Environmental Microbiology

Wastewater Treatment, Water Purification, and Waterborne Microbial Diseases

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Wastewater Microbiology and Water Purification

- Public Health and Water Quality
- Wastewater and Sewage Treatment
- Drinking Water Purification

Wastewater Microbiology and Water Purification

Water

- Potential common source of infectious diseases
- Can also be a source for chemically induced intoxications
- Ensuring water purity is essential for public health
- Treatment and purification schemes use microorganisms to identify, remove, and degrade pollutants

Public Health and Water Quality

Coliforms and Water Quality

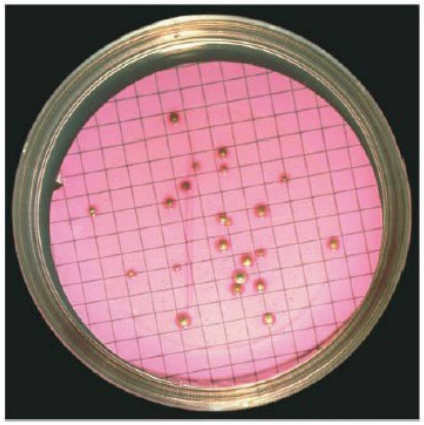
- Water can be sampled for the presence of specific indicator microorganisms
- The coliformgroup of microorganisms
- Includes *Enterobacter*, *Escherichia coli*, and *Klebsiella pneumoniae*
- They are a widely used indicator for fecal contamination in water

Public Health and Water Quality

Testing for Coliforms and *E. coli*

- All tests assay the growth of organisms recovered from the water samples
- Common methods for enumerating the samples:
- Most-probable-number (MPN) procedure
- Membrane filter (MF) procedures
- *E. coli* can be distinguished from total coliforms with defined substrates (e.g., MUG, IBDG)

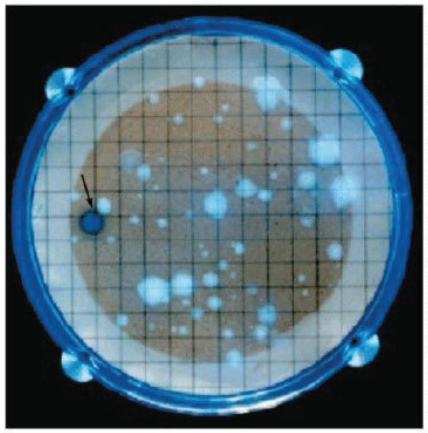
Coliform colonies growing on a membrane filter



T. D. Brock

A drinking water sample was passed through the filter. The filter was then placed on eosin–methylene blue (EMB) medium that is both selective and differential for lactose-fermenting bacteria (coliforms). The dark, shiny appearance of the colonies is characteristic of coliforms. Each colony developed from one viable coliform cell present in the original sample.

Total coliforms and *Escherichia coli*.



USA Environmental Protection Agency

A filter exposed to a drinking water sample was incubated at 35°C for 24 hours on MI media and examined under UV light. The single *E. coli colony appears dark blue* (arrow). The other colonies are coliforms that fluoresce and appear white to light blue.

Public Health and Water Quality

A commonly used method for performing coliform counts is the IDEXX Colilert test system. Utilizes proprietary substrates

- Sample with no coliforms remains clear
- Coliforms turn media yellow
- *E. coli* turns media fluorescent blue

The IDEXX Colilert water quality test system

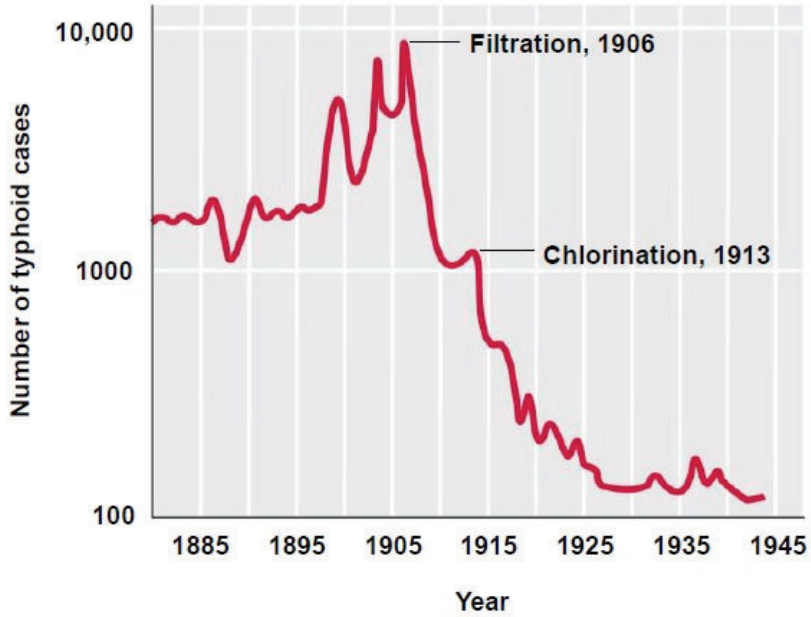
Colilert reagents are added to 100-ml water samples. After incubation for 24 h at 35-37°C, the samples develop yellow color if they contain coliform bacteria (right). Samples containing *Escherichia coli develop yellow color* and also develop blue fluorescence (left). Samples negative for coliform bacteria remain clear (center).

