

# Microbiology of Distribution Systems

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# Outline

**Significance of distribution system microbiology**

**the “Players”** (Microbiology 101)

**the Problems** (possible controls?)

**the Future**

# I) Microbes in the Pipes: Significance

Payment, 1991-1997:

“distribution system could be playing an important role in the contamination of populations”

1) Are a reservoir for human / animal disease

- even though *in compliance* with current regulations

2) Microorganisms in water also can cause biofouling and decay.

## the positive side

Microorganisms are natural and essential constituents of water

All *naturally* occurring water contain microbes; majority non-pathogenic, non-corrosive

**Microbes:** use water as medium for growth and survival  
vehicle for distribution

**Water:** “uses” microbes contribute to chemical quality (Fe, S, Hg, As)

enable C, N, H cycling ( $\text{CO}_2$ ,  $\text{CH}_4$ , denitrification)

control / eliminate pollutants (hydrocarbons, pesticides)

**Humans:** microbes in water contribute to / replenish normal flora  
- help maintain disease resistance

## II) the “Players”

- Representative protozoa, bacteria, viruses
- In order of decreasing size (filterability) and increasing chlorine sensitivity

# Water-borne parasitic protozoa

## *Giardia lamblia*

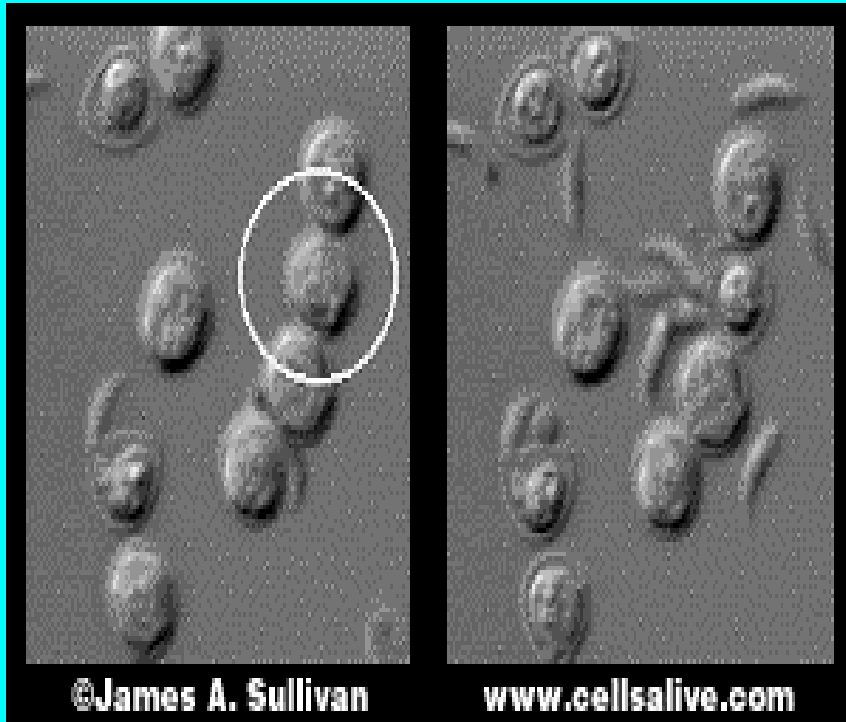
- size: 10 –12  $\mu\text{m}$
- removed by filtration
- thick-walled cysts in water
- resistant to chlorine
- infections: motile trophozoite
- treatable diarrhea
- do not multiply in water
- reservoir settling decreased counts, viability



