

The Risk for Disease Transmission From Manure to Humans

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Outline

- **What are the pathogens**
- **What are their clinical signs**
- **Sources of the pathogens**
- **Survival of waterborne pathogens**
- **Detection of pathogens in water**
- **Elimination of waterborne pathogens**
- **Biofilms in water distribution systems**
- **Prevention of waterborne contamination**
- **Issues to be addressed in regulation development**

Pathogens in Water

- Water contains numerous microbes (ubiquitous) only some are pathogenic
- Bacteria
 - Coliform Bacteria
 - Coliform Antagonists
 - Antibiotic Resistant Bacteria
 - Disinfectant Resistant Bacteria
- Protozoans
 - Giardia, Cryptosporidium, Toxoplasma
- Fungi and Yeast

Infectious Bacteria in Drinking Water

- *Campylobacter jejuni*
- Enteropathogenic *E. coli*
- *Salmonella* spp
- *Shigella* spp
- *Vibrio* spp
- *Yersinia enterocolitica*
- *Helicobacter pylori*
- *Legionella*
- *Mycobacterium* spp

Infectious Viruses in Drinking Water

- Adenoviruses
- Enteroviruses
- Hepatitis A
- Norwalk virus
- Vibrio spp
- Reovirus
- Rotovirus
- Coxsackie

Infectious Protozoa in Drinking Water

- Balantidium coli
- Entamoeba coli
- Giardia
- Cryptosporidium
- Toxoplasma
- Cyclospora
- Isospora

Infectious Helminths in Drinking Water

- Ancylostoma (hookworms)
- Ascaris (roundworms)
- Trichuris (whipworms)
- Taenia (Tapeworms)
- Echinococcus (Tapeworms)

Coliforms in Water

- Citrobacter
- Enterobacter
- Escherchia coli
- Klebsiella

Waterborne Outbreaks - Bacteria

Agent	Outbreaks	Cases	Deaths
Shigella	52	7,462	6
Salmonella	38	25,286	8
Campylobacter	5	4,773	0
E. coli (toxigenic)	6	1,431	8
Cholera	1	17	0
Vibrio	1	17	0
Yersinia	1	16	0

Waterborne Outbreaks - Viral

Agent	Outbreaks	Cases	Deaths
Hepatitis A	51	1,626	14
Norwalk	16	3,973	0
Rotavirus	1	1,761	0

Waterborne Outbreaks - Parasitic

Agent	Outbreaks	Cases	Deaths
Giardia	84	22,897	14
Cryptosporidium	16	3,973	0
Entamoeba	3	39	2

Giardia in Canadian Water Samples

Sample	# Sampled	Positive	% Positive
Raw drinking water	1173	245	20.9
Treated drinking water	423	77	18.2
Raw Sewage	164	119	72.6
Total	1760	441	25.1

Cryptosporidium in Canadian Water Samples

Sample	# Sampled	Positive	% Positive
Raw drinking water	1173	53	4.5
Treated drinking water	423	15	3.6
Raw Sewage	164	10	6.1
Total	1760	78	4.4

Clinical Signs of Waterborne Pathogens

- Diarrhea, vomiting, abdominal pain
- Fever
- Food allergies
- System failure (kidney, cardiovascular)
- Antibiotic resistance
- Death

Factors which Influence Disease

- Host
 - Age
 - Children
 - Elderly
 - Naive
 - Immunosuppressed
 - Infections (HIV)
 - Cancer patients
 - Organ transplant

Factors which Influence Disease

- Pathogen
 - Virulence
 - infective dose
 - invasion
 - proliferation
 - virulence factors (toxins, adhesions etc)
 - Survival
 - environmental
 - ability to avoid host elimination
 - competition with other microorganisms

