

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

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Abstract background: Waterborne outbreaks of diarrhoeal illness reported worldwide are mostly associated with *Cryptosporidium* sp. and *Giardia* sp. Lake and river waters contaminated with (oo)cysts are major routes of human exposure making essential the development of preventive strategies for water safety. Since monitoring of water contamination with (oo)cysts 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..

Abstract 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 with Dynal procedure (Dynabeads), followed by detection of (oo)cysts by immunofluorescence microscopy after staining with FICT-labelled monoclonal antibody. Cysts viability was also confirmed by nucleic acid dye (DAPI) staining.

Abstract Results: The results show that *Giardia* cysts are present at least in 83% of the sampled beaches. Presence of *Cryptosporidium* oocysts was not lower than 74%. Additionally, seasonal differences on (oo)cysts amount were perceived. A dendrogram analysis highlighted different clusters which evidence patterns among the sampled beaches. Principal Component Analysis also indicates distinct weights for land use, physical-chemical and microbiological parameters in these different clusters.

Abstract Conclusions: The results of the present study indicate that *Giardia* sp. and *Cryptosporidium* sp. are widely distributed and should be considered as a public health issue. Moreover, beach clusters turn out to be a helpful tool to assess the public health risk.

Keywords (4-6words): *Giardia*, *Cryptosporidium*, risk assessment, fluvial beaches