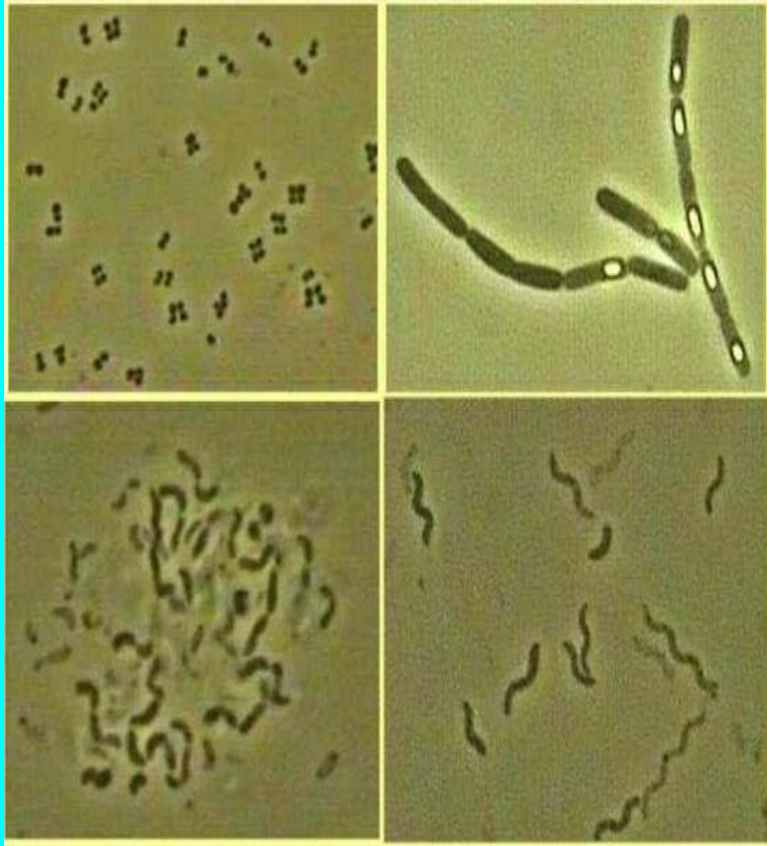
# Water-borne parasitic protozoa

## *Cryptosporidium parvum*

- size: 4 – 6  $\mu\text{m}$   
(filtration inconsistent)
- thick-walled oocysts
- UV, ozone sensitive
- infections: non-motile  
sporozoites (invasive)
- ID<sub>50</sub>: 10-1000 oocysts
- diarrhea lasts 1-2 weeks
- not antibiotic treatable
- do not multiply in water
- OB source: human waste



# Water-borne Bacteria (heterotrophic)

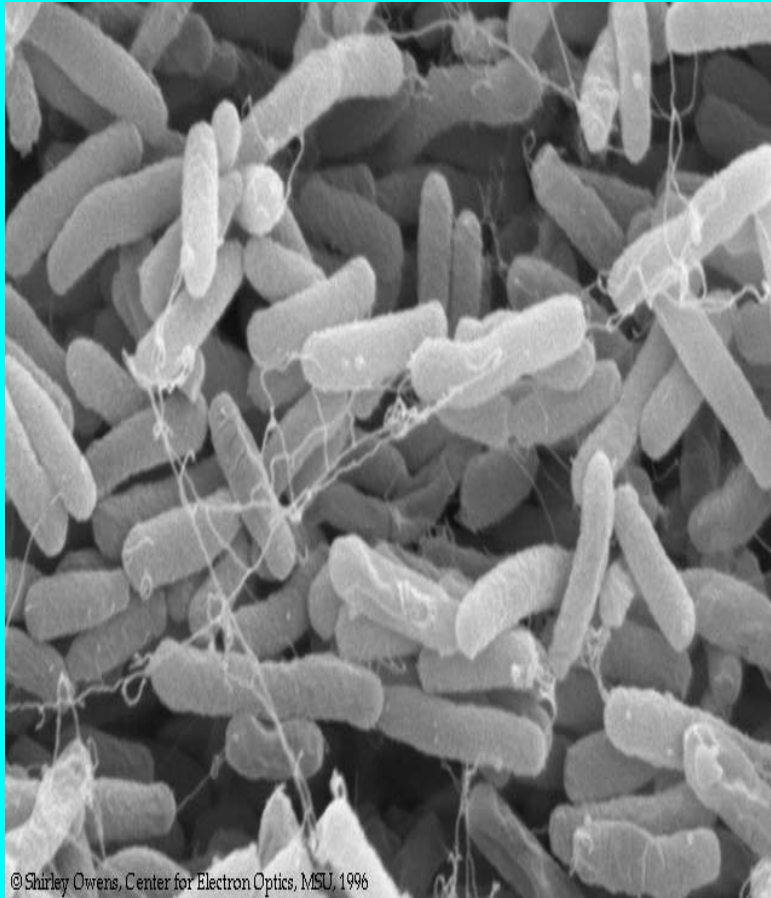


- diverse shapes (sphere, rod, comma, spiral)
- size: 0.2 – 4  $\mu\text{m}$
- most chlorine sensitive

Examples: *Enterococcus*  
*Klebsiella*, *Serratia*  
*Vibrio cholera*  
*Sprillum*