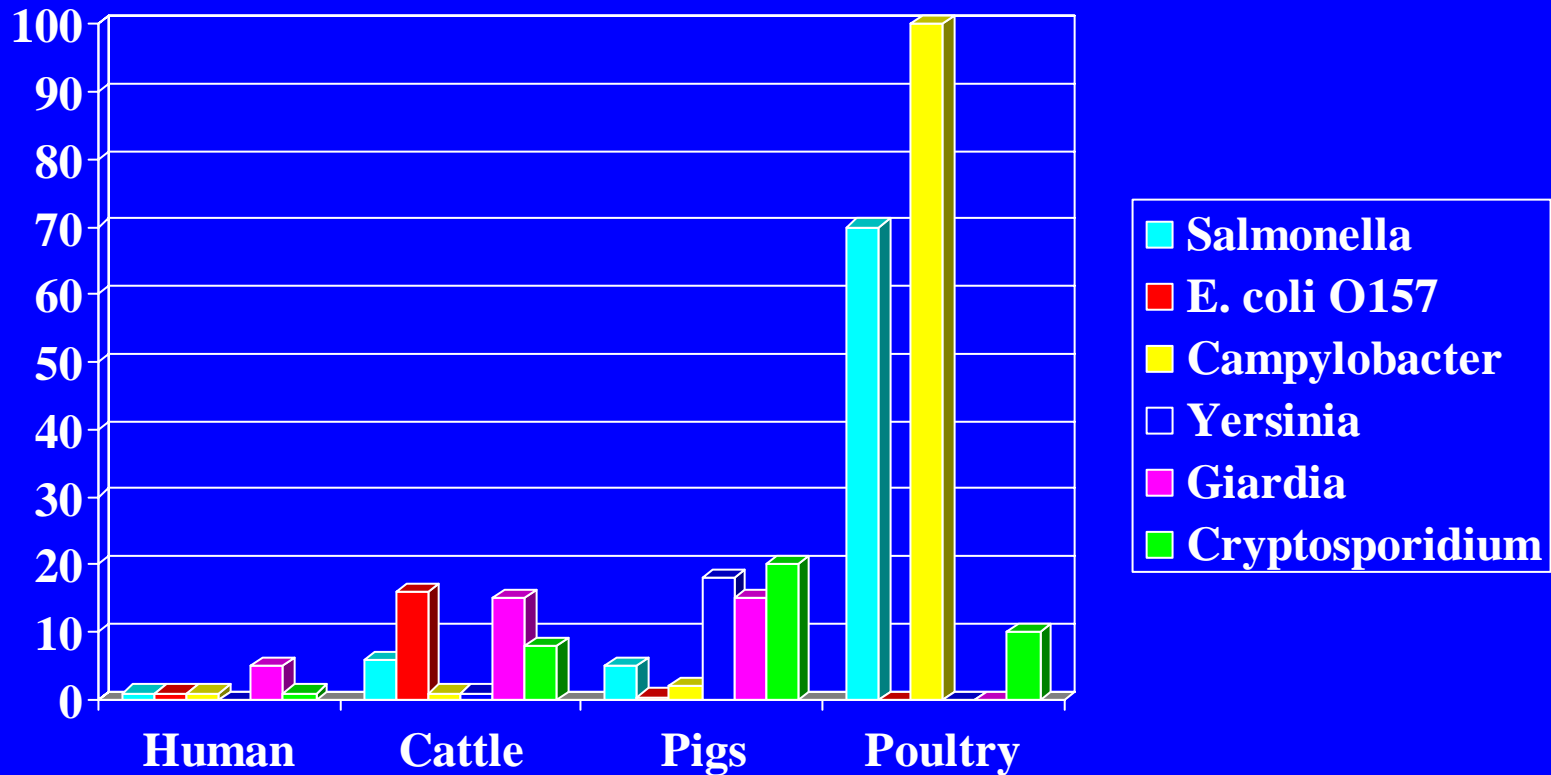
Origins of Waterborne Pathogens

- Humans
 - Sewage (effluent, septic fields)
 - Runoff
 - Food handling
- Companion Animals
- Farm animals
 - runoff
 - seepage
- Wildlife

Microbial Flora of Animal Feces (#/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

Prevalence of Enteric Pathogens



Waterborne Zoonoses

- Transmission of a disease form an animal to humans
- Not all animal pathogens are human infective (e.g. Cryptosporidium, Mycobacterium)
- Not all pathogenic species are human infective (e.g.. Cryptosporidium, Giardia, Salmonella, E. coli O157:H7)
- Zoonotic transmission is difficult to prove and difficult to disprove

Pathogen Pathways

- Sewage
 - Untreated
 - Processed
 - continual discharge
 - intermittent discharge
 - septic fields
 - runoff
 - Construction errors
 - misconnection of storm sewers
 - positioning of sewage discharge

