

Pollution source tracking - identifying indicator bacteria as human or bovine.

Bartholomew Masterson,
University College Dublin.

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Background (1)

Microbial pollution of water
resources is a threat to public health
and is a universal problem.

The microorganisms often are pathogens
that originate in animal wastes.

Microbial pollution of drinking-water,
and water used for aquaculture or for
recreation may cause risk to health.



Background (2)

To protect the public health, water-quality regulations are in force.

The regulations require “faecal indicator organism” concentrations to be monitored, and mandatory and desirable limits are set.

Water-quality regulations operate realistically today on a catchment basis.



Background (3)

Microbial pollution in a catchment reflects the land use, e.g. urban, agricultural, forestry, upland.....

The risk to public health comes from human sewage and from faecal material from farm animals and wildlife.



Background (4)

So, to manage catchment microbial water quality effectively, we need to know the animal source.

The indicator organisms used at present do not discriminate between human and non-human sources of faecal pollution.

Hence PA3!



Pilot Action 3

Developing Pollution Source Tracking



To develop a working method
to discriminate human and animal
microbial pollution of the
aquatic environment.



Supported by the European Union
Project co-financed by the ERDF



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A phased approach



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Phase 1

Systematic literature review: completed and published in *Environmental Forensics* December 2004.

International workshop, that identified the methods with best potential for development, held in Guilford (UK) in January 2004.



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Two methods were chosen for development in the four Member States participating in PA3.

- Bacteroides genotyping
- F+ coliphage genotyping



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Bacteroides (1)

Bacteroides species are anaerobic bacteria.

Associated with the intestinal tract of mammals.

Make up a significant portion of faecal bacteria.

Survive long in open waters up to 6 days.



Bacteroides (2)

Some species occur predominantly in either human or in animal hosts.

B. distasonis

B. thetaiotaomicron

B. vulgatus

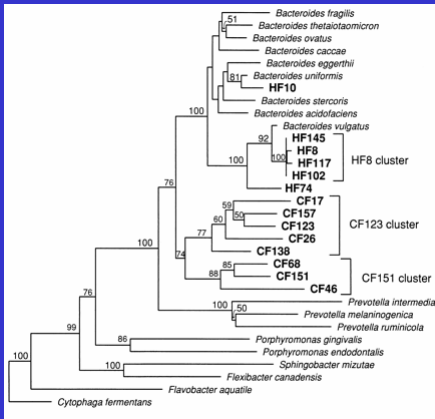
78% presence in human

7-11% presence in non-human samples



Bacteroides (3)

Host specific *Bacteroides* species identified that have genes with unique DNA segments.



← Human *Bacteroides*

← Cow *Bacteroides*

Method developed by Dr. K. Field at the Department of Microbiology, Oregon State University.



Bacteroides genotyping

Uses a polymerase chain reaction (PCR) method to detect animal-specific *Bacteroides* in water samples.

Based on primers specific for cow or human *Bacteroides* species.

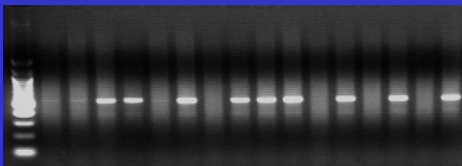


Image of a typical stained PCR electrophoresis gel



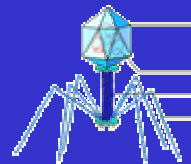
F+ coliphage genotyping (1)

The enumeration of total F+ RNA coliphages, an indicator for faecal contamination, uses a relatively inexpensive quantitative standard method (ISO10705).

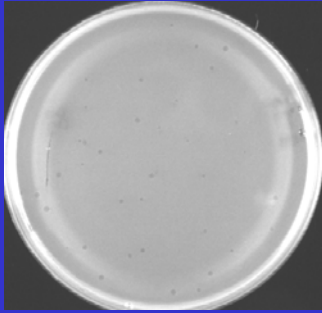


F+ coliphage genotyping (2)

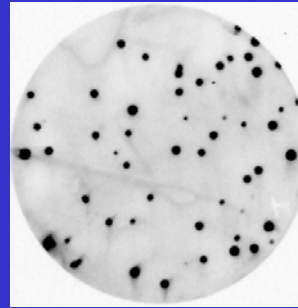
- Virus that infects *E. coli*
- Present in faecal pollution
- Resists stress factors
- Exists in four geno-groups
 - - MS2 and SP in animals
 - - GA and QB in humans



F+ coliphage genotyping (3)



Formation of plaques with indicator host strain



Transfer to membrane and Southern hybridisation with group-specific oligonucleotides



Phase 2: commission the chosen methods in the participating laboratories

Current status

- Commissioning completed.
- Validation report at final preparation stage.



Phase 3: testing the methods with environmental samples from previously-recognised polluted sites

Current status

- Fieldwork completed.
- Results informed planning of field trials during the 2005 bathing season.



Phase 4: full field trials in a variety of catchments in the participating countries

The field trials largely successful, but additional work in progress in the countries that had little rainfall during the bathing season.



Phase 5: reporting, dissemination, method adoption

Will commence in December 2005, to
be completed by April 2006.



Transnational participation

- United Kingdom (Lead Partner)
- Portugal
- France
- Ireland



United Kingdom



Andy Gawler
(Manager PA3)

Martin Walters



Portugal



Leonor Falcão
João Brandão
Jorge Machado
Baltazar Nunes
Raquel Rodrigues



France



Jean-Yves Piriou
Marie-Paule Caprais



University of CAEN

Alain Rincé



Ireland



Bart Masterson
Wim Meijer
Martin Thorp
Nora Carroll
Keith Real
Sarah Fraser

