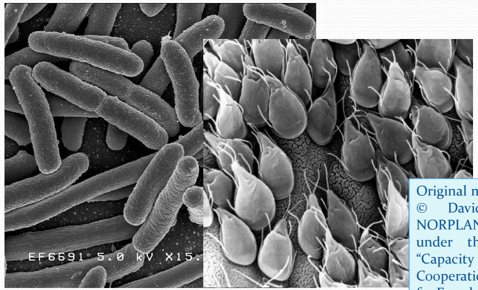
Groundwater Microbiology

by: David Banks, Hydrogeologist and thermogeologist



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NORAD supported project in MRRD:
Capacity Building and Institutional Cooperation in the field of Hydrogeology for Faryab Province , Afghanistan

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Bacteria

- Total heterotrophic plate counts are not necessarily good indicators of microbiological water quality. It is a good indicator of disinfection efficiency.
- Faecal bacteria are a better indicator of faecal contamination or pathogenic potential (faecal coliforms or streptococci)
- Total coliforms
    - Vegetative coliforms – 10-40%. In vegetation, soils, sediments, insects.
    - Faecal / thermotolerant coliforms – 60-90%, cultivated on lactose for 24 hrs at 44 C, with fermentation
  - 90% are typically Escherichia
    - One species is E. coli (generally harmless, lives in gut)

E. coli

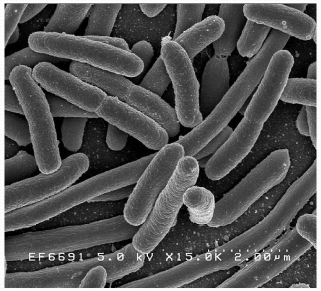
Gram negative, facultative anaerobic bacterium, around 2µm long.

Lives in mammalian gut.

E.coli and similar bacteria comprise only 0.1% of gut flora.

Are mostly harmless or even beneficial. A few strains cause disease.

Can survive for short time out of body. Transmitted by faecal-oral route.



E. coli. Public domain image

World Health Organisation

The WHO Drinking Water Guidelines (4th Edition) are non-binding…but form the background and scientific consensus for most national drinking water legislation.

In recent editions, the WHO Guidelines have moved away from highly prescriptive norms…

…and towards risk assessment health-based targets and water safety plans

- "It is not practical, and there are insufficient data, to set performance targets for all potentially waterborne pathogens, including bacteria, viruses, protozoa and helminths."

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Many parameters are indicators of pollution

- For example: coliform bacteria, faecal coliforms and faecal streptococci and even E. coli do not necessarily cause disease.
- But their presence may indicate contamination by faeces...
- ...and therefore a risk from other, more dangerous microorganisims
- Therefore we often analyse for faecal (thermotolerant) coliforms or faecal streptococci

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Indicator organisms

Selection criteria for indicator organisms include

- waterborne transmission established as a route of infection;
- occurrence in source waters;
- persistence in the environment;
- sensitivity to removal or inactivation by treatment processes;

WHO Guidelines

Table 7.9 Use of indicator organisms in monitoring

Microorganism(s)	Type of monitoring: Validation of process	Operational	Verification and surveillance
E. coli (or thermotolerant coliforms)	Not applicable	Not applicable	Faecal indicator
Total coliforms	Not applicable	Indicator for cleanliness and integrity of distribution systems	Not applicable
Heterotrophic plate counts	Indicator for effectiveness of disinfection of bacteria	Indicator for effectiveness of disinfection processes and cleanliness and integrity of distribution systems	Not applicable
Clostridium perfringens[a]	Indicator for effectiveness of disinfection and physical removal processes for viruses and protozoa	Not applicable	Not applicable[b]
Coliphages *Bacteroides fragilis* phages Enteric viruses	Indicator for effectiveness of disinfection and physical removal processes for viruses	Not applicable	Not applicable[b]

[a] Use of *Clostridium perfringens* for validation will depend on the treatment process being assessed.
[b] Could be used for verification where source waters are known to be contaminated with enteric viruses and protozoa or where such contamination is suspected as a result of impacts of human faecal waste.

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World Health Organisation

Table 7.10 Guideline values for verification of microbial quality[a] (see also Table 5.2)

Organisms	Guideline value
All water directly intended for drinking	
E. coli or thermotolerant coliform bacteria[b,c]	Must not be detectable in any 100 ml sample
Treated water entering the distribution system	
E. coli or thermotolerant coliform bacteria[b]	Must not be detectable in any 100 ml sample
Treated water in the distribution system	
E. coli or thermotolerant coliform bacteria[b]	Must not be detectable in any 100 ml sample

[a] Immediate investigative action must be taken if *E. coli* are detected.
[b] Although *E. coli* is the more precise indicator of faecal pollution, the count of thermotolerant coliform bacteria is an acceptable alternative. If necessary, proper confirmatory tests must be carried out. Total coliform bacteria are not acceptable as an indicator of the sanitary quality of water supplies, particularly in tropical areas, where many bacteria of no sanitary significance occur in almost all untreated supplies.
[c] It is recognized that in the great majority of rural water supplies, especially in developing countries, faecal contamination is widespread. Especially under these conditions, medium-term targets for the progressive improvement of water supplies should be set.

