The National Waters Inventory of Scotland

The Distribution of AMR Genes in Freshwater in Scotland

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The James Hutton Institute

Introduction

The National Waters Inventory of Scotland (NWIS) is an archive of water samples representing primarily end of catchment locations. It provides a national baseline of the state of Scotland's water resource, which is required to understand resilience to the accumulating catchment pressures of delivering national objectives for food and renewable energy production, water supply for people, livestock, ecology and industry.

AMR found in human and livestock populations can enter water bodies as a result of sewage discharges and agricultural run-off. AMR is further transferred in the environment via wildlife. The aquatic environment therefore becomes a mixing pot of inorganic and organic contaminants as well as faecal (possibly pathogenic) and indigenous microbes exchanging AMR genes from both natural and anthropogenic sources.

This project aims to define antibiotic resistance patterns in surface waters and determine environmental drivers of resistance.

Environmental DNA was extracted from the NWIS samples and used to look for tetracycline resistance genes, since tetracycline s are predominantly used for animal use. Our research questions are:

1. Does prevalence of tetracycline resistance genes vary spatially across Scottish surface waters?
2. If so, do hot-spots of tetracycline resistance genes correlate with large herd sizes?
3. Does prevalence of tetracycline resistance genes correlate with water quality and land use?

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Methods

Sample Collection and Water Filtration

1L of water was collected in sterile bottles and transported on ice to the lab. Repeat samples were collected over time from some of the sites during the two year sampling period. Samples that were in storage for four days or less were selected for further molecular analysis. This was based on unpublished data indicating that *E. coli* did not significantly decline until after four days storage; different water typologies did not affect this outcome. Water was filtered onto a 0.22 µm membrane filter using a vacuum pump. Negative filtration controls consisted of 1L of sterile MilliQ water. A final DNA archive of 207 samples (excluding negatives) was achieved.

Molecular Methods

Environmental DNA was extracted from the membrane filters using the Dneasy PowerWater kit (Qiagen). Environmental DNA quantity was measured using the Qubit assay, and quality was checked by the measurement of the 260/280 ratio (Nanodrop) and visualisation on an agarose gel. DNA was normalised to 5 ng/µl. Tetracycline resistance genes, tetO[1]and tetW[2], were amplified from environmental DNA using the SYBR green assay . PCR negatives consisted of sterile reagent grade water as a template. A Cq value of less than 5 was considered to be negative.

Land Use and Herd Size Mapping

Land use was produced by summarising the Land Cover of Scotland (LCS88) produced by the Hutton. Animal numbers come from 2km x 2km summaries of the Scottish Agricultural Census data produced by EDINA (Edinburgh University) .

Results

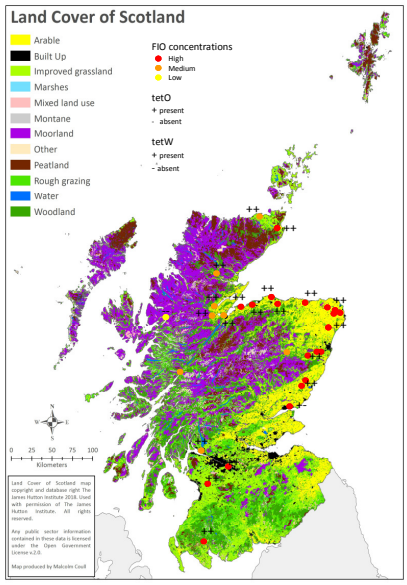

Figure 1 Land use and land cover in Scotland

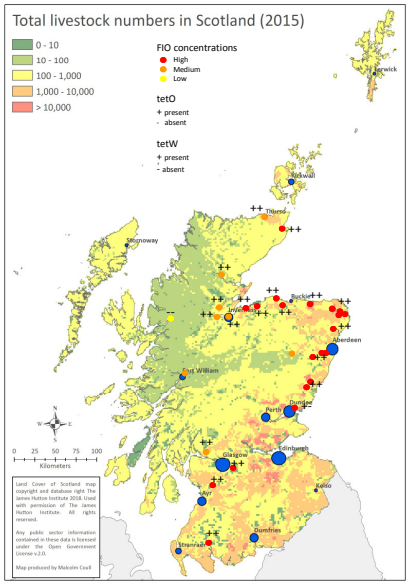

Figure 2 Livestock and population densities in Scotland

Conclusions

- Preliminary results suggest that:
  - The prevalence of tetO is correlated with water quality, such that it is absent when water quality is high.
  - The prevalence of tetW may also correlate with water quality, but there are complicating factors that warrant further study.

References

1. Malhotra-Kumar et al (2005). Antimicrob. Agents and Chemother. 49: 4798-4800.
2. Aminov et al (2001). Appl. Env. Microbiol. 67: 22-32.