

# Investigation of bacterial pathogen sources and transfer hydrodynamics in rural catchments.

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In Ireland, bacterial pathogens from continuous point source pollution and intermittent pollution from diffuse sources can impact both drinking water supplies and recreational waters. This poses a serious public health threat. Observing and establishing the source of faecal pollution is imperative for the protection of water quality and human health. Conventional culture methods to detect such pollution via faecal indicator bacteria have been extensively utilised but do not determine the source of pollution.

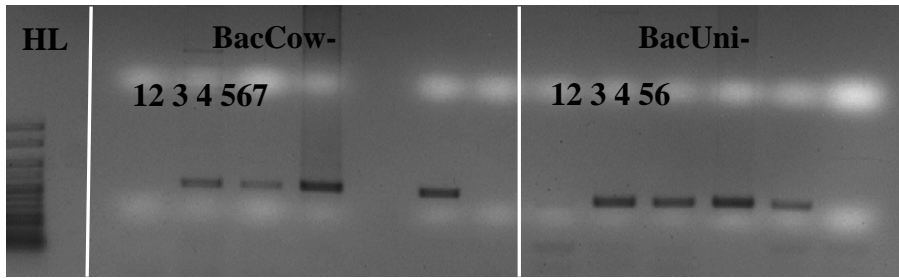
To combat this, microbial source tracking, an important emerging molecular tool was applied to detect host-specific markers in faecally contaminated waters. The aim of this study was to target ruminant and human-specific faecal *Bacteroidales* and *Bacteroides* 16S rRNA genes within rural river catchments in Ireland. Monitoring the movement of bacterial pathogens within the catchment area in storm and non-storm periods is imperative for investigating hydrological transfer dependencies.

The two catchment areas selected and monitored for this project are located in Cregduff, Co. Mayo and Dunleer, Co. Louth. These two sites differ on numerous levels. Cregduff is underlain with well-drained soil on karstic bedrock. Dunleer, however, consists of mostly poorly drained tillage soils in 70% of the catchment. This results in high nutrient losses both of phosphorus via overland flow in poorly drained soils and nitrogen leaching on the more freely drained soils in the east of the catchment. There is little surface drainage in the Cregduff catchment as this type of karst landscape is subject to hydrologic movement via groundwater pathways. The Dunleer catchment is not dominated by groundwater pathways as the River White flows through it and divides into multiple tributaries. The type of farming also varies in the two catchments. Cregduff is predominantly animal farming with little tillage. Dunleer is the opposite and is dominated by tillage/ crop farming with little animal farming.

Large (5–20L) untreated water samples were collected from the two catchment sites during storm and non-storm periods. Samples were filtered through 0.2µm nitrocellulose filters to concentrate bacterial cells and total nucleic acids were extracted. Animal and human stool samples were also collected from the catchments to determine assay sensitivity and specificity following nucleic acid extraction. Aquifer response to seasonal events was assessed by monitoring coliforms and *E. coli* occurrence using the IDEXX Colisure® Quanti Tray®/2000 system in conjunction with chemical and hydrological parameters. A universal primer targeting *Bacteroidales* (BacUni-UCD), ruminant specific *Bacteroidales* (BacCow-UCD) and *Bacteroides* primers (BoBac) and a human specific *Bacteroidales* primer (BacHum-UCD) were assayed against both faecal and water samples.