Public Health and Water Quality

Public Health and Drinking Water Purification

- Effective water treatment practices that reduced the incidence of waterborne disease were not in place until the twentieth century
- Coliform-counting methods were developed and adapted around 1906
- Water purification started with filtration
- About 1913, chlorine came into use as a disinfectant for large water supplies

The effect of water purification on the incidence of waterborne disease



The graph shows the incidence of typhoid fever in Philadelphia, Pennsylvania. Note the dramatic reduction in the incidence of typhoid fever after the introduction of both filtration and chlorination.

Wastewater and Sewage Treatment

Wastewater

- Domestic sewage or liquid industrial waste

Wastewater treatment

- Relies on industrial-scale use of microbes for bioconversion

Following treatment, the discharged treated wastewater (effluent water) is suitable for

- Release into surface waters
- Release to drinking water purification facilities

Wastewater and Sewage Treatment

Wastewater treatment facility

- Its goal is to reduce organic and inorganic materials in wastewater to a level that no longer supports microbial growth and to eliminate other potentially toxic materials
- The efficiency of treatment is expressed in terms of a reduction in the biochemical oxygen demand (BOD)
- Amount of dissolved oxygen consumed by microbes to completely oxidize all organic and inorganic matter in a water sample

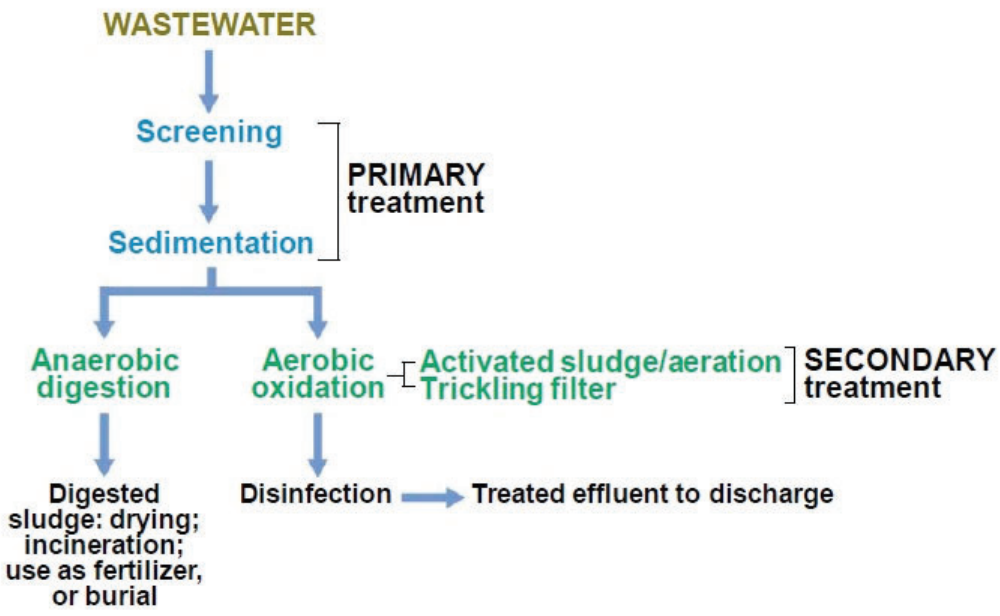
Wastewater and Sewage Treatment

- Wastewater treatment is a multistep operation employing both physical and biological processes
- Primary, secondary and sometimes tertiary treatments are used

Primary treatment

- Uses physical separation methods to separate solid and particulate organic and inorganic materials from wastewater

Wastewater treatment processes



Effective water treatment plants use the primary and secondary treatment methods shown here. Tertiary treatment may also be used to reduce BOD levels in effluent water to undetectable levels.