## Water-borne bacterium: *Escherichia coli* (coliforms)



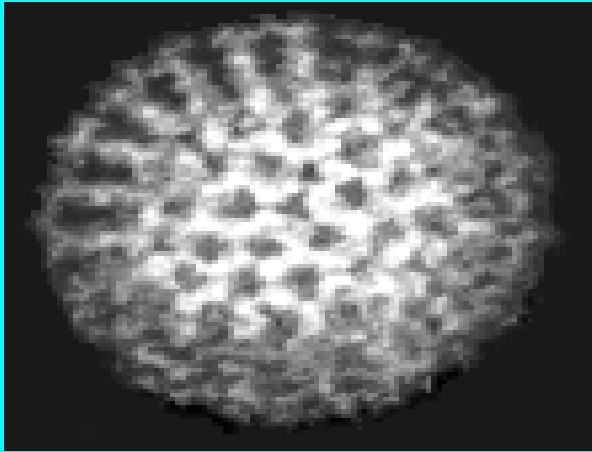
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- both pathogenic and non-pathogenic forms in water

### Pathogenic type:0157:H7

- attaches to wall of intestine
- forms potent toxin
  - bloody diarrhea, kidney damage, death
- half-life: approx. 8 days (ground water)
- acid resistant

# Water-borne viruses

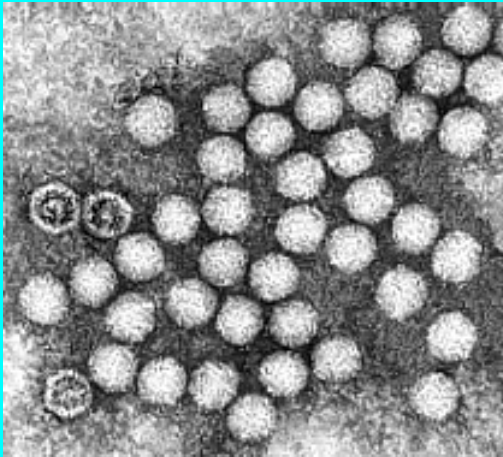


## Small round viruses (SRV)

- 0.02 - 0.07 $\mu$ m diameter
- chlorine resistant
- untreatable gastroenteritis

## ← Rotavirus

- diarrhea in children



## ← Enterovirus

- diarrhea children/adults
- est half-life: 2 – 8 hours

### III) the Problems

High quality water leaving treatment plant subsequently may be subject to several processes that degrade this quality:

a) disinfection proficiency

b) regrowth

c) nitrification

d) biofilms

e) intrusion events

## Problems cont'd

### a) Variation in treatment proficiency, transient failures

- breakthrough inoculation → reseedling, increase nutrients, color
- microbes: original complement (esp viruses, protozoans)

*Control:* effluent monitoring, secondary disinfection

## Problems cont'd

b) Regrowth: post treatment increase in counts within system

- disinfectant resistance, nitrification, biofilm sloughing  
→ loss of bacterial quality, new biofilm formation, increased corrosion, taste, odor, grazing macroinvertebrates
- microbes: coliforms, Gm+, heterotrophs, film formers

*Control*: limit biodegradable organic material (BOM) entering system  
with coagulation, filtration (carbon, biologic, membrane)

action level: effluent AOC > 150 ug/liter, BDOC > 0.5 mg/l

NB. chlorination, ozone >> BOM in water

## Problems cont'd

- 3) Nitrification: increase in nitrite/nitrate content via microbial activity
- secondary disinfection with ammonium chloride (**chloramination**)
  - less reactive than Cl (fewer DBPs), penetrates biofilms, but promotes growth of ammonium / nitrite oxidizing bacteria [AOB,NOB] (*Nitrosomonas*, *Nitrospira*) that deplete chloramine, foster growth of heterotrophic bacteria → loss of water quality. Occurs with residuals of 0.2 -1.1 mg Cl<sub>2</sub>/l, 0.3 – 0.6 mg N/l

*Control*: monitor effluent pH, NH<sub>4</sub> conc, AOB densities  
routine flushing, breakpoint chlorination  
action level: effluent: 50 ug nitrite / nitrate/l