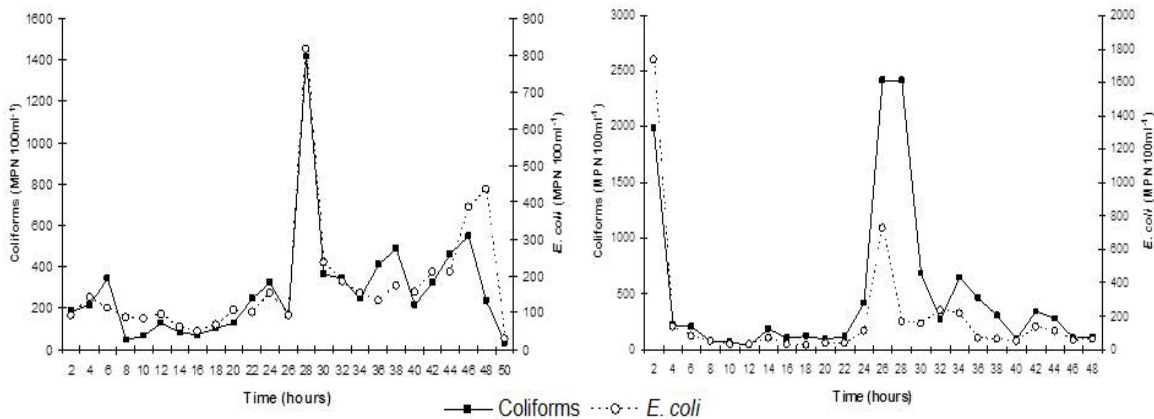
## Results

Positive amplification was achieved by BacUni-UCD and BacCow-UCD from water samples taken from three sample points along the river in Dunleer. At the Dunleer spring however, neither assay detected faecal contamination. (Figure 1.1)



**Figure 1.1 BacCow-UCD and BacUni-UCD amplicons of water samples from Dunleer.** HLV: Hyperladder™ V 500bp marker. BacCow-UCD & BacUni-UCD Lanes 1: Spring, Lanes 2: Sample 1, Lanes 3: Sample 2, Lanes 4: Sample 3. BacCow-UCD Lane 5: Blank, Lane 6: Positive Control, Lane 7: Negative Control. BacUni-UCD Lane 5: Positive Control, Lane 6: Negative Control

Colisure data for summer and winter events in Cregduff determined that coliforms and *E. coli* peaks were higher in winter. Peak values of  $2.42 \times 10^3$  100 ml<sup>-1</sup>, were seen for coliforms and  $1.73 \times 10^3$  100ml<sup>-1</sup> for *E. coli* for the winter event. Lower peak values of  $1.42 \times 10^3$  100 ml<sup>-1</sup>, were seen for coliforms and  $8.16 \times 10^2$  100 ml<sup>-1</sup> for *E. coli* for the summer event. When comparing breakthrough events following heavy rain, higher pulses were recorded in winter than in summer. Regardless of season the aquifer still responds rapidly to heavy rainfall events.



**Figure 1. Colisure data of (a) Summer and (b) winter events in Cregduff.** Both graphs show coliforms and *E. coli* for summer and winter events over a 48-hour time period.

The universal primer *BacUni-UCD* detected *Bacteroidales* in all Cregduff faecal samples while ruminant primers *BacCow-UCD* and *BoBac* detected *Bacteroidales* and *Bacteroides* in bovine and ovis faecal samples. For Dunleer, *BacCow-UCD* and *BoBac* detected *Bacteroidales* and *Bacteroides* in bovine, ovis and Capra faecal samples respectively. *BacUni-UCD* and *BacCow-UCD* detected faecal contamination in three of the four water samples from Dunleer. The colisure data shows the difference between summer and winter events in Cregduff, winter seeing higher peak values of coliforms and *E. coli*. A significant correlation ( $P < 0.0001$ ) was shown between phosphorus and turbidity for both Cregduff and Dunleer. Conventional PCR assays, as those shown in Figure 1.1, are useful as a qualitative technique but are not quantitative. In order to quantitatively determine faecal contamination in water using host specific *Bacteroidales* and *Bacteroides* markers, the more sensitive technique of Real-Time PCR will be utilised. It is also possible that this method may complement *E. coli* as a faecal indicator. It is necessary to ascertain the relationship between the occurrence of faecal pollution, pollution source and hydrology in order to create an accurate representation of contamination in environmental water. The microbial source tracking techniques and hydrodynamic model that will be designed by this project will be of vital importance for water quality control in Ireland.

## References

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