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

A thesis presented in partial fulfillment of the requirements for the degree of
Master of Science in Microbiology
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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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LIST OF ABBREVIATIONS

| | |
|--------------------|--|
| 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(oxyethylenitrilo)-tetraacetic acid |
| ELISA | Enzyme linked immunosorbent assay |
| GIT | Gastrointestinal tract |
| HB _s Ag | 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 | Morbidity 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 |

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