## Problems cont'd

### 3) Biofilms: organized community bacteria adhering to physical surface

- enables microbe to resist flush, survive nutrient deprivation
- cause physical damage (corrosion), reduces proper function (flow rates)\*, source of pathogens (regrowth), color/odor/taste
- microbes: coliforms, aerobes (oxidizers), anaerobes (reducers)  
resistant to disinfectants (1-2 mg  $\text{Cl}_2/\text{l}$ ,) antibiotics\*

# Pipe Encrustation

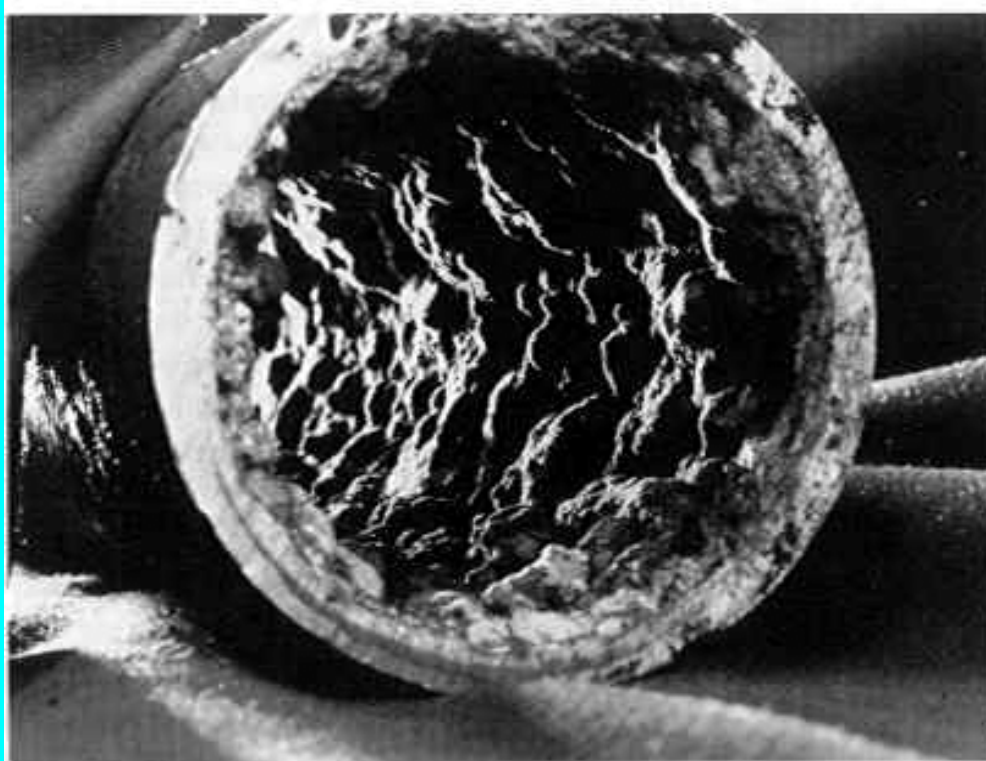
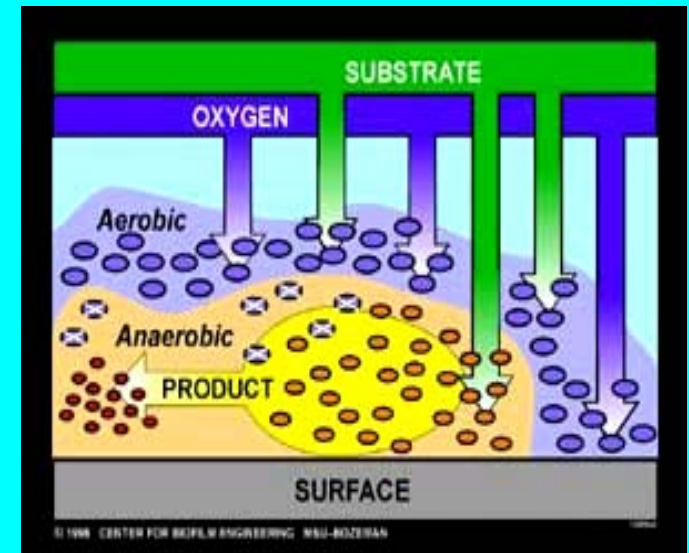
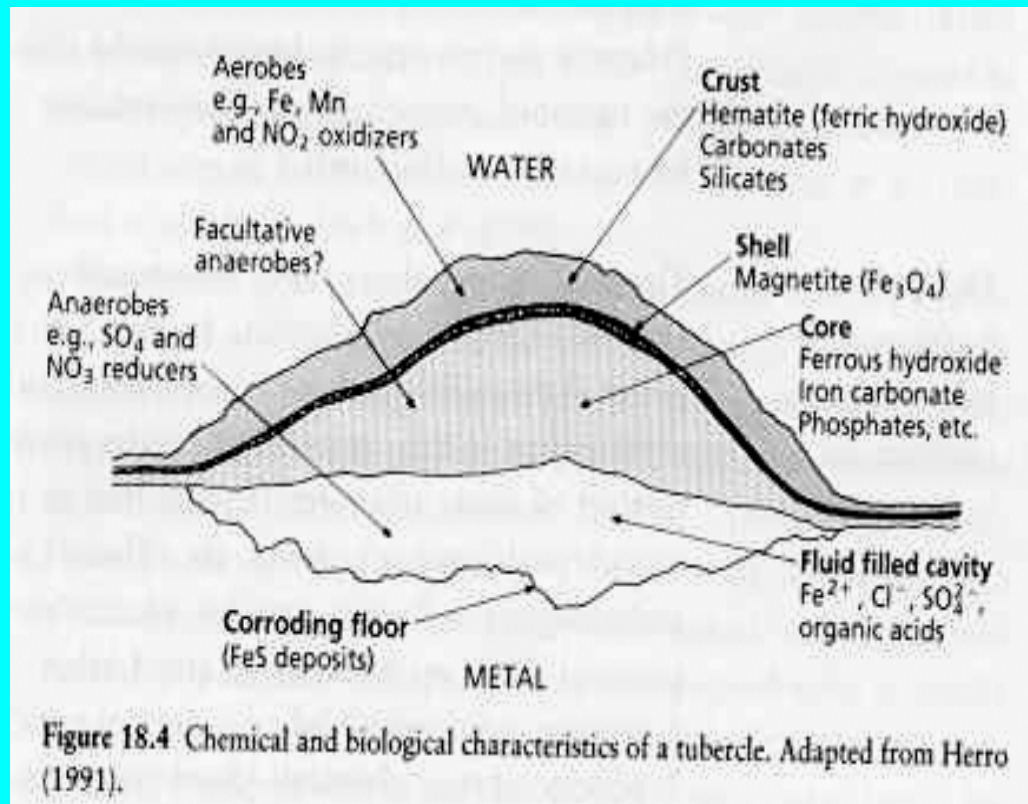


