ISOLATION AND IDENTIFICATION SOME FOOD-BORN VIRUSES

By

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B.Sc. (Open Education Center), Fac. of Agriculture, Ain Shams Univ. (2009)

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ABSTRACT

Naglaa Abozied Seif EL-Alfy: Isolation and Identification of some Food-borne Viruses. Unpublished M. Sc. Thesis, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, 2016.

In recent years it has been recognized that viruses are an important cause of food-borne disease, viruses do not grow or multiply in or on foods, but foods may become contaminated with human viruses and transmit infection. Five samples from drainage water were collected from El-Rahawy (Giza); Sabal (El menofiya); Tala (Gharbiya); Baher El-baker (Port Saied) and El- Manzalla (Daqahliah), in addition to fifteen samples from vegetables (Lettuce, watercress and green onion) obtained from the sites of drainage water, and twenty clinical stool samples obtained from ill childrens, Razi mokattam in greater Cairo and Abo El-Reish Hospital, Cairo, Egypt. The samples were extracted and concentrated for Rotavirus and Enterovirus detection by molecular, serological and biological methods. The Rotaviruses were assayed qualitatively and quantitatively by (Real time PCR) in concentrated drainage water and it was detected in Baher El-baker and El- Manzalla but not detect in drainage water of Sabal, Tala and El-Rahawy. It was also detected in concentrated extract of vegetables and two clinical stool samples. The Enteroviruses were assayed qualitatively and quantitatively by (Real time PCR) in concentrated drainage water and it was found in Tala and El-Rahawy but not detected in drainage water of Sabal, Baher El-baker and El- Manzalla, as well as it was detected in concentrated extracts of vegetables.

On the other hand concentrated extracts of vegetable samples and drainage water samples which gave negative results with ELISA and Rapid chromatographic Immunoassay card (RCIC) tests. But the concentrated clinical stool samples gave positive results with RCIC

except eight samples. So, all negative samples were inoculated on Vero, Caco-2 and HEp-2 cell line for propagation of Rotavirus and Enterovirus and assayed again using ELISA and RCIC tests for Rotavirus and Enterovirus antigen detection in propagated samples. It was found that, the water samples and clinical stool samples gave positive results with RCIC test while the extracts of vegetables crops gave positive result with ELISA tests. The isolated Rotavirus and Enterovirus on cell lines were identified depending on some biological properties. It was found that, the titer of Rotavirus and Enterovirus particles estimated by plaque assay on Caco-2 and HEp-2 cells were ($9.5x10^6$) and ($1.2x10^4$) PFU/ml respectively. The viruses caused also death of survival cells, round cells and giant cells. Electron microscope showed that, Rotavirus and Enterovirus particles are clarified from infected cell culture have naked, spherical with Icosahedra symmetry. The amplification products of *vp6* gene of Rotavirus using conventional RT-PCR was in expected size 231 bp and the amplification products of *vp1*gene of Enterovirus using conventional RT-PCR was in expected size 242bp.

The action sequence of nucleotides molecular genetics vp6 and vp1 rotavirus and Enterovirus using DNA Sequencer. As has been the work of an analysis of the sequences through Bio Informatics using specialized programs. The work of the similarity for sequences of Enterovirus and Rotavirus Egyptian isolate between isolates recorded in the gene bank and the percentage of similarity using phylogenetic tree. Based on MSA analysis, the phylogenetic tree was performed and shows four clusters in which the Egy.

Key Words.

Rotavirus, Enterovirus, Drainage water, Food-borne viruses, RT-PCR, rt-RT-PCR, cell line, Electron microscope, Haematoxylin and Eosin stain, ELISA,

Rapid chromatographic Immunoassay card, sequencing, alignment, phylogenetic tree.

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