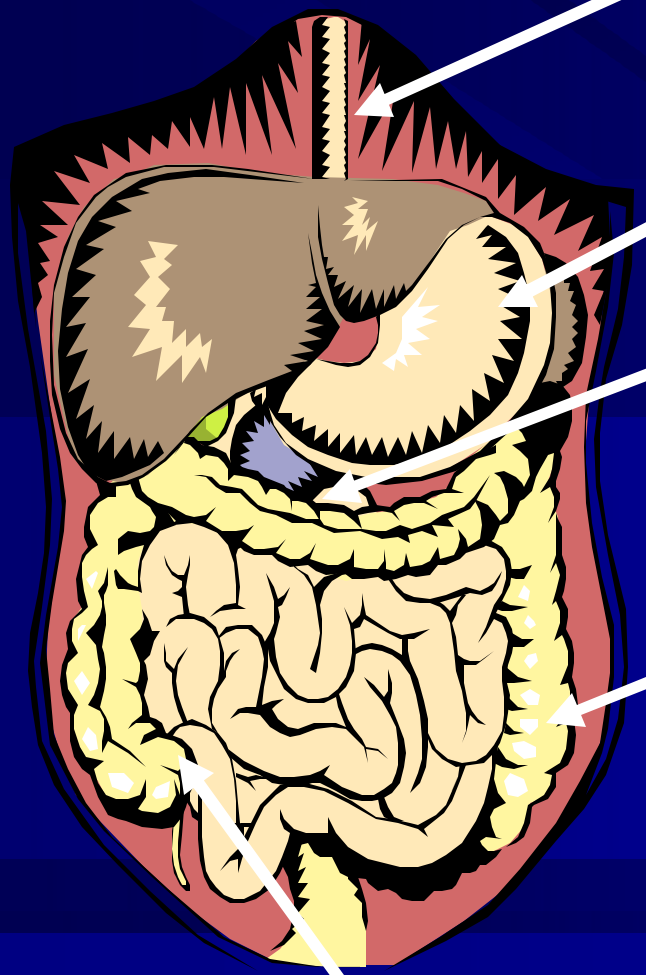
Numbers are given as percent of pathogen in the environment (feces, water and soil) that have the capacity to infect humans and animals.

The Intestinal Zone

Supplying the World Billions of Microorganisms Each and Everyday for 1000’s of years..

What is in Fecal Material?

- Feces are 75% water and 25% solid matter
- The solid matter is composed of:
  - Dead bacteria
  - Live bacteria
  - Undigested food (fiber)
  - Waste material from blood
  - Cellular linings
  - Fats
  - Salts
  - Substances released from the intestines and liver (mucus)



Few Bacteria

Few Bacteria (*Helicobacter pylori)*

Gram (+) 10^5 to 10^7 cell mL^{-1}

Lactobacilli & *Enterococcus faecalis.*

Gram (+) & (-) 10^{11} cell mL^{-1}

Anaerobic *Bacteroides*, *Bifidobacterium*
Many others

Gram (+) & (-) 10^8 cell mL^{-1}

Coliforms, *Bacteroides,* Lactobacilli, Enterococci

Normal Bacteria in Large Intestine/Feces

Bacteroides fragilis, Bacteroides melaninogenicus, Bacteroides oralis, Lactobacillus, Clostridium perfringens, Clostridium septicum, Clostridium tetani, Bifidobacterium bifidum, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Salmonella enteritidis, Salmonella typhi, Klebsiella sp., Enterobacter sp., Proteus mirabilis, Pseudomonas aeruginosa, Peptostreptococcus sp., Peptococcus sp., Methanogens

The Masses

- Approximately 40% to 55% (by weight) of a human's fecal material is comprised of bacteria (live).
- Assuming bacteria have an average weight of 1 x 10^{-12} g (one trillionth of a gram) per bacteria (dry wt) this would translate to 4 x 10^{11} (400 billion) bacteria per 1 g of feces.
- If 0.1% are *E. coli*, then (400 million) per gram of feces are *E. coli*

Microbial Flora in Fecal Material (#/gram)

Animal	Coliforms	Strep	Clostridium	Bacteroides	Lactobacilli
Human	13,000,000	3,000,000	1,580	5,000,000,000	630,000,000
Cow	230,000	1,300,000	200	< 1	250
Sheep	16,000,000	38,000,000	199,000	< 1	79,000
Pig	3,300,000	84,000,000	3,980	500,000	251,000,000
Chicken	1,300,000	3,400,000	250	< 1	316,000,000
Dog	23,000,000	980,000,000	251,000,000	500,000,000	39,600
Cat	7,900,000	27,000,000	25,100,000	795,000,000	630,000,000

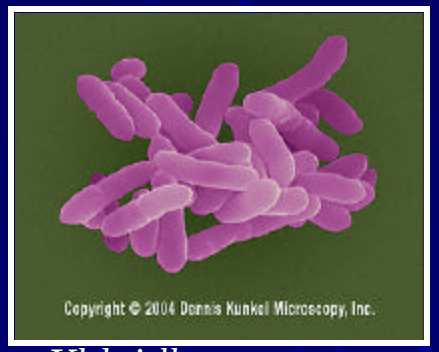
Human and Animal Pathogens in Manure, Olson, M. E.

