Studies to distinguish between human and animal faecal pollution using F-RNA coliphages and faecal sterols

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Abstract

Human enteric viral infections are considered to be predominantly associated with human wastes, as opposed to animal wastes, and a distinction between these has benefits for water quality control and risk assessment. A variety of techniques have been described to distinguish between human and animal faecal pollution of water. F-RNA (male-specific) coliphages have been classified into four sero-groups and evidence has been presented that two of these sero-groups are specific for human excreta and the other two for animal excreta. Certain chemical compounds such as the faecal sterols cholesterol and coprostanol yielded valuable results in attempts to distinguish between faecal pollution of human and animal origin.

In this study the application of F-RNA coliphages and faecal sterols to distinction between human and animal excreta has been investigated. Faecal sterols were extracted from water and analysed by gas chromatography using published methods that were adapted for the detection and quantification of cholesterol and coprostanol. Wastewater containing predominantly animal excreta was collected from cattle, pig and chicken feedlots. Wastewater containing predominantly human excreta was collected from hospitals.

Results revealed that F-RNA coliphages isolated from wastewater from four different hospitals consisted almost exclusively of genotypes 2 and 3. Only F-RNA coliphage genotypes 1 and 4 were detected in all three wastewater samples from cattle feedlots, while F-RNA coliphage genotypes 1, 3 and 4 were detected in all three chicken feedlot wastewater samples. Five wastewater samples from pig feedlots contained typically F-RNA coliphage genotypes 3 and 4.

Cholesterol and coprostanol were detected in ranges of 28 to 1 013 µg/ℓ and 19 to 1 441 µg/ℓ in wastewater, respectively. Coprostanol concentrations were more than double the cholesterol concentration in wastewaters from hospitals and pig feedlots in the five samples analysed. The opposite applied to wastewater from cattle and chicken feedlots, where cholesterol concentrations in all seven samples were higher than coprostanol concentrations. Analysis of wastewater from a poultry feedlot yielded a high cholesterol:coprostanol ratio and the presence of predominantly F-RNA coliphage genotype 4, confirming the specificity of these determinants for animal wastes.

The results of this study confirmed earlier reports on the specificity of F-RNA coliphage genotypes 1 and 4 for animal wastes, and genotypes 2 and 3 for human excreta, in a part of the world where investigations using these methods are limited. The same applies to the higher ratio of coprostanol: cholesterol in human excreta, and the higher ratio of cholesterol: coprostanol in animal excreta. The observations suggested that further optimisation of applying these indicators in combination may lead to the development of procedures for the meaningful distinction between faecal pollution of human and animal origin in quantitative terms.

Keywords: cholesterol, coprostanol, ratios, genotyping, F-RNA phages, wastewater

Introduction

The maintenance of the microbiological quality of water systems used for drinking, recreation, and the harvesting of seafood is critical, as faecal contamination of these systems can pose risks to human health as well as result in economic losses (Jagals et al., 1995; Sinton et al., 1998; Scott et al., 2002). Although limited epidemiological data are available, human faeces are generally perceived to constitute a greater human health risk than animal faeces (Sinton et al., 1998; Schaper and Jofre, 2000; Scott et al., 2002). Understanding the origin of faecal pollution is paramount in assessing associated health risks as well as the actions necessary to remedy the problem while it exists (Griffin et al., 2000; Scott et al., 2002). The identification and apportioning of human and animal faecal inputs to natural water cannot be done with great confidence by isolated methods and it has been suggested that both microbiological and chemical determinations be used to generate more reliable data (Sinton et al., 1998). Microbial determinations include using faecal streptococci: faecal coliform ratios, *Bifidobacteria*, phages infecting *Bacteroides fragilis* HP40 specific to humans, *Rhodococcus coprophilus* specific for animals and F-RNA phage subgroups (Sinton et al., 1998; Scott et al., 2002). The limited number of chemical determinations researched includes the faecal sterol profiles, caffeine known to be excreted by humans from the diet and even linear alkyl benzenes specific to humans (Sinton et al., 1998; Scott et al., 2002).

Enteric bacteriophages like the F-RNA phages are widely used as indicators of enteric viruses and research has shown that these viral indicators could be used to discriminate between human and animal faecal pollution (Havelaar and Hogeboom, 1984; Havelaar et al., 1986; Furuse, 1987; Sinton et al., 1998; Leclerc et al., 2000; Scott et al., 2002). The male-specific F-RNA phages have been classified into four groups based on their phylogenetic diversity and it has been reported that groups 1 and 4 occurred in animal wastewater while groups 2 and 3 usually predominated in human wastewater (Osawa et al., 1981; Havelaar et al., 1986; Furuse, 1987; Havelaar et al., 1990; Hsu et

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