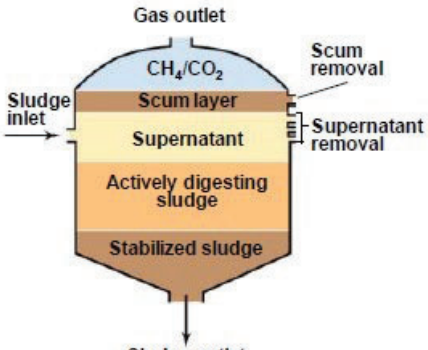
Wastewater and Sewage Treatment

Secondary treatment

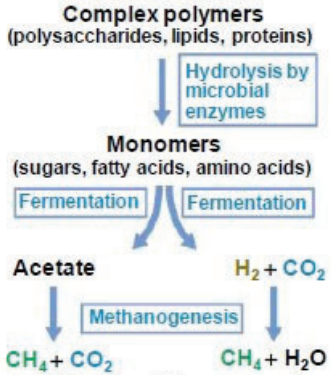
- Anaerobic secondary treatment involves a series of digestive and fermentative reactions carried out by various microbes under anoxic conditions
- The process is carried out in large enclosed tanks (sludge digesters or bioreactors)

Anaerobic treatment

(a) Anaerobic sludge digester. Only the top of the tank is shown; the remainder is underground.



(b) Inner workings of a sludge digester



(c) Major microbial processes in anaerobic sludge digestion. Methane (CH_4) and carbon dioxide (CO_2) are the major products of anaerobic biodegradation.

Wastewater and Sewage Treatment

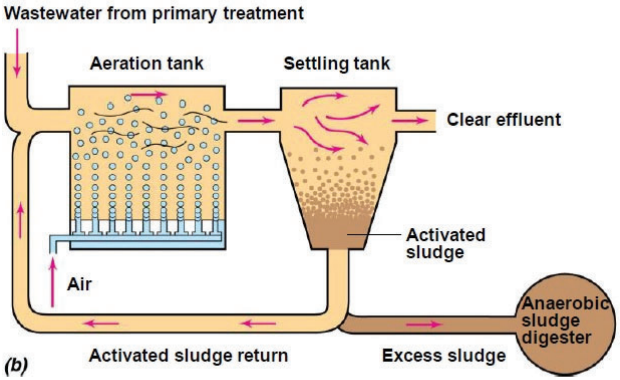
Secondary Treatment (cont'd)

- Aerobic secondary treatment uses digestive reactions carried out by microbes under aerobic conditions to treat wastewater with low levels of organic materials
- Activated sludge and the trickling filter are the most common decomposition processes

Aerobic wastewater treatment processes (aeration tank)

(a) Aeration tank of an activated sludge system in a metropolitan wastewater treatment plant. The tank is 30 m long, 10 m wide and 5 m deep.

Aerobic wastewater treatment processes (activated sludge process)



Wastewater flow through an activated sludge system. Recirculation of activated sludge to the aeration tank introduces microorganisms responsible for oxidative degradation of the organic components of the wastewater

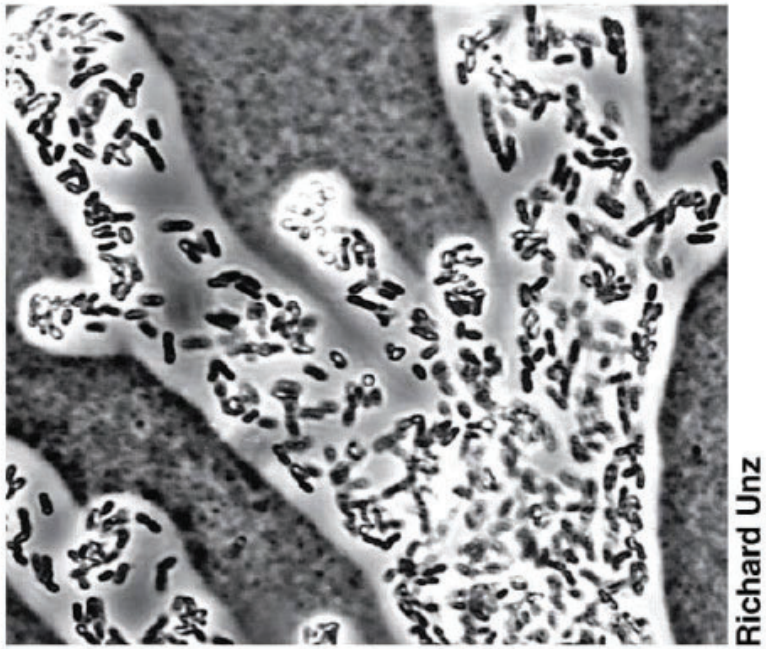
Aerobic wastewater treatment processes (trickling filter)