Figure 18.2 Distribution system pipeline heavily encrusted with iron and manganese oxide deposits. From Herro (1991).



# Biofilm: tubercle anatomy / activity



## Biofilms cont'd

*Control:* use antifouling / anti-adhesive surface^ (iron vs cPVC)  
chemical biocides (monochloramine)^  
physical removal of tubercles  
reduce AOC (nutrients)^

^ all influence rate and amount of formation

Recognition: sporadic, unexplained change in counts, color, turbidity  
seasonal flushing

## Problems cont'd

### 4) Intrusions: introduction of microbes from outside system

a) accidental: pressure loss, breaks/repairs, cross-connection, seepage, flooding

b) intentional: bioterrorism (deliberate tampering)

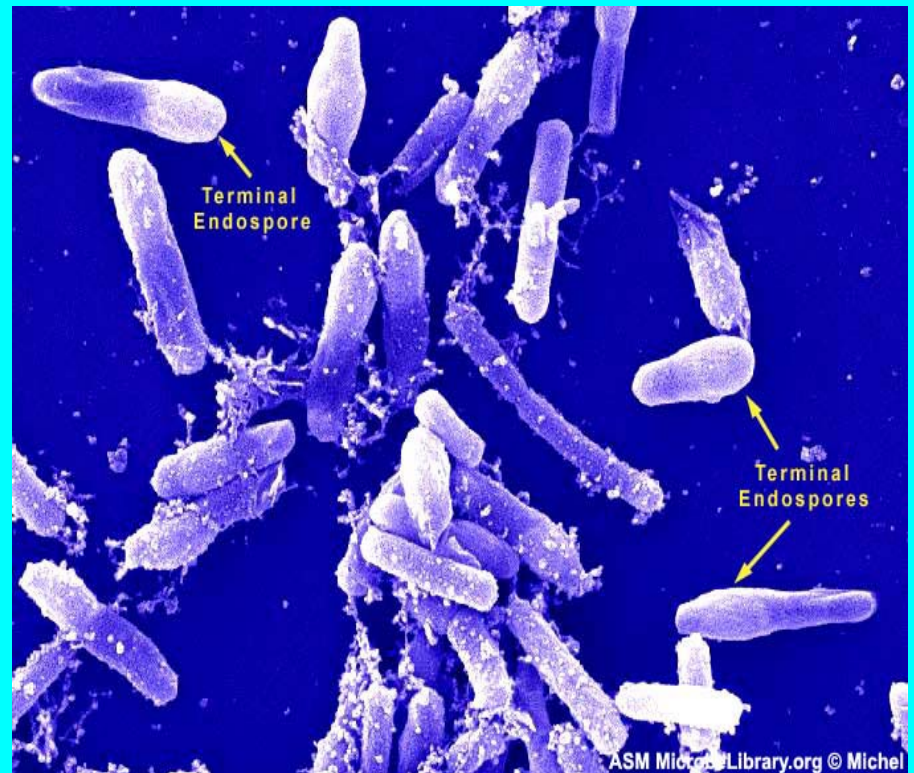
- toxigenic bacteria (eg. *E.coli* 0157:H7<sup>^</sup>, *B. anthracis*)\*
- parasitic protozoans (*Cryptosporidium parvum*)<sup>^</sup>
- enteropathogenic viruses (Norwalk<sup>^</sup>?)\*

*Control*: good SOPs, communication, controlled access  
rapid detection, response plan

# Confirmed Bioterrorism Agent

## *Bacillus anthracis* (anthrax)

- spores survive soil, water, air
- toxins: skin, GI tract, lungs (resp.> 60% mortality, 48h)
- ID<sub>50</sub>: 2000 spores/person
- vaccine, antibiotics useful
- spores Cl resistant



# Potential Bioterrorism Agent

## Norwalk virus

- small round, contain RNA
- infections associated with water, aerosols
- incubation period 24-48h
- diarrhea and vomiting
- no vaccines, no antivirals
- chlorine sensitive

## IV) the Future

### a) Monitoring / detection

- non-culturable microbes: post-concentration particle counts?  
(acoustic energy concentration, microfluidic/capillary sampling)
- screen for specific pathogens? (molecular probes, PCR)

### b) Disinfection

- greater use of UV irradiation, ozonation?
- soft X-ray sources?

