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MOLECULAR DETECTION OF ENTEROVIRUSES IN ENVIRONMENTAL SAMPLES AND STUDY OF RECOMBINATION BETWEEN CIRCULATING STRAINS.

ABSTRACT

The genus of Enteroviruses belongs to Picornaviridae family. According to the degree of their genetic relatedness, they are divided into four species: HEVA, HEVB, HEVC and HEV-D Enteroviruses are non enveloped viruses with a 7.500 nt single stranded positive RNA genome protected by an icosahedral capsid. The enterovirus genome consists of three major regions: the 5′untranslated region (5′UTR), the open reading frame (ORF) and the 3′untranslated region (3′UTR). ORF is translated into a single polyprotein and is then processed to generate four structural (VP1-VP4) and seven non-structural (2A-2C and 3A-3D) proteins.

Enteroviruses are transmitted by fecal-oral route and multiply in gastrointestinal tract. Infected people, symptomatic or asymptomatic, shed enteroviruses in large amounts in the environment by their faeces. Despite the reduction of viral amount following sewage treatment, enteric viruses can contaminate environmental waters causing a potential risk for public health by rejoining the food chain. The environmental surveillance constitutes a great tool for estimating the extent and the duration of poliovirus circulation in a population.

Enteroviruses, like other RNA viruses, have high mutation rate due to the lack of proofreading activity during genome replication. In addition, recombination plays a paramount role in the evolution of enteroviruses. Genetic exchanges between enteroviruses can give rise to new viral genotypes that may be extremely virulent and dangerous for public health. Environmental surveillance could be a useful tool for the study of enterovirus evolution, as long as a great number of enteroviruses can be isolated from environmental samples.

The aim of the present thesis was the isolation of enteroviruses from environmental samples and the molecular study of any putative recombinant

strains. The first step was the selection of the appropriate method for the detection and the isolation of enteroviruses from environmental samples. The most appropriate methods that were the two phase separation method-cell culture and the electronegative filter-semi nested PCR used for the detection of enteroviruses in river water and sewage. Enteroviruses were detected in 30% of the sewage samples. Finally, two enteroviruses from river water and seven from sewage were isolated in cell cultures. The full genome analysis of the environmental strains and three poliovirus strains isolated from poliomyelitis cases, revealed that inta- and inter-serotypic recombination events have played a major role in their evolutionary history. The contribution of recombination to enterovirus evolution is substantial, giving rise to new multi recombinant genetic lineages with unknown properties.