(c) Trickling filter process. The booms rotate, distributing wastewater slowly and evenly on the rock bed. The rocks are 10-15 cm in diameter and the bed is 2 m deep.

Wastewater and Sewage Treatment

- In the activated sludge process, wastewater is mixed and aerated in large tanks and slime-forming bacteria (e.g., *Zoogloea ramigera*) grow and form flocs
- Most treatment plants chlorinate the effluent after secondary treatment to further reduce the possibility of biological contamination

A wastewater floc formed by the bacterium *Zoogloea ramigera*



Floc formed in the activated sludge process consists of a large number of small, rod-shaped cells of *Z. ramigera surrounded by a polysaccharide* slime layer, arranged in characteristic fingerlike projections in this negative stain with India ink.

Wastewater and Sewage Treatment

Tertiary treatment

- Any physiochemical or biological process employing bioreactors, precipitation, filtration, or chlorination procedures similar to those used for purification of drinking water
- Reduces the levels of inorganic nutrients (e.g., phosphate, nitrate, nitrite)
- Most complete method of treating sewage but not widely adopted due to costs

Drinking Water Purification

- Wastewater treated by secondary methods is not yet potable, or safe for human consumption
- It requires further treatment to remove pathogens, eliminate taste and odor, reduce chemicals (e.g., iron, manganese), and decrease turbidity
- A typical drinking water treatment installation purifies raw (untreated) water

Water treatment plant

(a) Aerial view of a water treatment plant. The arrows indicate direction of flow of water through the plant.

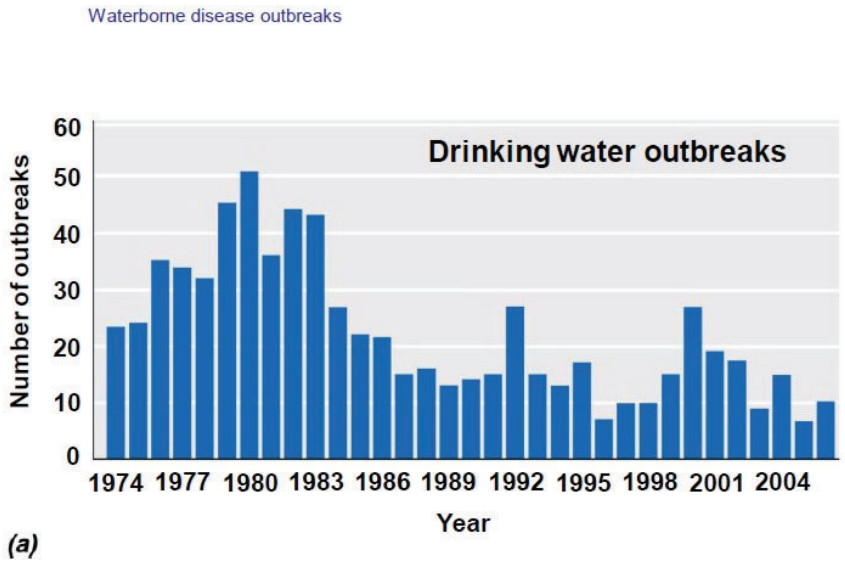
Waterborne Microbial Diseases

- Sources of Waterborne Infection
- Cholera
- Giardiasis and Cryptosporidiosis
- Legionellosis (Legionnaires' Disease)
- Typhoid Fever and Other Waterborne Diseases