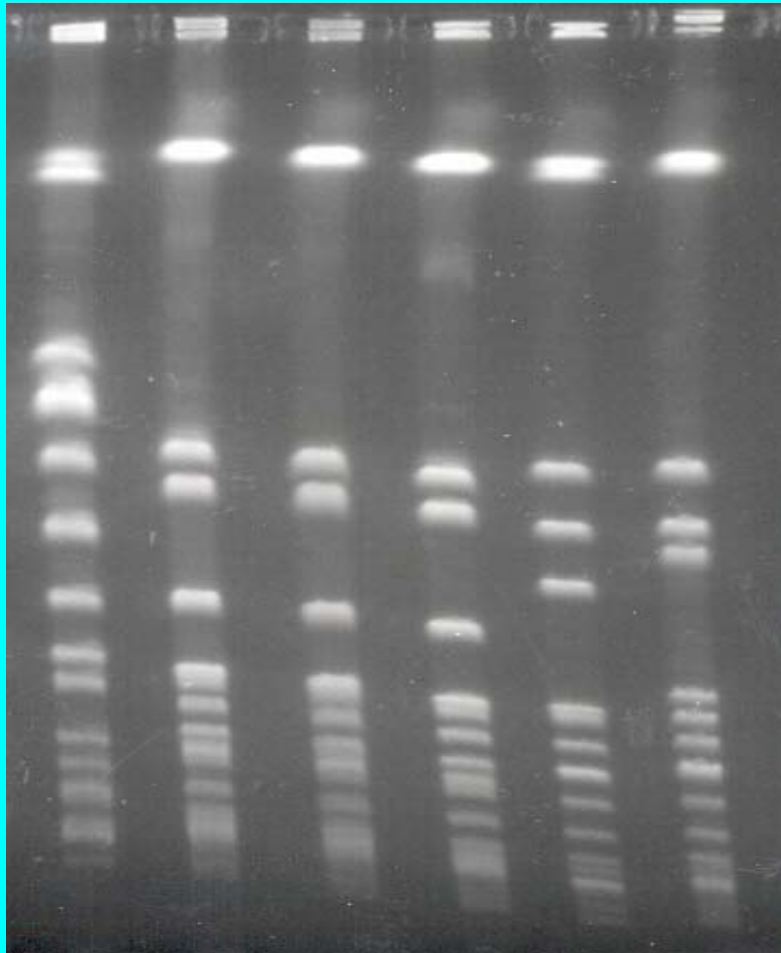
### c) Bacterial source tracking

- eliminate cause rather than deal with outcome (asses similarity) \*

### d) Regulation

- WHO recommends adoption of HACCP approach

# Detection / Confirmation of Contamination Source: DNA Typing



- Comparison of chromosome fragment banding patterns implicates cattle manure as source of *E. coli* polluting well water.

← Left to right: manure

“

well water

“

septic tank

“



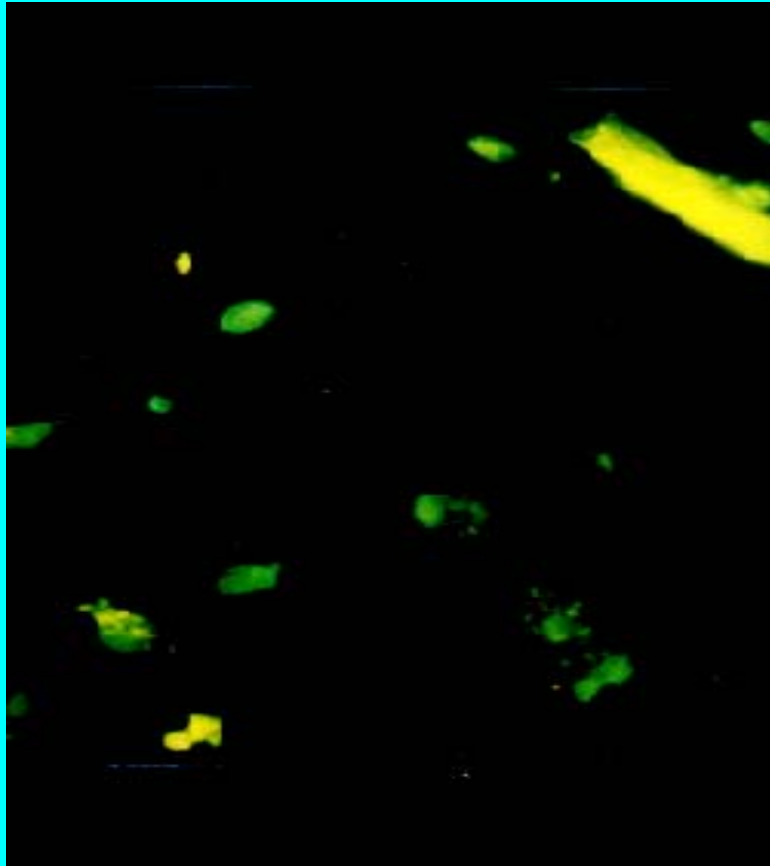


# Detection of Parasitic Protozoa



- Collect contents of >340 litres water in polypropylene wound-string filter
- Recover, concentrate, stain and examine under epifluorescence microscope

# Fluorescent Immunoassay for Protozoa



← *Giardia* cyst

← *Cryptosporidium* oocyst

← Cross-reacting algae

## WBB: Iron bacteria: *Gallionella*



- Filamentous, fragmented strands occurring at aerobic/anaerobic interface
- Also includes *Thiobacillus* and *Leptothrix*
- Aerobic oxidizers of soluble ferrous iron (FeII) to obtain energy → insoluble ferric (FeIII) oxyhydroxides
- Fe II fr. water → clogs  
“ pipes → pitting

# Microbiology 101: the “Basics”

Microorganisms: small, singled-celled, self-replicating life forms

Three major groups: Eubacteria (bacteria, Gram stain + or -)

Eukarya (algae, fungi, protozoa)

Archaea (methanogens)

Viruses (obligate parasites)

Exist as interactive communities in natural systems

- the **Prokaryotes** = Eubacteria, Archaea, viruses

- the **Eukaryotes** = protozoa, fungi, algae

Either aerobic (O<sub>2</sub> requiring) or anaerobic (O<sub>2</sub> independent)

Net negative charge at cell surface (adhere to solids)