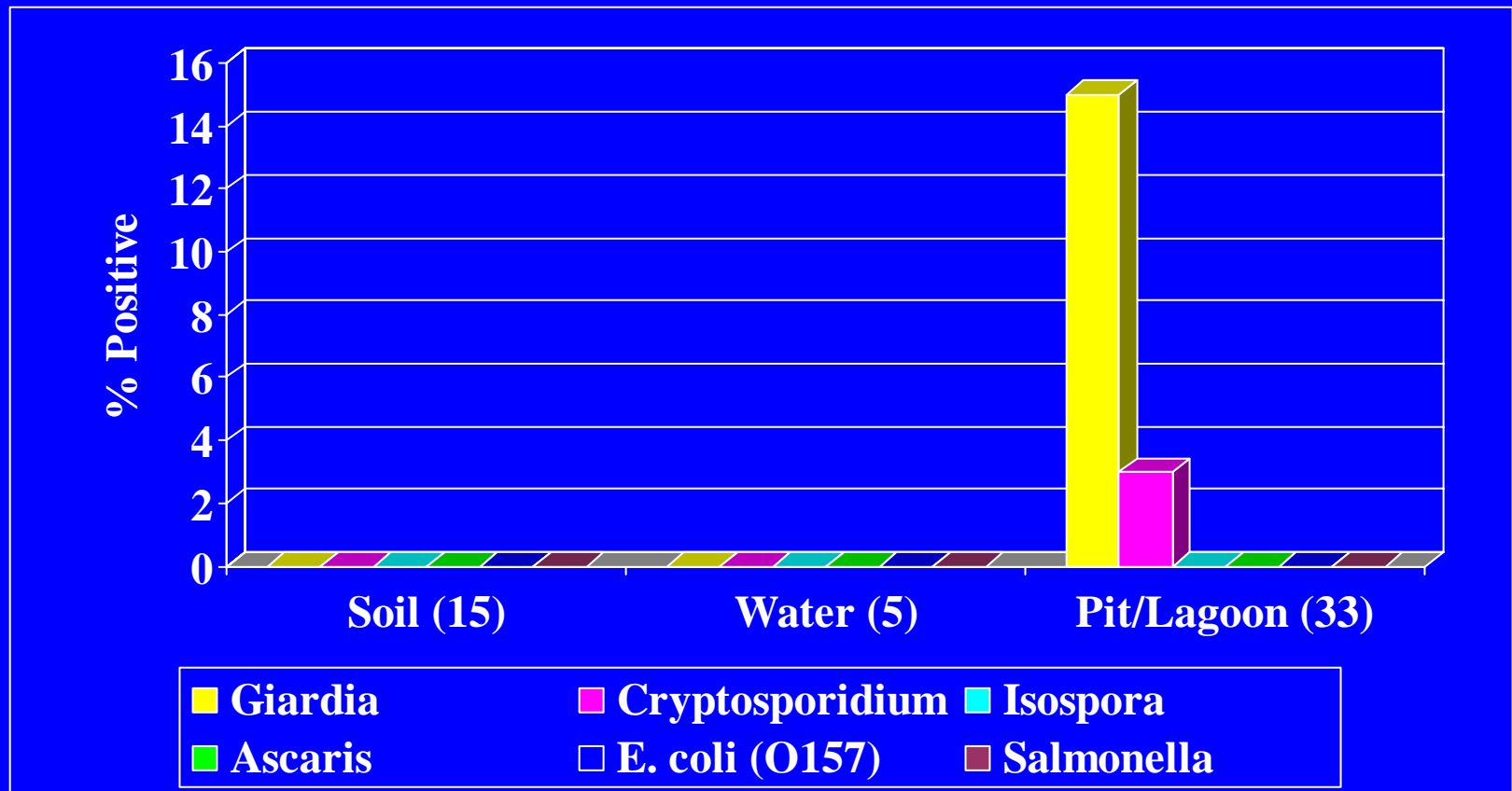
Pathogen Pathways

- Animal Waste
 - Companion Animal
 - storm sewers, exercise areas, access to raw water
 - Farm Animals
 - location of farms
 - access to raw water
 - manure disposal (frozen ground, excess, soil quality, slope of the land)
 - flooding