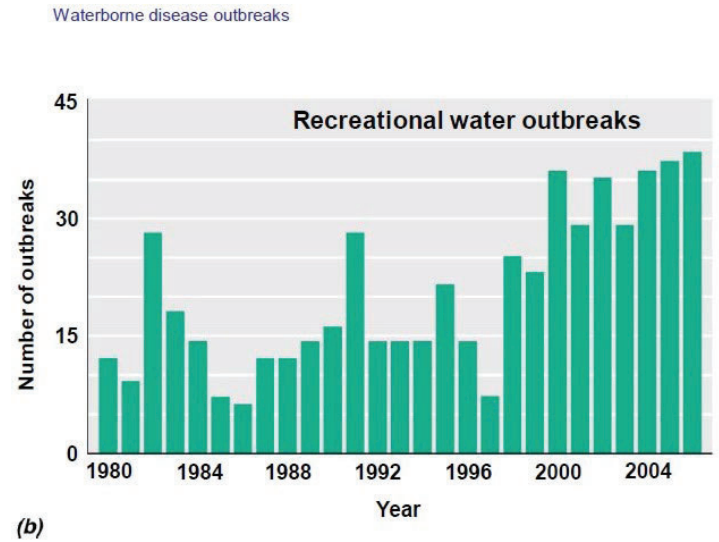
Sources of Waterborne Infection

Common sources of waterborne disease transmission include

- Potable water used for drinking and cooking
- May be improperly treated or of low quality
- May be from nonregulated sources (e.g., private wells)
- Recreational water from public ponds, lakes, swimming pools, etc.



Data were provided by the Centers for Disease Control and Prevention, Atlanta, Georgia, USA. Reported drinking water disease outbreaks from 1974 to 2006. Of 729 outbreaks, about 90% were due to biological agents (bacteria, viruses and protists)



Reported recreational water outbreaks from 1980 to 2006. Of 544 total outbreaks, almost all were due to biological agents.

Sources of Waterborne Infection

Microorganisms transmitted in water generally grow in the intestines and leave the body in feces

Feces then pollute water

- Numerous bacterial and protozoan pathogens can be transmitted in drinking water
- In the U.S. the number of disease outbreaks due to drinking or recreational water contamination is low
- Lack of adequate water treatment facilities and access to clean water contribute to the spread of infectious disease

Cholera

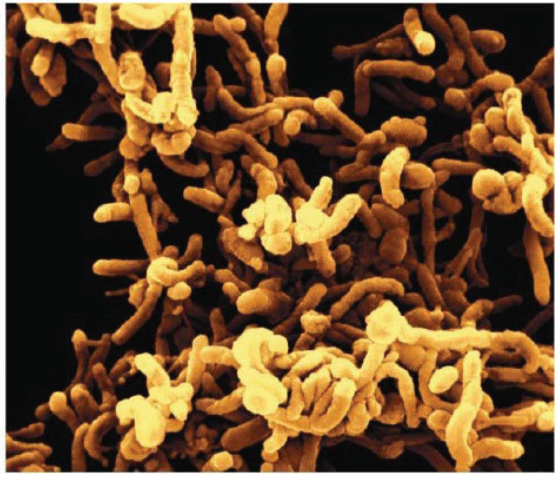
Cholera

- Severe diarrhea
- Caused by *Vibrio cholerae*
- Typically transmitted through ingestion of contaminated water
- Largely restricted to developing countries

Endemic in Africa, Southeast Asia, the Indian subcontinent, and Central and South America

- Can be controlled by application of water treatment

Cells of *Vibrio cholerae*



This colorized scanning electron micrograph shows a rod to curved rod morphology. The organism is about 0.3 μm in diameter and up to 2 μm in length.

Cholera

Pathogenesis

- *V. cholera* attaches to epithelial cells in the small intestine, where it grows and releases cholera toxin
- This enterotoxin causes severe diarrhea
- Can result in dehydration and death unless treated

Diagnosis

- Presence of *V. cholerae* bacilli in the feces of patients
- Prevention
- Immunization not recommended
- Public health measures are very important

Treatment

- Oral rehydration and electrolyte replacement therapy

Giardiasis and Cryptosporidiosis

- *Giardia intestinalis* causes giardiasis, an acute gastroenteritis
- *Cryptosporidium parvum* causes cryptosporidiosis
- These organisms are found in nearly all surface waters and are highly resistant to chlorine due to cyst formation

