

Microbial Source Tracking for the monitoring of faecal pollution in environmental waters and bioindicator organisms

Main author: BARBARA CIOFFI (ISTITUTO ZOOPROFILATTICO SPERIMENTALE DEL MEZZOGIORNO (IZSM))

Co-authors: Barbara Cioffi, Marina Monini, Simona Schiavo, Francesco Serra, Hiba Dakroub, Ilaria Di Bartolo, Sonia Manzo, Lucio De Maio, Esterina De Carlo, Giovanna Fusco, Maria Grazia Amoroso

INTRODUCTION

Most of the water contamination comes from slurry discharges by intensive livestock farms, the intensification of which in recent years has contributed to the increase in zoonotic diseases. Viruses are excreted at high concentrations in the faeces of infected humans and animals and of the healthy population. Considering that there are approximately 15 350 cattle farms, 1 298 water buffalo farms, 34 641 pig farms and 9 698 sheep and goat farms in the region of Campania, the identification of sources of faecal contamination through Microbial Source Tracking (MST) markers provides fundamental support for proper management of water. The aim of this study was to evaluate water faecal pollution in marine-coastal waters through the research of targets which make it possible to identify the origin of the contamination. We used specific MST targets to evaluate the circulation of human viruses (human adenovirus and JC polyomavirus) as well as animal viruses (bovine polyomavirus and ovine polyomavirus, porcine adenovirus) via quantitative real-time PCR. We also evaluated the Integrated Ecotoxicological Assessment (VEI) on bioindicator organisms, which allows for the univocal identification of environmental pollution sources.

METHODOLOGY

During our study, 310 seawater samples were collected, every two months, from 27 collection points in the Gulf of Naples, (from the mouth of the Volturno river to the city of Torre del Greco). These points are characterised by urban, industrial and rural discharges and located near mussel farms and natural banks.

Prior to viral detection, samples were concentrated from 10 l to 50 ml through filtration and 400 ml of concentrate underwent nucleic acid extraction using the Qiasymphony automatic extraction system. The presence of the following viruses was investigated by qPCR using specific primers and probes: human adenovirus, JC polyomavirus, bovine/buffalo polyomavirus and sheep/goat and porcine adenovirus.

The VEI (integrated eco-toxicological assessment) was carried out using a battery of assays with *Vibrio fisheri*, *Paracentrotus lividus* and *Dunaliella tertiolecta*. Data was integrated by means of a statistical procedure that attributes a weight to the various observed effects in order to derive a single ecotoxicological risk score (PT %) which indicates the level of risk observed (PT % < 5 = negligible, 5 < PT % < 10 = moderate, 10 < PT % < 20 = high, 20 < PT % < 50 = very high, PT % > 50 = extremely high).

RESULTS

In our samples, we revealed the presence of human adenovirus with a prevalence of 12 % (37/310), human polyomavirus (8 %) (25/310), bovine/buffalo polyomavirus (2.2 %) (7/310), while porcine adenovirus and ovine polyomavirus were never detected. Quantification analysis, carried out via standard curves, showed the following average values of genome copies per litre (gc/l): HAdV 1.2×10^5 , JCPyV 7.9×10^3 , BPyV 5.1×10^2 . Sequencing analysis detected HAdV serotype 41, subgenus F, showing 100 % nt. id. with AdV41 strains previously isolated in Northern Italy both in patients and water discharges. In the eco-toxicological evaluation, all the samples tested showed a VEI toxicity score which indicated the presence of high to extremely high risk. The most sensitive test, which always responded with a hormetic effect, was that with bacterium *V. fisheri*. The algal assay with *D. tertiolecta* mainly showed an evident inhibition of algal growth, sometimes even at 50 % dilution of the sample. The less sensitive test was with the sea urchin *P. lividus*, in the case of the fertilisation test and the embryotoxicity test.

DISCUSSION

Virological assays highlighted the presence of viral faecal indicators of predominantly human origin (HAdV and JCPyV, 20 %) even though animal targets (BPyV, 2.26 %) were also detected. These indicators showed us that discharges flowing into our coastal waters are mainly represented by urban wastewater, and to a minor extent by discharges at livestock farms. The sequencing data also confirmed the anthropogenic origin of the isolated strains, underlining the importance of searching for viral pathogens in the waters and also the surveillance of human cases. All the samples analysed for VEI presented a not-negligible risk. Some samples, although classified as 'excellent' for bathing purposes, always showed high or very high ecotoxicological risk. The VEI index therefore seems to be a valid and truly descriptive tool of the pollution status of the water body. Our results demonstrate that the MST tool is highly specific, sensitive and provides data on the origin of faecal contamination of water bodies in any geographical area, distinguishing human and animal origins.