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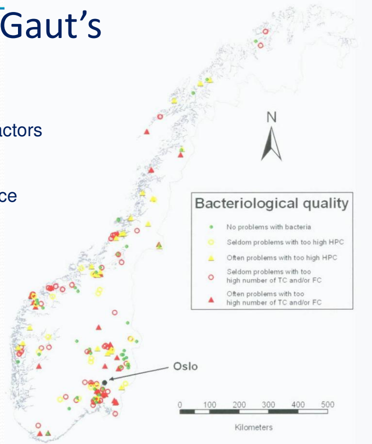
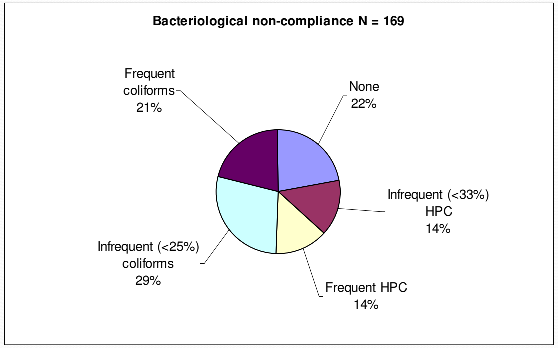
1999-2005 Sylvi Gaut's work in Norway

"Drinking water wells in bedrock: Factors influencing microbiological quality". Department of Geology, Norwegian University of Technology and Science

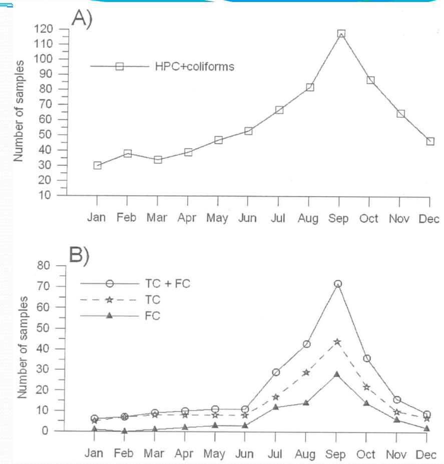
- Bacteriological non-compliance studied at 169 waterworks based on bedrock groundwater (largely untreated)



Of 169 waterworks, around half were non-compliant re. coliforms.



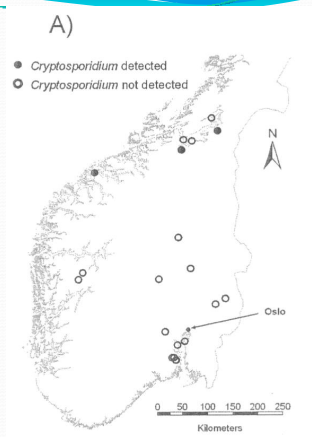
Seasonality



Typically contamination was highest at the end of summer

20 waterworks tested for *Cryptosporidium*

At 3 of 20, *Cryptosporidium* was detected.



Main factors in bacteriological quality

1. Surrounding land use (especially agriculture and latrines)
2. Distance to surface water
3. Well head completion
4. Thickness of superficial deposits (casing)

Protozoa

Giardia lambella & *Cryptosporidium parvum*

- Ability to encyst. Periodically shed in faeces from domestic and wild and animals
- Cysts or oocysts survive for prolonged periods (weeks or months)
- Cause gastrointestinal disorders. Pose a severe risk to immuno-compromised individuals such as AIDS sufferers.
- Person-to-person contact is the most important transmission route, fewer than ten cysts or oocysts are required to infect humans
- Contaminated water is also a significant infection pathway. Raw sewage can contain tens of thousands of cysts per litre.

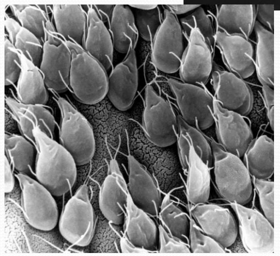
Protozoa

Giardia lambella

- ***Giardia***: cyst diameter 8-12 µm. Giardia is the most common cause of waterborne infection in the USA.