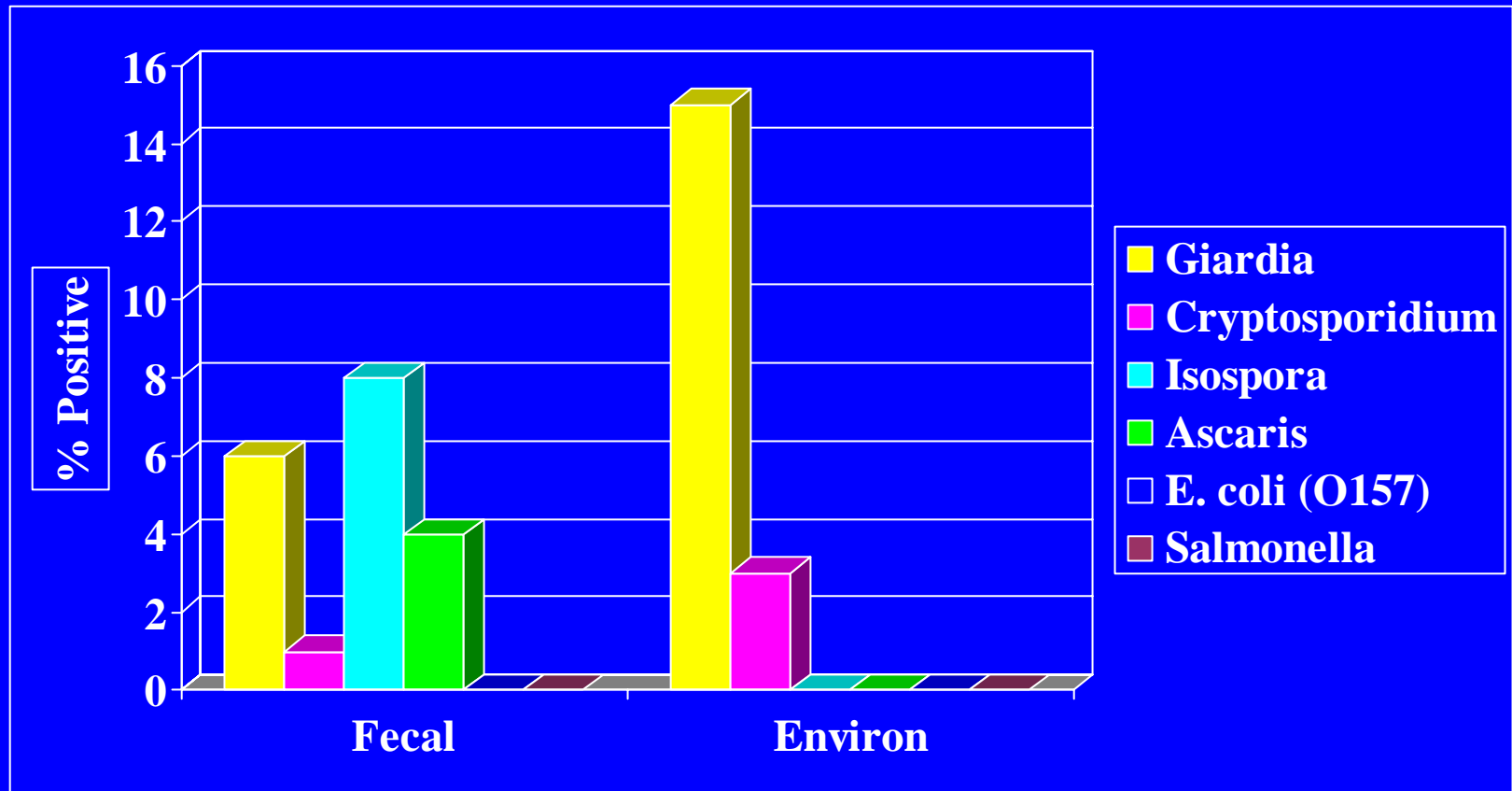
Pathogen Survival

Agent	Water	Soil	Feces
Giardia	4 mo	2 mo	1 mo
Cryptosporidium	> 1yr	> 1yr	>1 yr
Salmonella	> 6 mo	6 mo	6 mo
Campylobacter	2 mo	2 mo	2 mo
E. coli	> 1yr	>1yr	>1yr

Prevalence of Parasites and Bacteria in Soil, Water, Pit/Lagoon

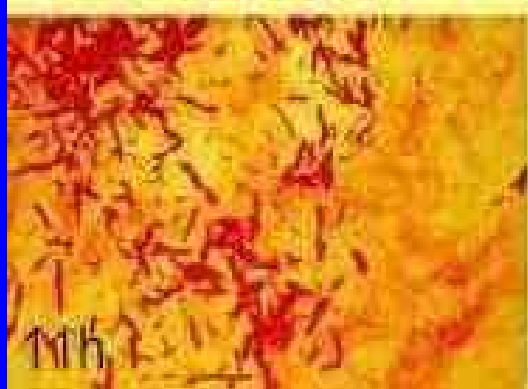
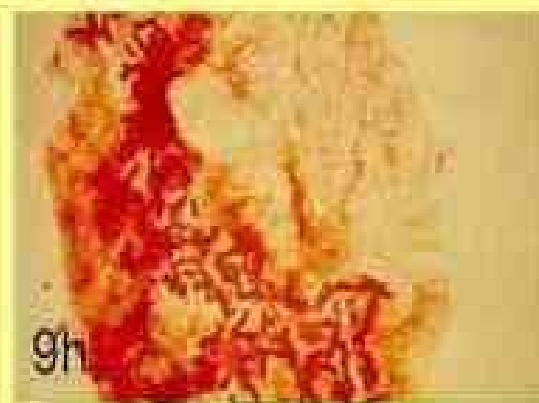
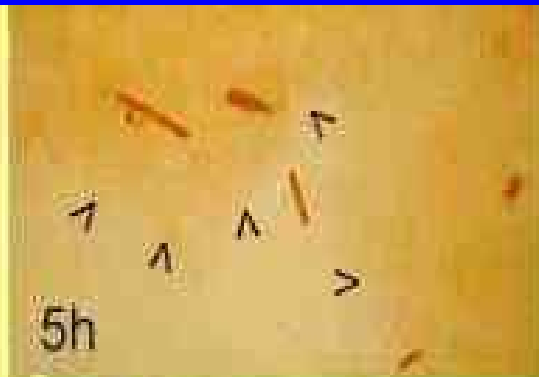


Overall Prevalence of Fecal (n=702) and Environmental Samples (n=53)

