NOVEL SCREENING METHODS FOR THE DETECTION OF *YERSINIA ENTEROCOLITICA* IN INFECTED BLOOD USED FOR TRANSFUSION

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Abstract

Between 1991-1996, 8 patients experienced rare life-threatening reactions that followed the transfusion of blood infected with *Yersinia enterocolitica*. The first reported case occurred in 1991 and was followed by seven others that directly caused or contributed to the death of 5 of 8 patients. *Y. enterocolitica* is a food and water borne infection of the gastrointestinal tract which in adults is often asymptomatic. An unknown number of those infected experience a period of self-limiting bacteraemia. The large volume of blood collected during donation phlebotomy may contain small numbers of bacteria that can increase in number during blood bank storage, producing potentially lethal levels of bacteria and toxin. Currently there are no reliable methods available to distinguish blood donations that present the greatest risk from those that present little risk.

This thesis, reports on the evaluation of two techniques to prevent the transfusion of blood infected with *Y. enterocolitica*. The first, a molecular method, was used to amplify bacterial DNA in blood by Polymerase Chain Reaction (PCR). A 425 bp product was amplified from DNA extractions of infected blood. Results showed that the technical complexities of the methodology, together with poor sensitivity and the need for large-scale donation sampling make PCR as applied for this purpose unattractive.

An Enzyme Linked Immunosorbent Assay was developed to detect current/recent infection with *Y. enterocolitica* in healthy blood donors. Polystyrene beads were coated with bacterial proteins to detect IgA antibody to *Y. enterocolitica* in human serum. The sera from donors of confirmed unit infections, paired sera from culture-proven *Y. enterocolitica* gastrointestinal tract infection and sera from volunteer blood donors were tested. Results showed that the sera of six bacteraemic blood donors tested contained elevated levels of IgA antibody. High rates of positivity (26/27), were detected in sera from culture-confirmed GIT infection and a rate of 4.04% seropositivity was found among 495 blood donors enrolled in a clinical trial. Results showed a strong correlation between IgA seropositivity, and recipient risk associated with the transfusion of blood heavily infected with *Y. enterocolitica*. The work demonstrated how the use of a simple screening test for recent infection, could be used to exclude high risk donations and improve the safety of blood transfusion in New Zealand.

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AIDS	Acquired Immune Deficiency Syndrome
AS-1	Additive solution - 1
BHI	Brain heart infusion
BHI-B	Brain heart infusion broth
bp	Base pair
CIN	Cefsulodin-irgasan-novobiocin
CMI	Cell mediated immunity
CPD	Citrate phosphate dextrose
CR-MOX	Congo red, magnesium oxalate
DIC	Disseminated intravascular coagulation
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethylenebis(oxyethylenenitrilo)-tetraacetic acid
ELISA	Enzyme linked immunosorbent assay
GIT	Gastrointestinal tract
HB_sAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HLA-B:27	Human leucocyte antigen B:27
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-1	Interleukin-1
INF-γ	Gamma interferon
kb	Kilobase
kD	Kilodalton
lcr	Low Ca^{++} restricted
LPS	Lipopolysaccharide
MIS	Mucosal immune system
MMWR	Morbid mortality weekly report
MWM	Molecular weight marker

MQ	Millipore filtered - type 1 laboratory reagent grade water
O/N	Overnight
PCR	Polymerase chain reaction
PTH	Post transfusion hepatitis
PV	Predictive value
pYV	Yersinia virulence plasmid
RO	Reverse osmosis - type 3 laboratory reagent grade water
RP	Released protein
sIgA	Secretory immunoglobulin A
SIS	Systemic immune system
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide electrophoresis
TNF	Tumour necrosis factor
TE	Tris EDTA
Tris	Tris-(hydroxymethyl) aminomethane
WWII	World war II
Yops	Yersinia outer membrane protein

LIST OF APPENDICES