Indicators Organisms in Mammalian Fecal Material

Indicator Organism	Feces Occurrence %	Numbers per g feces
E. Coli	100	10^7-10^9
Enterococci	100	10^5-10^6
Clostridium perfringes	13-35	10^6-10^7
F-specific coliphage	6	10^1-10^2

Köster et. al., Pathogens in (Drinking) Water

Coliforms



Klebsiella pneumonae

- Large bacterial group found in the environment and the feces of human and warm blooded animals. They are aerobic and facultative anaerobic, gram negative rod shape bacteria.

- Are comprised by two groups (procedural split):
  - Total Coliforms – they are commonly found in feces and also occur naturally in unpolluted soils and waters. This group includes the fecal coliforms and other species of the genera *Citrobacter*, *Enterobacter*, *Escherichia* and *Klebsiella*.
  - Fecal coliforms - are exclusively of fecal origin to be composed mainly of *E. coli.*

Representation of the Masses

- Fecal Coliforms:
  - Aerobic bacteria found in the colon and or in feces, used as indicators of fecal contamination.
- History:
  - US Department of Treasury (1914) promulgated drinking water bacteriological standard, 2 coliforms per 100 mL (*Interstate Waters only*)
    - Lowered to 1 coliforms per 100 mL (1925)
  - 1942, the US Public Health Service takes over
  - 1957, membrane filter process for analysis
  - 1974, Safe Drinking Water Act

What are Indicators?

E. coli

- Traditionally indicators are use to predict the presence of pathogens
- There is no direct (100%) tie between indicator number and the occurrence of enteric pathogens

Types of Indicators

- General Microbial indicators – indicates the efficacy of a process (ex. Chlorine disinfection)
- Index and model organisms- indicates the occurrence and behavior of pathogens
- **Indicators of Fecal -** indicates the presence of fecal contamination. They INFER the existence of pathogens

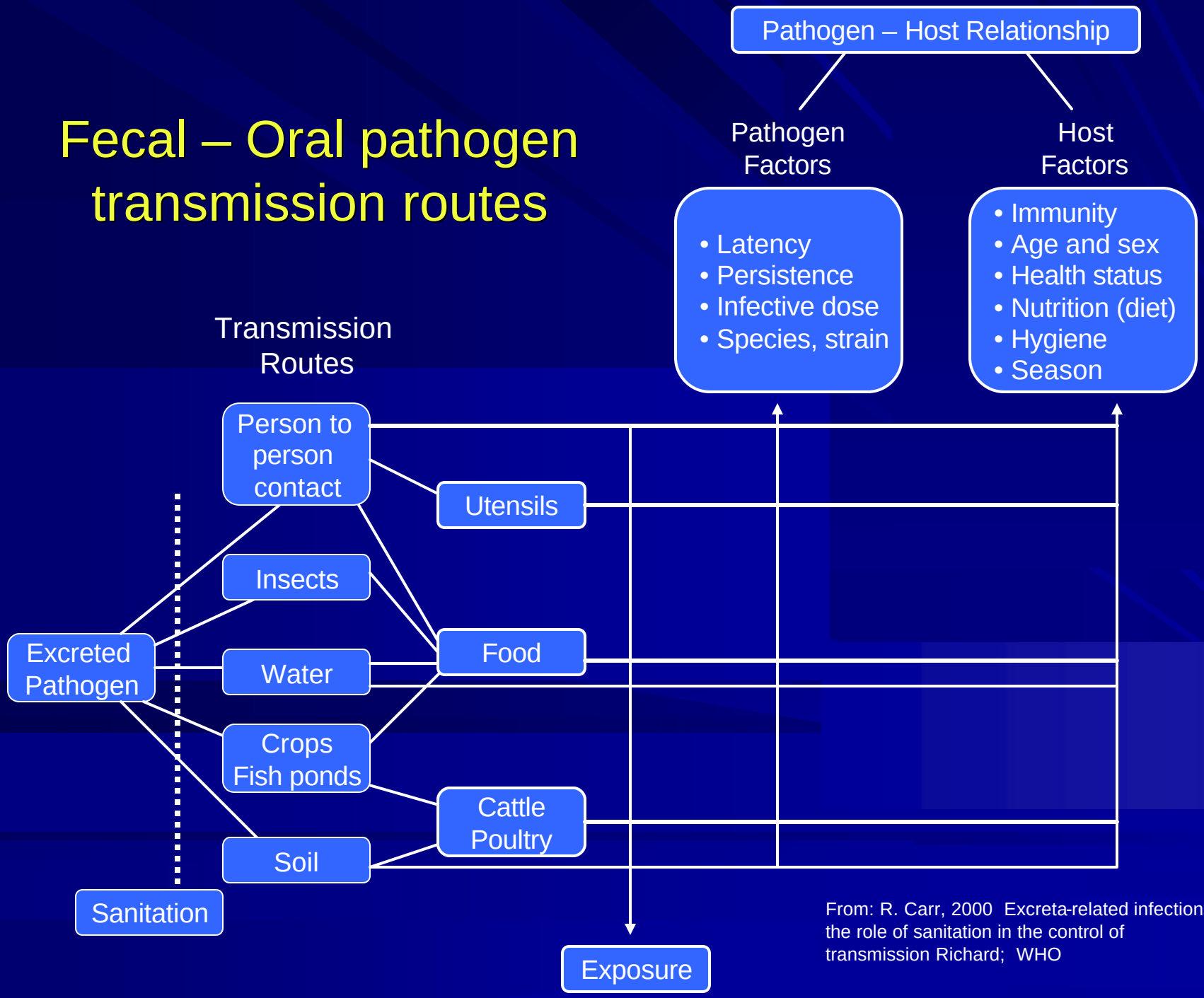
Characteristics of a good Fecal Source Indicator