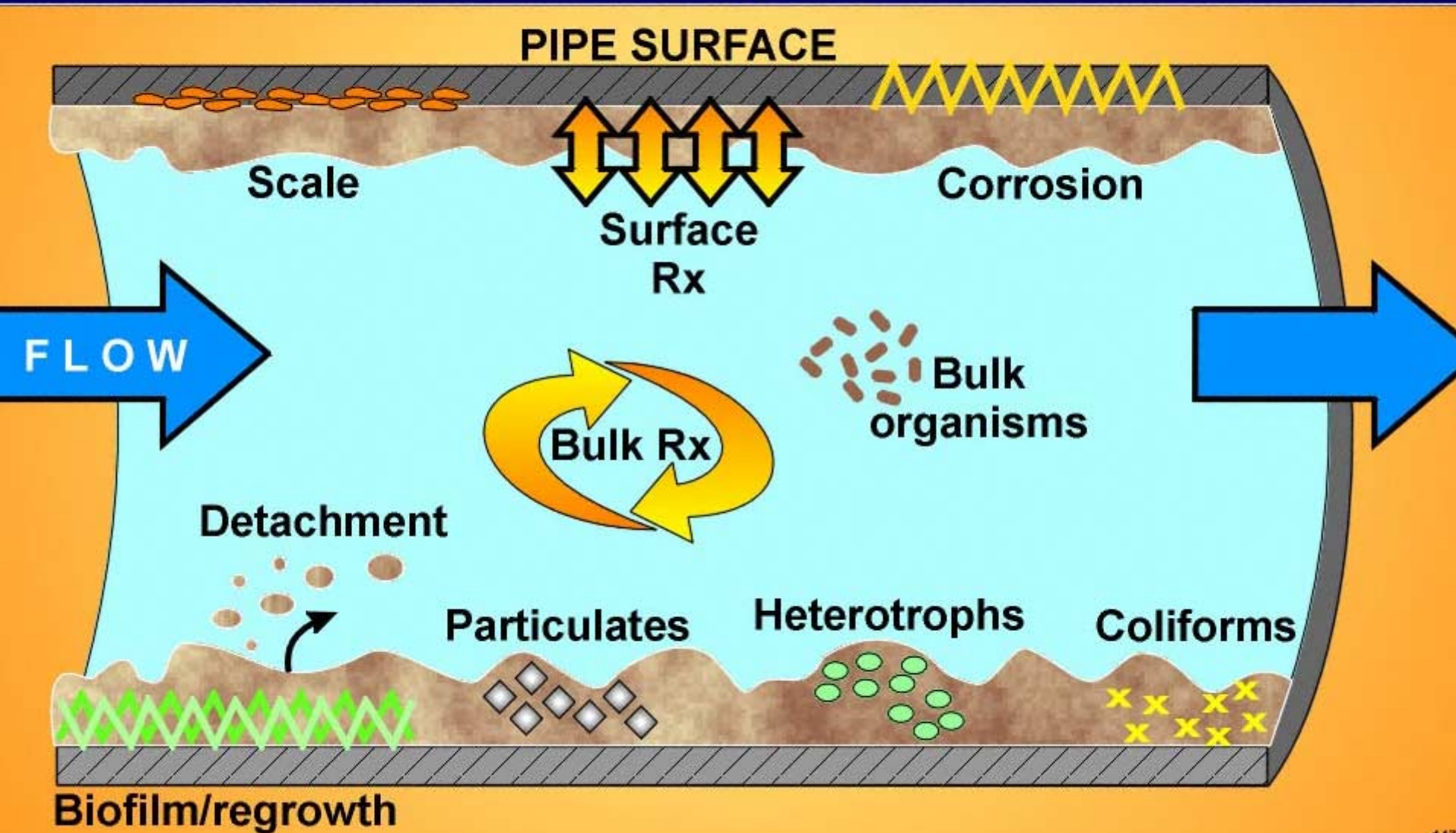


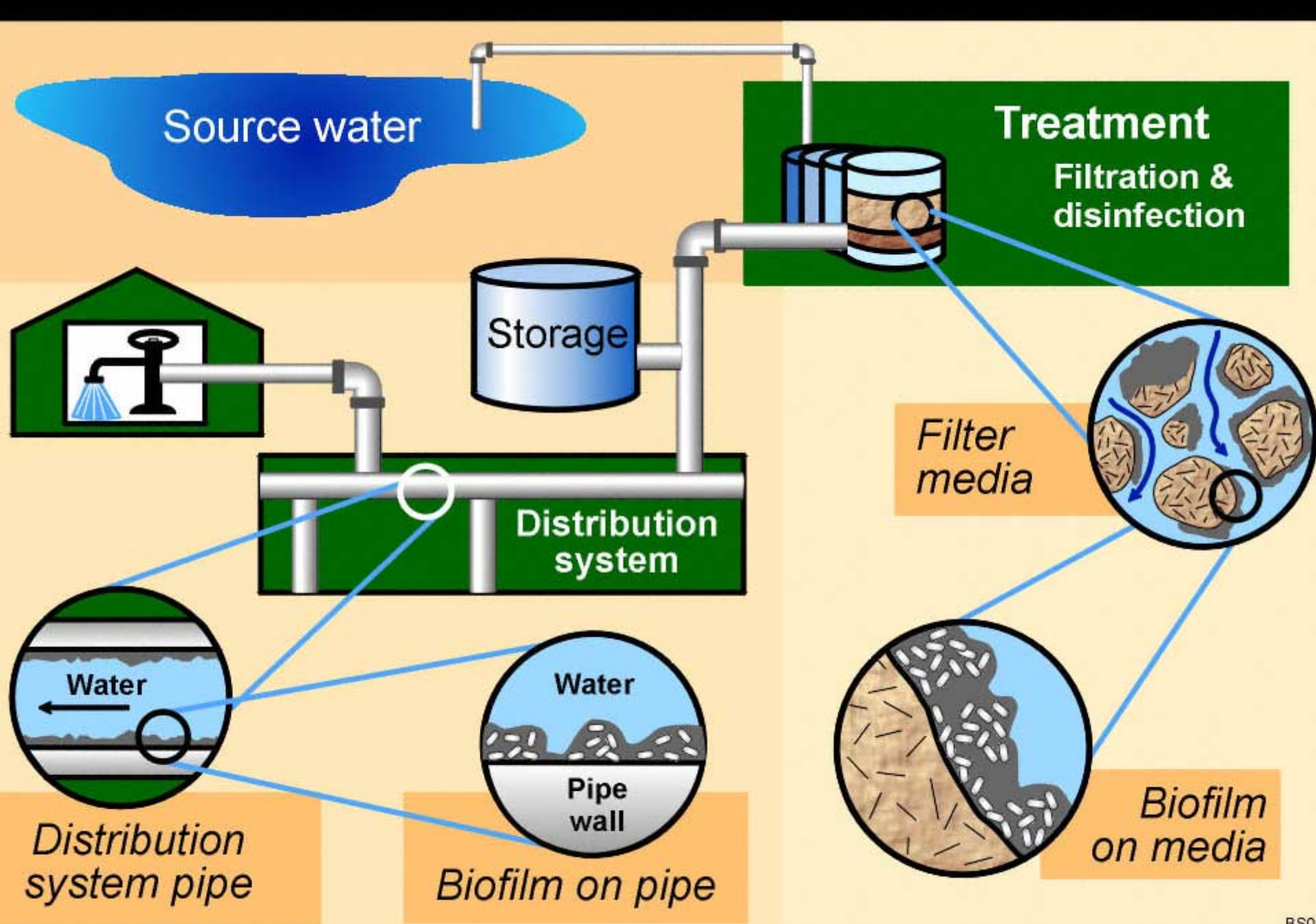
Biofilms and Pathogen Survival

- Adherent forms of bacteria, yeast and fungi
- encased in protective exopolysaccharide
- mixed pathogens
- highly resistant to antibiotics and biocides
 - 10,000 > planktonic forms
- site of transmission of antibiotic resistance
- Biocides efficacy and development based upon the ability to kill planktonic forms



The Distribution System as Reactor





Disinfectants

- **Resistance to Inactivation**
 - Protozoan > Viruses > Coliforms
- **Disinfectant Efficacy**
 - Ozone > chlorine dioxide > hypochlorous acid > hypochloride ion > chloramines

Detection of Waterborne Pathogens

- Standard methods (fecal coliforms, heterotrophic forms)
