APPLICATION OF ENTERIC VIRUSES IN THE DETECTION OF WATER POLLUTION

By

MOHAMED IBRAHIM HASAN AZZAM

B.Sc. Agric. Cooperative Sc., Higher Institute for Agric. Cooperation, 2003

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Approval Sheet

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ABSTRACT

Mohamed Ibrahim Hasan Azzam: Application of Enteric Viruses in the Detection of Water Pollution. Unpublished M.Sc. Thesis, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, 2010.

This study aims to evaluate the virological, bacteriological and physico-chemical properties of the Nile River at El-Rayah El-Menofy before (inlet) and after (outlet) treatment in three drinking water stations. Water samples were taken during the period from February 2007 to November 2009. The bacteriological analyses involved were coliphage assay as a potential indicator of sewage pollution; total viable bacterial counts (TVBCs); total coliforms (TC) and estimation of fecal coliforms (FC), fecal *streptococci*. This study also included the detection of human viruses (enteric viruses and H5N1) by both of RT-PCR and real-time-RT-PCR throughout four seasons.

The results of physicochemical tests revealed that, El-Bagour site (inlet and outlet) especially in warmer seasons (summer and spring) was suffering from chemical pollution. While both of Menof and Shibin El-Kom (inlet and outlet) are within the permissible standard limits. On the other hand, the bacteriological analyses showed that TVBCs for River water (inlet) ranged from $0.3X10^4$ to $240X10^4$ cfu/ml and from $0.2X10^4$ to $160X10^4$ cfu/ml at 22°C and 37°C, respectively, while for drinking water (outlet) ranged from 30 to100 cfu/100ml and from 20 to 80 cfu/100ml at 22°C and 37°C, respectively. Identification of *E.coli* isolates in inlet water samples were identified according to bergey's manual.

Bacteriophages infecting *Escherichia coli* were detected in both of sewage polluted samples and chlorinated water samples especially in warmer seasons (summer, spring and autumn). The phage concentration ranged from

$3X10^2$ to $8.0X10^9$pfu/ml. However, maximum counts were recorded during summer and the minimal were detected in winter. The results of the fecal indicators counts revealed that their densities increased from up to down stream. The result of the present investigation indicated that, the Nile River water at El-Rayah El-Menofy is subjected to sewage pollution and consequently high microbial contents were detected even after treatment in drinking water stations.

Water samples from the tested sites were subjected to using ultrafiltration process to detect enteroviruses and H5N1 using specific primers throughout four seasons. Enteroviruses were detected using RT-PCR and rt-RT-PCR in inlet water of El-Bagour and Shibin El-Kom stations in summer season only, while H5N1 was not detected in all sites through out four seasons. Transmission electron microscopy revealed that the phage particles had an isometric head and long-contractile tail. Some particles appeared to have a short tail with full heads. While enteric virus particles were found to be an isometric particles with 24–30 nm in diameter.

Key Words: Indicator Coliform bacteria, Pollution, Coliphages, Enteroviruses, H5N1, RT-PCR, real-time-RT-PCR, Drinking water, El-Rayah El-Menofy, Nile River.

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LIST OF ABBREVIATIONS

(A)	
A_{260}/A_{280}	Absorption at 260 nm /Absorption at 280 nm
ATC	Automatic temperature compansation
ATCC	American Type Culture Collection
et.al.,	And other (*et alii*)
APHA	American public health association
AI	Avian influenza
(B)	
Ba*	El-Bagour inlet
Ba**	El-Bagour outlet
bp	Base pair
BOD	Biochemical oxygen demand
(C)	
°C	Centigrade
cDNA	Complementary DNA
CFU	Colony forming unit
CLEQM	Central Laboratory for Environmental Quality Monitoring
cm	Centimeter
COD	Chemical oxygen demand
C_T	Threshold cycle
(D)	
DEPC	Diethylpyrocarbonate
dNTPs	deoxy nucleotide triphosphate
DO	Dissolved oxygen
DNA	Deoxy ribonucleic acid
DPI	Days post-inoculation
DTT	Dithiothreotol
(E)	
EB	Ethidium bromide
EC	Electrical conductivity
EDTA	Ethylene diamine tetra acetic acid
e.g.,	For example (*Exampli gratia*)
EM	Electron microscope
EMB	Eosin methylene blue

EPA	Environmental Protection Agency
EtoH	Ethanol
EV	Enteroviruses
(F)	
F	Forward
FAO	Food and Agriculture Organization
FC	Fecal coliform
Fig.	Figure
FS	Fecal *streptococci*
(G)	
g	gram
gal	gallon
(H)	
HA	Hemagglutinin
HPC	Heterotrophic bacterial population
HPAI	Highly pathogenic avian influenza
hrs	Hours
(I)	
IC	Ion chromatography
ICP-ES	Inductively coupled plasma-Emission spectrometry
ICTV	International Committee on Taxonomy of Viruses
i. e.	That is (*id est*)
Inst.	Institute
(K)	
KDa	Kilo Dalton
Kg	Kilo gram
Km	Kilo metre
Kv	Kilo volt
(L)	
L.	Liter
Lab.	Laboratory log to the base 10
Log_{10}	log to the base 10
(M)	
M	Molar

m	Meter
Me*	Menof inlet
Me**	Menof outlet
µg	Microgram
µm	Micrometer
µmhos	Micromose
mg	Milligram
MF	Membrane filter
MilliQ	ultra pure water
min.	Minute
µl	Microliter
ml	Milliliter(s)
mm	Millimeter(s)
mM	Millimolar
mmhos	Millimose
MMLV	*Moloney murine leukemia virus*
MPN	Standard most probable number
MUG	4-methyl umbelliferyl-β-D-glucuronide
M.Wt	Molecular weight

(N)

NA	Neuraminidase
Na_2HPO_4	Di-sodium hydrogen phosphate
nm	nanometer
N	Normality
No.	Number
NRC	National research centre
nt	Nucleotide
NTU	Nephelometric turbidity unit
NWRC	National Water Research Center
NWQC	National Water Quality Center

(P)

PAGE	Polyaccrylamide gel electrophoresis
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PFU	Plaque forming unit
pH	Hydrogen ion concentration
ppm	Parts per million
PTA	Phosphotungstic acid
%	Percentage

(R)