Public domain image: CDC / Janice Carr

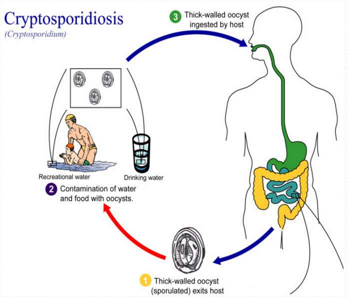
Public domain image: CDC / Stan Erlandsen



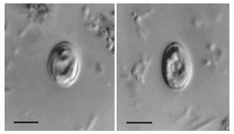
Protozoa

Cryptosporidium parvum

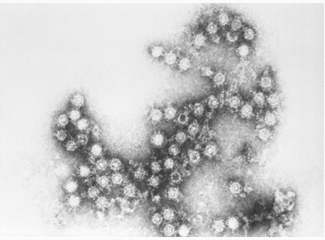
- ***Cryptosporidium***. Oocyst diameter 4-6 µm. 1993 outbreak in Milwaukee affected c. 400,000 people and cost over 96 million USD (WHO 2004). UV disinfection or membrane filtration can remove / inactivate oocysts.



Public domain image: CDC / Scale bar = 5 µm



Viruses



Enetrovirus: Stanford Health Laboratory

Viruses
- tiny. Several tens of nanometres
- e.g. polio, hepatitis

Helminths

Public domain image: CDC

Worms
- including tapeworms, schistosomiasis, elephantiasis, hookworm

Table 7.1 Pathogens transmitted through drinking-water[a]

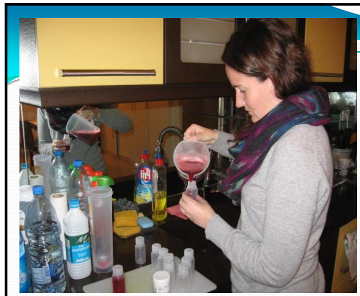
Pathogen	Health significance[b]	Persistence in water supplies[c]	Resistance to chlorine[d]	Relative infectivity[e]	Important animal source
Bacteria					
Burkholderia pseudomallei	High	May multiply	Low	Low	No
Campylobacter jejuni, C. coli	High	Moderate	Low	Moderate	Yes
Escherichia coli – Pathogenic[f]	High	Moderate	Low	Low	Yes
E. coli – Enterohaemorrhagic	High	Moderate	Low	High	Yes
Francisella tularensis	High	Long	Moderate	High	Yes
Legionella spp.	High	May multiply	Low	Moderate	No
Leptospira	High	Long	Low	High	Yes
Mycobacteria (non-tuberculous)	Low	May multiply	High	Low	No
Salmonella Typhi	High	Moderate	Low	Low	No
Other salmonellae	High	May multiply	Low	Low	Yes
Shigella spp.	High	Short	Low	High	No
Vibrio cholerae	High	Short to long[g]	Low	Low	No
Viruses					
Adenoviruses	Moderate	Long	Moderate	High	No
Astroviruses	Moderate	Long	Moderate	High	No
Enteroviruses	High	Long	Moderate	High	No
Hepatitis A virus	High	Long	Moderate	High	No
Hepatitis E virus	High	Long	Moderate	High	Potentially
Noroviruses	High	Long	Moderate	High	Potentially
Rotaviruses	High	Long	Moderate	High	No
Sapoviruses	High	Long	Moderate	High	Potentially
Protozoa					
Acanthamoeba spp.	High	May multiply	High	High	No
Cryptosporidium hominis/parvum	High	Long	High	High	Yes
Cyclospora cayetanensis	High	Long	High	High	No
Entamoeba histolytica	High	Moderate	High	High	No
Giardia intestinalis	High	Moderate	High	High	Yes
Naegleria fowleri	High	May multiply[h]	Low	Moderate	No
Helminths					
Dracunculus medinensis	High	Moderate	Moderate	High	No
Schistosoma spp.	High	Short	Moderate	High	Yes

WHO drinking water guidelines 4th edition

Sampling and analysis for bacteria requires

(a) Sterile techniques
(b) Sterile filtration of water samples
(c) Incubation under specific conditions
(d) Counting

Sampling and filtration



Delagua / Oxfam kit
preparing sterile nutrient broth

Coliform bacteria like this special "soup"

Incubation

1. Put filter paper in petri dish
2. Put petri dishes in incubator
3. Wait 60 minutes to 4 hours before switching on incubator
4. Incubate for c. 16-18 hours at 44°C (for faecal coliforms)

Counting colonies

1. Count all **yellow** colonies of diameter 1-3 mm. These are **thermotolerant coliforms**.
2. Ignore red/pink/blue/grey colonies
3. Result= No. of colonies observed / divided by sample volume