- Selective media
 - e.g.. E. coli O157:H7, Salmonella
- Magnetic beads
 - concentrate pathogen by binding pathogen to antibodies on a bead that are attracted to a magnet
- Immunoassays
 - labeled antibody stains pathogen
- Flow Cytometry
 - automated detection based on size and fluorescence

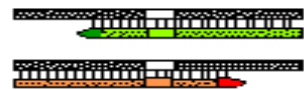


Molecular Detection of Waterborne Pathogens

- Molecular Detection
 - polymerase chain reaction (PCR)
 - can detect one organism
- Molecular Typing
 - Pulse gel electrophoresis
 - PCR + sequencing
 - PCR + restriction endonuclease analysis

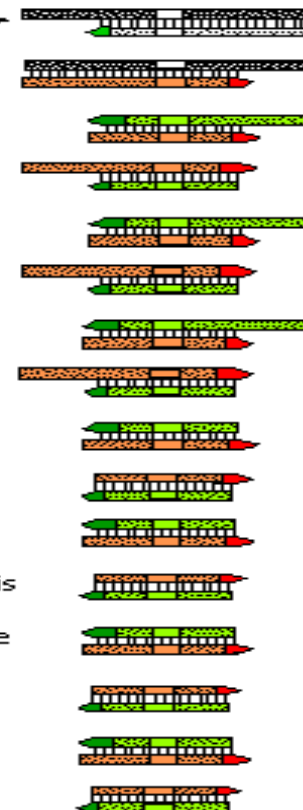
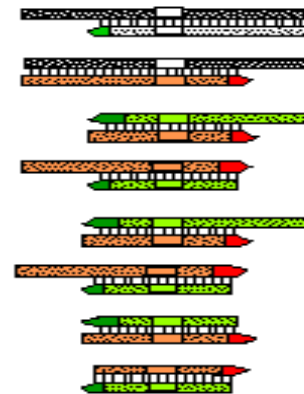
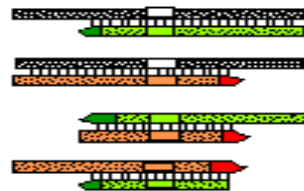
POLYMERASE CHAIN REACTION

