A reverse vaccinology approach to identifying subunit proteins for use in vaccines against *Brachyspira pilosicoli* infections in humans and animals

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Dedicated

To

My loving parents who first sparked my interest in science

To

My beloved wife, Zahra, and our children, Amin and Mahdi,

who has been a great source of inspiration and encouragement

A. Movahedi

THESIS DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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ABSTRACT

The anaerobic intestinal spirochaete *Brachyspira pilosicoli* is the causative agent of "intestinal spirochaetosis" (IS), a disease of humans and a number of animal species. IS has been reported in adults and children worldwide but the prevalence in people living in poor hygienic conditions, indigenous populations, homosexual males, and in immunocompromised people is much higher than in other populations. IS is also widespread in pigs and chickens, and causes significant economic impact in the associated industries. To date attempts to develop a vaccine against *B. pilosicoli* to protect humans and animals have not been successful.

In this study a reverse vaccinology approach was used, in which 24 putative open reading frames (ORFs) derived from a partial genome sequence of *B. pilosicoli* were subjected to *in silico* and laboratory screening processes to identify potential efficacious vaccine antigens. *In silico* analysis of the ORFs using a range of bioinformatics algorithms assigned 12 ORF products as periplasmic, outer membrane, or secretory proteins, and these were given a high priority as potential vaccine candidates. The 12 selected ORFs were amplified from a human strain of *B. pilosicoli* (Wes-B) and cloned. Products from nine ORFS were successfully over-expressed in an *Escherichia coli* expression system, and then purified using affinity chromatography.

In an *in vitro* immunogenicity trial all the recombinant proteins except for NAV-P27 were strongly recognised in Western immunoblots by a mouse serum raised against *B. pilosicoli* strain WesB, and by a subset of convalescent sera from pigs naturally and experimentally infected with *B. pilosicoli*. In an analysis of *in vivo* immunogenicity, the post-immunisation mouse sera raised against each recombinant protein reacted strongly with each specific proteins, and also recognised the native

protein in extracts of *B. pilosicoli* strain WesB. Sequence analysis of four randomly selected ORFs showed that these were highly conserved amongst the genomes of different human and swine strains of *B. pilosicoli*. Evaluation of all the data obtained in the reverse vaccinology approach resulted in selection of four ORF products (NAV-P3, NAV-13, NAV-22 and NAV-31) as being potentially protective antigens to be analysed for their further efficacy.

These four recombinant proteins were assessed for their efficacy as vaccine components in a mouse model of IS, where the animals were challenged with a human strain of *B. pilosicoli*. The proteins all induced systemic and local antibody responses, and tended to reduce spirochaete colonisation following experimental infection. These proteins used individually or in combination now have the potential to be further developed into a new vaccine to prevent *B. pilosicoli* infections.

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PUBLICATION ARISING FROM THIS THESIS

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Movahedi, A., Hampson, D.J. (2008) Recent advances in identifying bacterial antigens for vaccine development. *Veterinary Microbiology* 131: 1-13

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Proceeding of international conference:

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Movahedi, A., Hampson, D.J. (2004) Distribution, sequencing and expression of the *clpX* gene as a histidine-tag protein. School of Veterinary and Biomedical Sciences, Murdoch University, Western Australia, (Awarded the Invitrogen Life Technologies prize as the best poster on Molecular Microbiology).

Movahedi, A., Hampson, D.J. (2005) Reverse vaccinology and construction of recombinant protein library to develop a potential vaccine against human strains of *Brachyspira pilosicoli*. School of Veterinary and Biomedical Sciences, Murdoch University, Western Australia.

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ABBREVIATIONS

List of commonly used abbreviations in the thesis:

Abbreviation	Expansion
aa	Amino acid
A_{600}	600 nm Absorbance
ABI	Applied Biosystems Inc.
AP	Alkaline phosphatase
ATCC	American Type Culture Collection
ATP	Adenosine-5'-triphosphate
bp	Base pair
BSA	Bovine serum albumin
dd water	Double distilled water
dil.	Dilution
DNA	Deoxyribonucleic Acid
DNAse	Deoxyribonuclease
DTT	Dithiothreitol
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylenediaminetetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
FCA	Freund's incomplete adjuvant
FIA	Freund's complete adjuvant
Fig.	Figure
FCS	Foetal calf serum
g	Gram
h	Houre
HRP	Horse radish peroxidase
Ig	Immunoglubolin
IM	Intramuscular
IP	Intraperitoneal
IPTG	Isopropyl-β-D- thiogalactopyranoside
kbp	Kilobase pair
kDa	Kilo Daltons
L	Litre
LB	Luria-Bertani
LPS	Lipopolysaccharide
M	Molar
mM	Millimolar
MCS	Multiple cloning site
mg	Miligram

List of commonly used abbreviations in the thesis (continued):

Abbreviation	Expansion
min	Minute
ml	Mililitre
MW	Molecular Weight
ng	Nanogram
Ni-NTA	Nickel-nitriolotriaceic acid
nm	Nanometer
OD	Optical density
Oligo.	Oligonucleotide
OMP	Outer membrane protein
ORF	Open Reading Frame
PCR	Polymerase chain reaction
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline containing 0.05% (v/v) Tween-20
PM	Post mortem
PMSF	Phenylmethylsulphonyl fluoride
pmol	Picomolar
ppm	Parts per million
RNAse	Ribonuclease
rRNA	Ribosomal ribonucleic acid
RT	Room temperature
S	Second
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SC	Subcutaneous
spp.	Species
TAE	Tris-acetate EDTA buffer
TB	Terrific broth
TBS	Tris Buffered saline
TBST	Tris Buffered saline containing 0.05% (v/v) Tween-20
TE	Tris EDTA buffer
TEMED	N,N,N´, N´-Tetramethyl-ethylendiamine
TSA	Trypticase soy agar
UV	Ultraviolet
v	Volume
w	Weight
µg	Microgram
µl	Microliter
µm	Micrometre
µM	Micromolar