- Non Pathogenic
- Rapidly detected and easily enumerated
- Have survival characteristics similar to the pathogen
- Can be associated with the presence of pathogenic organisms
- Should not replicate in the environment
- Should be present in greater numbers than the pathogens

Factors Affecting the Bacterial Number in Feces

- Host (genetics) and Host Diet
- Medications (antibiotics)
- Diseases (as source of disease agents)
- Digestive system (established populations)
- Probiotics

Fecal – Oral pathogen transmission routes



From: R. Carr, 2000 Excreta-related infections and the role of sanitation in the control of transmission Richard; WHO

Impacted Waters

- Recreational waters (beaches)
- Agricultural waters (irrigation and stock waters)
- Drinking water facilities (raw water)
- Wells (public and private)

Sources of Fecal/Microbial Material in Water

- Runoff from:
  - Agricultural land and cattle feed lots
  - Fecal material from domestic animals
  - Poorly functioning septic tanks
  - Wastewater applications
  - Fecal material from wildlife
- Finding these inputs ⇒ Microbial Source Tracking

"Detecting Microbial Contamination in Water and Soil"

1:00	Introduction to EPI-Net *Dr. Ron Turco*
1:10	Pathogens in the Environment *Dr. Ron Turco*
1:25	Fecal source tracking: past, present, and future *Dr. Valerie J. Harwood* (*Dr. Jorge Santodomingo)*
2:15	Validation of Performance in Library-Dependent Microbial Source Tracking Studies *Dr. Valerie J. Harwood*
3:05	Break
3:20	Small Successes: Library Independent Methods to Identify Microbial Pollution Sources *Dr. Von Sigler*
4:10	Watershed-based microbial source tracking case study: From implementation and solutions to research and method evaluation Dr. Nancy White

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Log number g^{-1}

Animal	*E. coli*	*C. perfringens*	*Strep.*	Bacteroid.	*Lactobaci.*
Cattle	4.3	2.3	5.3	0	2.4
Sheep	6.5	4.3	6.1	0	3.9
Horses	4.1	0	6.8	0	7.0
Pigs	6.5	3.6	6.4	5.7	8.4
Chickens	6.6	2.4	7.5	0	8.5
Rabbits	2.7	0	4.3	8.6	0
Dogs	7.5	8.4	7.6	8.7	4.6
Cats	7.6	7.4	8.3	8.9	8.8
Mice	6.8	0	7.9	8.9	9.1
Humans	6.7	3.2	5.2	9.7	8.8

Todar, K., 2002. The Bacterial Flora of Humans. Department of Bacteriology, University of Wisconsin-Madison.
Internet address: http://www.bact.wisc.edu/Bact303/Bact303normalflora
http://textbookofbacteriology.net/normalflora.html