DNA region of interest.



primer

1. DNA is denatured. Primers attach to each strand. A new DNA strand is synthesized behind primers on each template strand.



2. Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

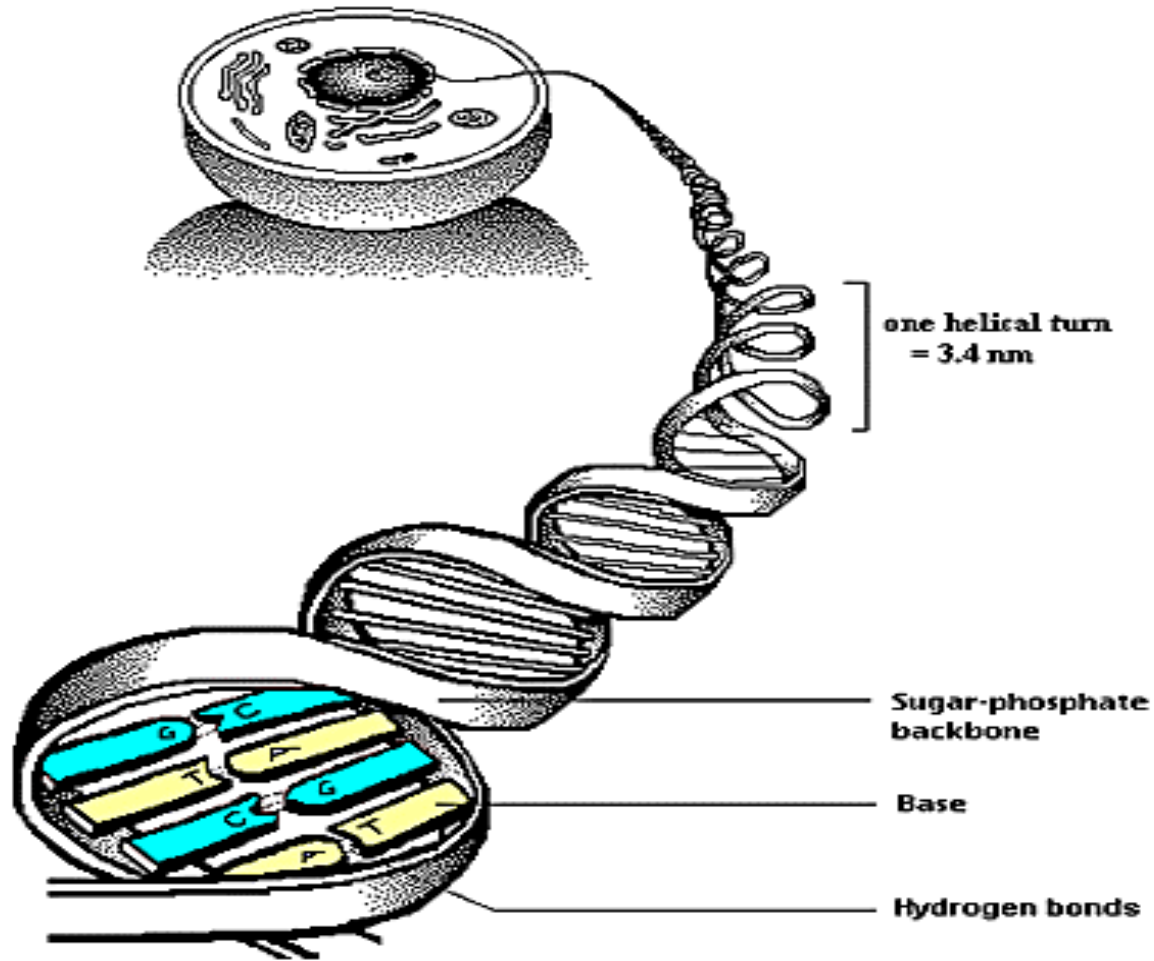
3. Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