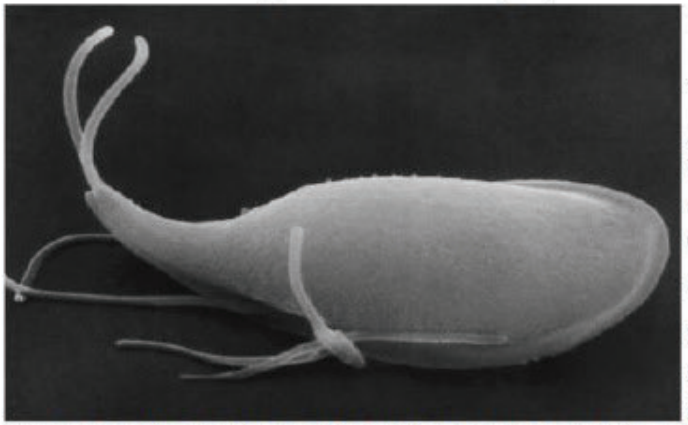
Giardiasis and Cryptosporidiosis

Giardia intestinalis

- A flagellated protest that is usually transmitted to humans in fecally contaminated water
- The protest cells produce a resting stage (cyst) that is resistant to desiccation and chemical disinfection
- Following ingestion, cysts germinate and attach to the intestinal wall, causing symptoms of infection
- Many individuals exhibit no symptoms and can act as carriers

The parasite *Giardia*

Scanning electron micrographs.



D. E. Feely, S. L. Erlandsen, and D. G. Case

(a) A motile trophozoite*. The trophozoite is about 15 μm in length.

S. L. Erlandsen

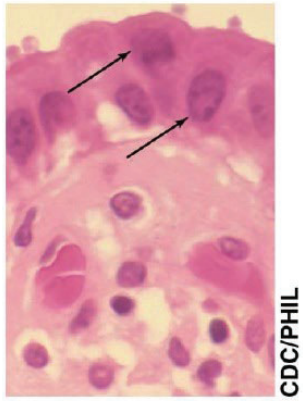
(b) A giardial cyst. The cyst is about 11 μm in length.

Giardiasis and Cryptosporidiosis

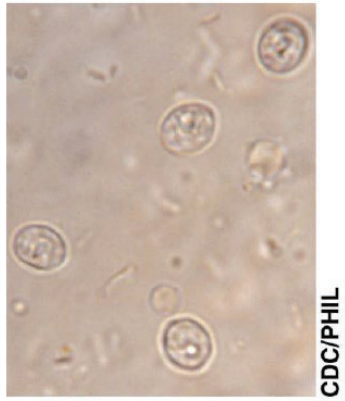
Cryptosporidium parvum

- Protist that lives as a parasite in warm-blooded animals
- Produces thick-walled cells (oocysts) that are shed in the feces of infected animals
- The oocysts are transmitted in fecally contaminated water
- Oocysts are highly resistant to chlorine and UV radiation; thus sedimentation and filtration methods are most effective at removal
- Cryptosporidiosisis usually a self-limiting mild diarrhea
- But can lead to more serious disease in compromised individuals

Cryptosporidium



(a) The arrows point to two of the many intracellular trophozoites embedded in human gastro-intestinal epithelium. The trophozoites are 2-5 µm.



(b) *The thick-walled oocysts are* 3-5 µm in diameter in this fecal sample.

Legionellosis (Legionnaires' Disease)

Legionella pneumophila

- Causes legionellosis
- Normally transmitted in aerosols
- Gram-negative bacterium with complex nutritional requirements
- Common in terrestrial and aquatic habitats
- Relatively resistant to heating and chlorination

Legionella pneumophila

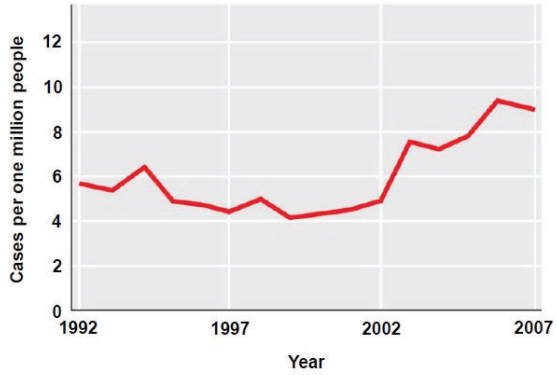
Colorized scanning electron micrograph of *L. pneumophila* cells. Cells are 0.3-0.6 μm in diameter and up to 2 m in length.

