MOLECULAR TYPING OF ENTEROVIRUSES

by

JOHANNA CHRISTINA VIVIER

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This thesis is dedicated to my parents

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JOHANNA CHRISTINA VIVIER

PROMOTER: Prof WOK Grabow

CO-PROMOTER: Dr MM Ehlers

DEPARTMENT: Medical Virology, Faculty of Medicine, University of Pretoria

DEGREE: M.Sc (Medical Virology)

SUMMARY

The enteroviruses comprise a large genus belonging to the family Picornaviridae. Sixty-six immunologically distinct serotypes are known to cause infections in humans. Infection has been associated with a wide range of diseases including aseptic meningitis, myocarditis, respiratory illness and insulin dependant diabetes mellitus. In view of the major health impact of enteroviruses, sensitive and reliable techniques for the detection and identification of this large group of closely related viruses are essential. Traditionally enteroviruses have been detected by isolation in cell culture, and their serotypic identity has been established by neutralization of infectivity with serotype-specific antisera. This procedure is cumbersome, laborious and insensitive.

The basic objective of this study was to use advanced molecular techniques to develop a rapid, simple and inexpensive assay for the detection and serotyping of enteroviruses in water sources and clinical specimens and to apply these techniques in research on the diagnosis and incidence of enterovirus infection.

The molecular techniques, optimised and assessed in this study, proved suitable for the rapid and sensitive detection and typing of enteroviruses in clinical specimens and environmental samples. The polio-nonpolio RT-multiplex PCR proved useful for the rapid and sensitive detection of polioviruses and for its distinction from nonpolio enteroviruses in environmental water samples. In addition to the sensitivity and specificity of the poliovirus detection protocol, the poliovirus detected can be subjected to strain specific typing with the Sabin specific triplex RT-PCR. Restriction enzyme analysis allowed the rapid identification of nonpolio enteroviruses, yielding

valuable information on viruses circulating in the environment and reducing the requirement for complete nucleotide sequence analysis. Sequencing within the 3'end of the VP1 region of the enterovirus genome proved useful for the typing of enteroviruses that were not typeable with restriction enzyme analysis.

The integrated cell culture (ICC) nested PCR approach, followed by restriction enzyme analysis proved more sensitive than the RT-multiplex PCR and cell culture techniques for the detection of low numbers of enteroviruses in drinking water samples and clinical specimens. Serotyping results indicated a high incidence of coxsackie B viruses (CBV) in water samples and clinical specimens. The drinking water supplies concerned constitute a risk of CBV infection of 3.91 x 10^{-3} (treatment unit A) and 7.4 x 10^{-3} (treatment unit B) which is an order of magnitude higher than the yearly acceptable risk of one infection per 10 000 consumers proposed for drinking water supplies.

This study has shown that PCR followed by restriction enzyme analysis was a sensitive tool for the detection and typing of enteroviruses in clinical and water samples. Results indicated that molecular techniques proved suitable to replace conventional detection and typing methods.

Key words: Enteroviruses, coxsackie A viruses, coxsackie B viruses, echoviruses, polioviruses, serotyping, polymerase chain reaction, restriction enzyme analysis, sequencing, risk assessment.

MOLEKULÊRE TIPERING VAN ENTEROVIRUSSE

deur

JOHANNA CHRISTINA VIVIER

PROMOTOR: Prof WOK Grabow

MEDE-PROMOTOR: Dr MM Ehlers

DEPARTEMENT: Geneeskundige Virology, Mediese Fakulteit, Universiteit van Pretoria.

GRAAD: M.Sc (Medical Virology)

OPSOMMING

Enterovirusse bestaan uit 66 immunologies verskillende serotipes wat infeksies in mense kan veroorsaak. Infeksie word geassosieer met 'n wye verskeidenheid siektes waaronder aseptiese meningitis, miokarditis, respiratoriese siektes en diabetes mellitus. Weens die gesondheidsimpak van enterovirusse, is vinnige, sensitiewe en spesifieke opsporings- en tiperings tegnieke 'n noodsaaklikheid. Enterovirusse word tradisioneel opgespoor deur isolasie in selkulture en getipeer deur neutralisasie met serotipe-spesifieke antisera. Hierdie metodes is egter duur en tydrowend.

