Genotyping of F-RNA coliphages isolated from wastewater and river water samples

A Sundram[1]*, N Jumanlal[1] and MM Ehlers[2]
[1]*Umgeni Water, PO Box 9, Pietermaritzburg 3200, South Africa*
[2]*Department of Medical Virology, University of Pretoria/NHLS, Pretoria 0001, South Africa*

Abstract

Faecal contamination of water sources can only be managed if the sources of pollution are identified and thus tools to distinguish between human and animal faecal sources are needed. Male-specific F-RNA 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. The sero-groups are readily detectable as genotypes with the application of molecular techniques, notable hybridisation tests using gene probes specific for each genotype. A standard ISO method was used for the detection of F-RNA coliphages. Wastewater containing predominantly animal excreta was collected from cattle, pig and chicken feedlots. Wastewater containing predominantly human excreta was collected from hospitals. The F-RNA genotyping study was qualitative and focussed on determining which groups of F-RNA phages were present in different wastewater and environmental samples. The results were in agreement with earlier reports on the specificity of F-RNA phage Genotypes 1 and 4 for animal wastes, and Genotypes 2 and 3 for human excreta. Besides being detected in human wastewater, F-RNA Group 3 was detected in both chicken and pig wastewater indicating that this Group was not specific to humans. The results showed that F-RNA phage Group 2 was the only group detected in seven of the twenty-six positive samples from the Dorpspruit, Slangspruit and uMsunduze Rivers, suggesting human faecal input. All other river water samples contained mixtures of F-RNA phage groups suggesting that the faecal input could not exclusively be ascribed to humans or animal sources alone. This suggested that both human and animal sources were responsible for contamination of receiving water at these sampling sites. The research proved that the genotyping of F-RNA phages was feasible in practice and could be used to assist in identifying the source of faecal pollution.

Keywords: genotyping, F-RNA phages, wastewater, river, human, cattle, pig, chicken, Groups 1 – 4

Introduction

The direct discharge of domestic waste, leaching from poorly maintained septic tanks, and improper management of farm waste are suspected to be the major sources of water-borne diseases (Isobe et al., 2004). There is a greater health risk when humans are exposed to water polluted with human faeces, as opposed to animal faeces (Jagals et al., 1995; Scott et al., 2002). Information on the origin of faecal pollution is important because it gives an indication of the pathogens that may be expected, the risk of infection and the treatment that may be needed to control the transmission of the disease (Jagals et al., 1995; Sinton et al., 1998; Scott et al., 2002). It is now well known that the presence of F-RNA phages in a water sample generally indicates pollution by human or animal faeces (IAWPRC, 1991; DWAF, 1996; Leclerc, 2000; Schaper and Jofre, 2000).

The male-specific F-RNA phages have been classified into four groups on the basis of their serological and physiochemical properties including MS-2, f2, and JP501 in Group 1; GA, BZ13 and JP34 in Group 2; Qβ, VK and TW18 in Group 3 and SP, F1 and TW28 in Group 4 (Havelaar and Hogeboom, 1984; Furuse, 1987; Beekwilder et al., 1996; Leclerc, 2000). These four groups of F-RNA phages have been fully characterised, with Groups 2 and 3 found to be highly associated with human faecal contamination and Groups 1 and 4 more specific for animal contamination (Osawa et al., 1981; Havelaar et al., 1986; Furuse, 1987; Havelaar et al., 1990; Hsu et al., 1995; Sinton et al., 1998; Schaper and Jofre, 2000; Schaper et al., 2002; Scott et al., 2002; Cole et al., 2003). It has been reported that more than 90 to 95% of phages detected from sewage and environmental samples using *Salmonella typhimurium* (*S. typhimurium*) WG49 as the host, were F-RNA phages (Havelaar and Hogeboom, 1984; Debartolomeis and Cabelli, 1991; Hsu et al., 1995; Grabow et al., 1997; Uys 1999). In addition, *S. typhimurium* WG49 was successfully used for the detection of all four genotypes and was recommended for studying the presence and distribution of genotypes of F-RNA phages in environmental samples (Schaper and Jofre, 2000).

Researchers have successfully developed genotyping techniques to group the F-RNA phage isolates to help distinguish between faecal pollution of human or animal sources (Hsu et al., 1995; Beekwilder et al., 1996; Griffin et al., 2000). Hsu and coworkers (1995) indicated that highly specific nucleic acid probes for the detection of F-RNA phages could be used as an alternative to sero-typing.

Earlier research found that all isolates from animal faecal sources were grouped as Sero-Groups 1 and 4 with the exception of isolates from pig and piglet faeces which often contained Groups 2 and 3 F-RNA phages, suggesting that group classification would not always distinguish between human and porcine faecal contamination (Osawa et al., 1981; Furuse, 1987; Havelaar et al., 1990; Hsu et al., 1995; Cole et al., 2003). This occurrence could be explained by the dietary and living conditions of pigs, which have historically involved exposure to human faecal

* To whom all correspondence should be addressed.
☎ +2733 341 1342; fax: +2733 341 1501;
e-mail: Ashogan.Sundram@umgeni.co.za
Received 6 June 2005; accepted in revised form 7 November 2005.