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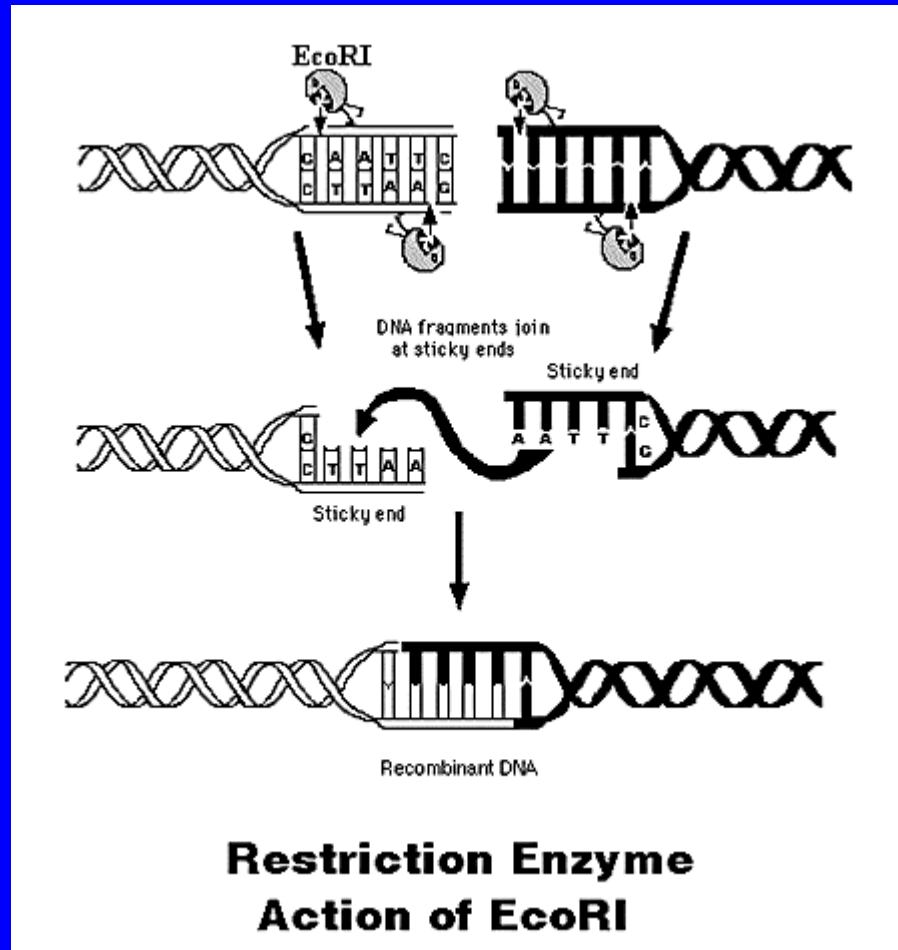
5. Continued rounds of amplification swiftly produce large numbers of identical fragments. Each fragment contains the DNA region of interest.

Gene Sequences

THE STRUCTURE OF DNA



Restriction Enzymes





A B C D E F G H I J

A = ladder

B = Calf *C.parvum*

C = Calf *C.parvum*

D = Calf *C.parvum*

E = Human *C.parvum*

F = Human *C.parvum*

G = Human *C.parvum*

H = Cat *C.felis* ??

I = Pig *C.suis* ??

J = Steer 356

Elimination of Waterborne Pathogens

- Must understand the biology of the pathogen
- Selection of appropriate processes and biocides
- Must consider biofilms within distribution systems
- Contact times, organic, turbidity
- Consider resistance

Prevention of Waterborne Diseases

- Humans
 - sewage processing and disposal
- Agriculture
 - responsible animal husbandry and manure disposal
- Wildlife
 - control exposure if possible

Costs of Waterborne & Foodborne Diseases

- Human Disease
 - clinical disease
 - disease susceptibility
- Animal Disease
 - clinical disease
 - production losses
- Food/water quality
- Tourism

Influence of Water Quality on Animal Performance

- Study Design
 - 2 pastures (60 acres)
 - 20 cows/20 calves in each pasture
 - Direct Watering vs Pumped Watering
 - Measurements
 - Weight gain
 - Water chemistry
 - Water bacteriology
 - Water parasitology

Influence of Water Quality of Animal Performance - Results

- Pumped Water
- Cows = 1.45 kg/day
- Calves = 2.34 kg/day
- superior chemistry
- low coliforms
- no parasites
- Benefits = \$712.00
- Direct Watering
- Cows = 1.07 kg/day
- Calves = 2.23 kg/day
- poorer chemistry
- coliforms
- *Giardia*
- no *Cryptosporidium*

Farming Practices

- Calving location away from drainage
- Calves < 4 weeks should be kept off of ranges
- Isolate animals with diarrhea (diagnosis)
- Protect running water
- Fence dugouts
- Treatment/vaccination
- Children/immunosuppressed adults
- Human water supplies
- Responsible manure management

Conclusion

- Waterborne pathogens are here and are a serious health treat to humans and animals
- serious health and economic impact
- reduction by waste management
- understand pathogen survival
- improve detection and identify contamination source
- pathogen elimination form water and distribution systems

Future Directions

- Re-examine sewage disposal
- manure management regulations/guidelines
- improve diagnostic methods
- develop practical regulations
- educate
- consumer confidence and awareness