Die doel van hierdie studie was om 'n sensitiewe, koste- en tydeffektiewe metode te ontwikkel vir die opsporing en tipering van enterovirusse in omgewings- en kliniese monsters.

Die molekulêre metodes wat geoptimiseer en geëvalueer is in hierdie studie, is effektief vir die vinnige en sensitiewe opsporing en tipering van enterovirusse in kliniese en water monsters. Poliovirusse is suksesvol getipeer deur die polio-nie-polio RT-PKR, gevolg deur die Sabin spesifieke tripleks RT-PKR. Beperkingsensiem analise was 'n effektiewe metode vir die tipering van nie-polio enterovirusse. Basispaar-opeenvolging-bepaling van die VP1 gedeelte van die enterovirus genoom was 'n waardevolle hulpmiddel om enterovirusse te tipeer wat nie deur beperkingsensiem analise getipeer kon word nie.

Die geïntegreerde selkultuur n-PKR blyk 'n meer sensitiewe metode te wees as die multipleks-PKR vir die opsporing van enterovirusse in drinkwater en kliniese monsters. Coxsackie B virusse was die mees algemene virusse wat opgespoor is in kliniese en water monsters gedurende

die studie periode. Die insidensie van coxsackie B virusse in drinkwater is gebruik as basis vir risiko analise. Resultate dui daarop dat die drinkwater 'n jaarlikse risiko van 3.91 x 10^{-3} (suiwerings eenheid A) en 7.4 x 10^{-3} (suiwerings eenheid B) vir CBV infeksie inhou. Hierdie waardes is 'n orde groter as die waarde van 1 infeksie per 10 000 verbruikers, voorgestel deur die US EPA vir drinkwater.

Hierdie studie het bewys dat PKR gevolg deur beperkingsensiem analise 'n sensitiewe metode is vir die vinnige opsporing en tipering van enterovirusse in water en kliniese monsters. Die resultate dui daarop dat molekulêre metodes konvensionele opsporings en tiperings metodes kan vervang.

Sleutelwoorde: Enterovirusse, coxsackie A virusse, coxsackie B virusse, echovirusse, poliovirusse, serotipering, polymerase ketting reaksie, beperkingsensiem analise, basispaar-opeenvolging-bepaling, risiko analise.

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LIST OF ABBREVIATIONS

AC	Antigen capture
AFP	Acute flaccid paralysis
AM	Aseptic meningitis
BGM	Buffalo Green Monkey
Bp	Base pairs
CA	Coxsackie A
CB	Coxsackie B
cfu	Colony forming units
CNS	Central nervous system
CPE	Cytopathogenic effect
DALY	Disability adjusted life years
DNA	Deoxyribonucleic acid
E	Echo
EOR	Efficiency of recovery
EV	Enterovirus
h	Hour
HIV	Human immuno-deficiency virus
IDDM	Insulin dependant diabetes mellitus
l	litre
LBM	Lim Benyesh-Melnick
M	Molar
min	minutes
NIV	National Institute of Virology
n-PCR	Nested PCR
NPEV	Non-polio enterovirus
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PFU	Plaque forming units
ppm	Parts per million
PPM	Paralytic poliomyelitis
PV	poliovirus
RD	Rhabdosarcoma
RE	Restriction enzyme
RFLP	Restriction fragment length polymorphism
RIA	Radio Immuno Assay
RNA	Ribonucleic acid
RT	Reverse transcription
SABS	South African Buro of Standards
SSCP	Single strand conformation polymorphism
TCID	Tissue culture infectious dose
Unt	untypeable
US EPA	United States Environmental Protection Agency
UTR	Untranslated region
UV	ultraviolet
VP	Viral protein
WHA	World Health Assembly
WHO	World Health Organization

LIST OF SYMBOLS

C	Concentration
R	Recovery
I	Fraction of pathogens that is capable of infection
DR	Inactivation efficiency of the treatment process
Vc	Daily individual consumption of unboiled drinking water
λ	Lambda, Poisson parameter
Pi	Probability of infection
r	Dose response parameter
$P\chi$	Probability of one or more infections over period χ
χ	Number of days of exposure
V	volume