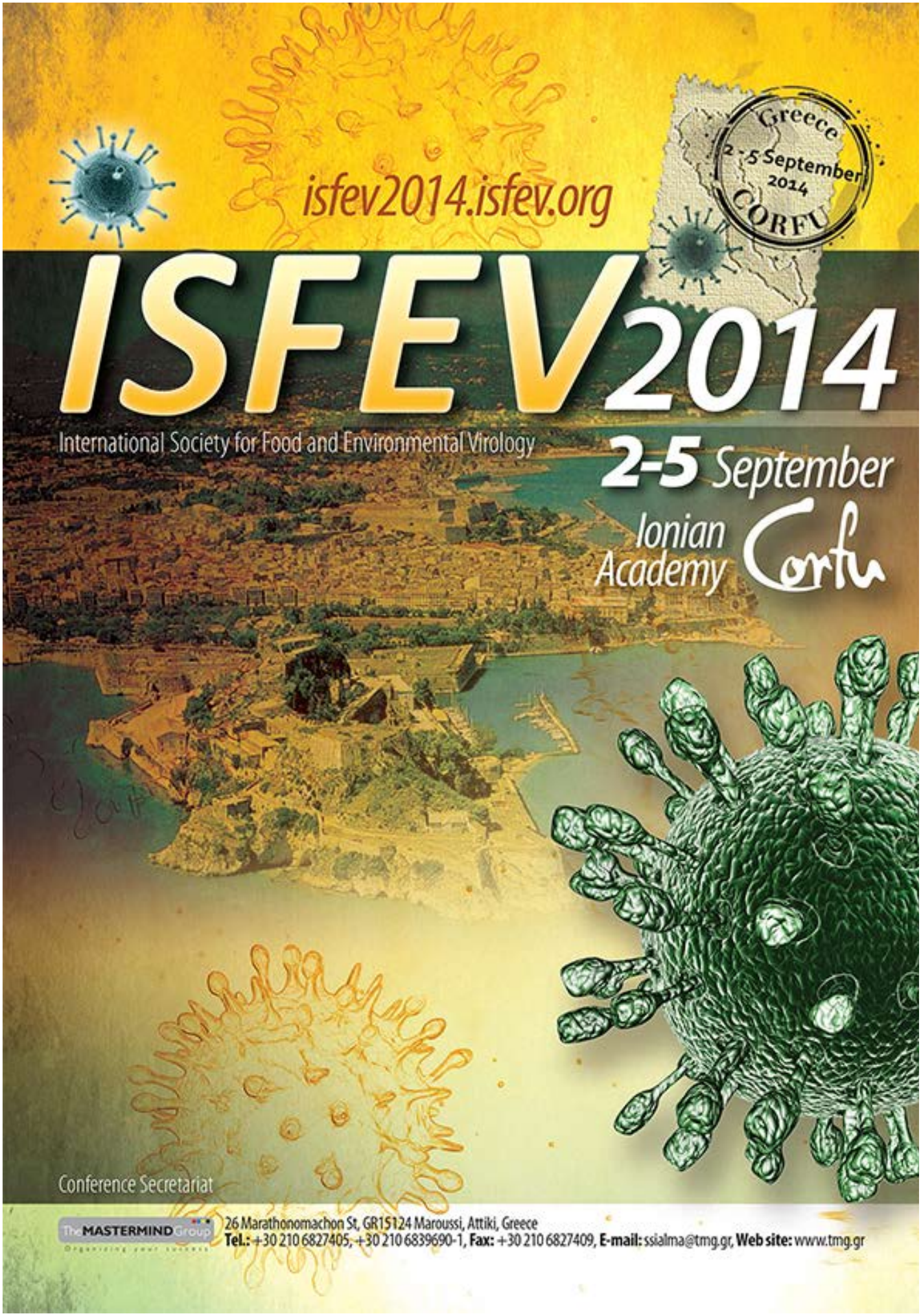
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10.15-10.30 O42: "The possible role of Acanthamoeba polyphaga in spreading and protection against chemical disinfection of Human Adenovirus type 5 in water"

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Introduction.

Adenoviruses are responsible for a wide range of health effects, including respiratory, gastrointestinal and urinary infections. They are excreted in large numbers in human feces and it is known to be present in aquatic environments, as river and sea water, but also in treated drinking-water supplies and sewage. For this reason, many studies suggest Adenoviruses as indicators of viral contamination in water and of the viral removal efficiency in wastewater depuration plants because of its high resistance to some treatment and disinfection processes. Free-living amoebae have been recovered from similar water reservoirs, and it has been shown that they may act as reservoirs or vehicles of various microorganisms living in the same environment by phagocytosis, without kill them.

Objectives. In this work it was studied the interaction between Human Adenovirus type 5 (HAdV 5) and Acanthamoeba polyphaga (AP) in water environment in order to highlight the role of protection from chemical disinfection of internalized viruses.

Methods. In the first part of the study a series of experiments were performed to standardize a methodology for virus-amoeba “co-cultivation”. Acanthamoeba polyphaga (ATCC strain 1501/18) was cultured axenic in PYG medium and incubated at 24°C. The acanthamoebae were taken from the medium and inoculated on a 24-well plate to provide a monolayer till the mean concentration of 105 cells/ml. Meanwhile an aliquot of Human Adenovirus type 5 (ATCC strain VR-5) was added to an A549 cell culture flasks to grow the viruses. After 5 days and a visible cytopathic effect, the virus titer of 3.16 x 104 DCP50/ml was counted by Karber method titration.

After these experiments, a series of solutions formed by water, AP and HAdV both free than in infected A549 cells, were co-cultured together for 1 day at 25°C and the viral uptake was assessed by direct immunofluorescence kit (IF) (17-020, Argene, France).

In a second series of experiments, the efficacy of sodium hypochlorite disinfection against AP and HAdV either singly, by cultural methods, or when co-cultured, as above method, was assessed. In particular 3 different concentration in water were tested: 5; 2.5 and 1 mg/L for 24h contact time.

Results. The data obtained by the co-culture trials demonstrated that HAdV 5 was incorporated into the host amoeba in water only when was in infected A549 cells, confirming, as published by other authors, that amoebae have a preference of prey size.

In singly disinfection tests, the results were similar to known data with AP more resistant than HAdV to chemical disinfection: amoeba still remained alive with 5 mg/L sodium hypochlorite while the viruses loss the infectivity with 2.5 mg/L. In co-cultured trials, at this disinfectant concentration, we found HAdV in AP cytoplasm.

Conclusion. The results of the study confirm and underline the possible role of protection of Acanthamoeba polyphaga for Human Adenovirus type 5 against chemical disinfection in water environment especially when virus is in infected cells. More deeply studies in co-cultured experiments can clarify if HAdV infectivity is still present in amoebae after disinfection, revealing a new system of viral resistance in water environment.