Towards a risk assessment for *Giardia* sp. and *Cryptosporidium* sp. in Portuguese fluvial beaches: a seasonal sampling over two years

C. Júlio a, I. Ferreira b, S. Martins c, C. Sá c, H. Ângelo a, J. Guerreiro a, R. Tenreiro c

a Instituto Nacional de Saúde Dr. Ricardo Jorge, Departamento de Doenças Infecciosas, LNR de Infeções Gastrointestinais, Av. Padre Cruz, 1649 – 016 Lisboa
b Universidade de Lisboa, Faculdade de Ciências, Centro de Oceanografia, Lisboa.
c Universidade de Lisboa, Faculdade de Ciências, Centro de Biodiversidade, Genómica Integrativa e Funcional (BioFIG), Lisboa.

INTRODUCTION

Waterborne outbreaks of diarrheal illness reported worldwide are mostly associated with *Cryptosporidium* sp. and *Giardia* sp. (1). Lake and river waters contaminated with (o)ocysts are major routes of human exposure (2), making essential the development of preventive strategies for water safety. Since monitoring of water contamination with (o)ocysts is not routinely performed in Portugal, this study aims to unveil the possible associations between Portuguese fluvial beach characteristics and risk for public health caused by different genotypes of *Giardia* sp. and *Cryptosporidium* sp..

MATERIAL AND METHODS

Nineteen beaches were selected according to land use and environmental parameters and sampled, on winter and summer, for the presence of *Giardia* sp. and *Cryptosporidium* sp., as well as faecal indicators and physicochemical parameters. Immunomagnetic separation was performed according the US EPA Method 1623 (3) with Dynal procedure (Dynabeads, Iberlab & Immunoreag), followed by detection of (o)ocysts by immunofluorescence microscopy after staining with FITC-labelled monoclonal antibody. Cysts viability was also confirmed by nucleic acid dye (DAPI) staining.

RESULTS

*Cryptosporidium* sp. and *Giardia* sp. are largely disseminated through Portuguese rivers, with *Giardia* sp. cysts present at least in 83% of the sampled beaches and *Cryptosporidium* sp. oocysts not lower than 74%.

Seasonal differences in *Giardia* sp. cysts were perceived, with more than 50% of total cysts identified for the second winter. Nevertheless, *Cryptosporidium* sp. oocysts are broadly scattered along the sampled periods.

Although the bathing water quality analysis showed that different classifications may be attributed depending on the applied Bathing Water Directive (76/160/EEC or 2006/7/EC), 70% of concordant classifications were unveiled.

Considering characteristics such as land use, sewage discharge and water quality, different clusters were highlighted. The dendrogram analysis evidence patterns among sampled beaches, establishing three major groups in terms of overall quality.

The bathing water analysis based on 2006/7/EC showed that an "Excellent" categorized beach is unlikely to have (o)ocysts presence.

The beach grouping analysis and association according to their characteristics points to their usefulness as a new cost-effective risk assessment methodology.

CONCLUSIONS

*Cryptosporidium* sp. and *Giardia* sp. are visually spread throughout the Portuguese rivers, with *Giardia* sp. cysts present in at least 83% of the sampled beaches and *Cryptosporidium* sp. oocysts not lower than 74%.

Seasonal differences in *Giardia* sp. cysts were perceived, with more than 50% of total cysts identified for the second winter. Nevertheless, *Cryptosporidium* sp. oocysts are broadly scattered throughout the sampled periods.

Although the bathing water quality analysis showed that different classifications may be attributed depending on the applied Bathing Water Directive (76/160/EEC or 2006/7/EC), 70% of concordant classifications were unveiled.

Considering characteristics such as land use, sewage discharge and water quality, different clusters were highlighted. The dendrogram analysis evidence patterns among sampled beaches, establishing three major groups in terms of overall quality.

The bathing water analysis based on 2006/7/EC showed that an "Excellent" categorized beach is unlikely to have (o)ocysts presence.

The beach grouping analysis and association according to their characteristics points to their usefulness as a new cost-effective risk assessment methodology.

This work is supported by Project PTDC/SAU-ESA/71609/2006.