SESSIONE 17 EVOLUZIONI EPIDEMIOLOGICHE E DIAGNOSTICHE IN PARASSITOLOGIA

APPLICATION OF MODERN SEQUENCING TECHNOLOGIES TO INVESTIGATION OF OUTBREAKS CAUSED BY PROTOZOAN PARASITES IN ITALY

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Introduction

Human outbreaks due to parasites of the genera Cryptosporidium and Giardia have been reported in many European countries, and globally. These events are often associated with exposure to contaminated water, although foodborne outbreaks can also occur, particularly by consumption of raw vegetables. Both humans and animals contribute to environmental contamination with Cryptosporidium and Giardia, and large genetic variability exists among isolates of these parasites. Traditionally, investigation into the nature of the causative agent has relied upon PCR and sequencing of a few genetic markers to provide information at the spcies or genotype level. In recent years, the introduction of high-throughput sequence technologies has provided new, powerful tools for outbreak investigation, since the suspected agent(s) can be fully characterized at the genome level. However, application of this technology to outbreaks caused by parasites is still very limited.

Materials and Methods

To characterize parasite isolates at the genome level, (oo)cysts needs to be purified from clinical, water or food samples prior to DNA extraction. This is usually obtained by immune-magnetic separation with parasite-specific anitbodies. The genomic DNA is then extraced and checked for residual bacterial contamination by a generic 16S rDNA PCR. Due to teh limited amount that is normally obtained, a Whole Genome Amplification step is performed. For Next Generation Sequencing (NGS) experiments, libraries are prepared and sequenced on Illumina platforms. Raw sequence reads are processed to identify Single Nucleotide Polymorphisms (SNPs) and structural variants.

Results

Here, we describe the results of a comparison of Giardia duodenalis genomes from a waterborne outbreak that occurred in the Bergamo province in January 2018. The outbreak involved 75 individuals, and classical genotyping showed that all patients were infected with Assemblage A, sub-assemblage A2, which is a genetic variant commonly found in humans but not in animals. The genomes of different isolates were investigated for the presence and distribution of SNPs, and this showed that few hundreds SNPs were specific for each isolate (i.e., singletons or private SNPs). Thus, outbreak genomes of Giardia were extremely similar, although not identical. Clustering analyses, which included unrelated genomes from Canadian outbreaks and sporadic cases of giardiasis from Europe, fully supported the close relatedness of the outbreak samples.

Conclusions

The example described shows that application of high-throughput sequence technologies can support epidemiologic investigation by providing extensive genetic characterization of the parasite. The availability of additional genomes and the development of ad-hoc bioinformatics pipelines are necessary for a more widespread and informative application of this methodology to parasitic outbreaks.