Legionellosis (Legionnaires' Disease)

Legionella pneumophila (cont'd)

- Common in cooling towers and evaporative condensers of large air conditioning systems
- The pathogen grows in the water and is disseminated in humidified aerosols
- Infection is not spread from person to person
- The prevalence of legionellosis is increasing and infections are often underreported
- Treatment with antibiotics is effective
- Prevention can be accomplished by improving maintenance and design of water-dependent cooling and heating systems and water delivery systems

Incidence of Legionnaire's disease in the United States



In 2007, there were 2716 reported cases. Data are from the Centers for Disease Control and Prevention, Atlanta, Georgia

Typhoid Fever and Other Waterborne Diseases

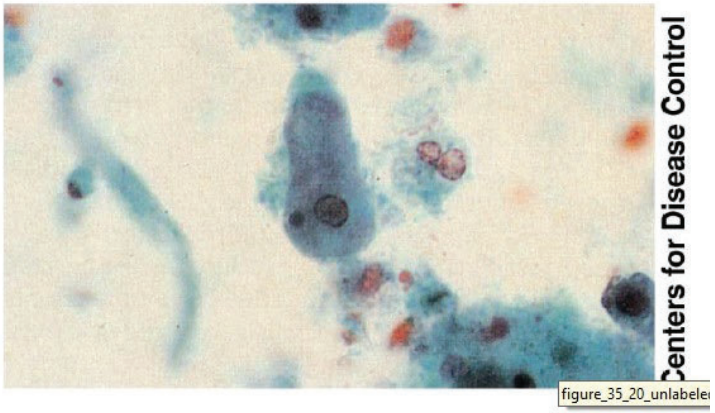
- Worldwide, the most important pathogenic bacteria transmitted by water are *Salmonella enterica* serovar *Typhi* and *Vibrio cholerae*
- *S. enterica* ser. *Typhi* causes typhoid fever and has been virtually eliminated in developed countries due to water treatment
- Viruses can also be transmitted in water and cause human disease
- For example, entero viruses such as poliovirus, norovirus, and hepatitis A are shed into water from feces
- Viruses can survive in water for relatively long periods but are inactivated by disinfection with chlorine

Typhoid Fever and Other Waterborne Diseases

Entamoeba histolytica

- A pathogenic protest transmitted to humans primarily through contaminated water and sometimes food
- Anaerobic and produces resistant cysts
- Infection can be asymptomatic or lead to diarrhea and/or dysentery
- If untreated, invasive cells can invade the liver and occasionally the lungs and brain
- Amoebicidal drugs are not universally effective

The trophozoite of *Entamoeba histolytica*, the amoeba that causes amebiasis



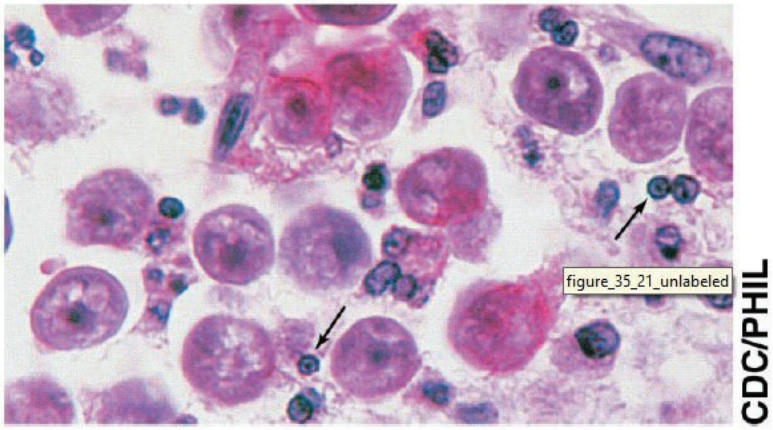
The small red structures are red blood cells. The trophozoites range from 12 to 60 μm in length.

Typhoid Fever and Other Waterborne Diseases

Naegleria fowleri

- A free-living amoeba found in soil and water runoff that can also cause amebiasis
- Infections usually result from swimming in warm, soil-contaminated water sources (e.g., hot springs or lakes)
- Enters the human body through the nose and burrows directly into the brain, causing extensive hemorrhage and brain damage (meningoencephalitis)
- Drug treatment is effective if infections are identified early

Trophozoites of *Naegleria fowleri* in brain tissue



This amoeba causes meningoencephalitis. Oval to round and amoeboid (irregularly shaped) trophozoites (arrows) are present as dark-stained structures with densely stained nuclei. There is extensive destruction of the surrounding brain tissue. Individual trophozoites are 10–35 μm long.