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# **Larval paralysis factor of sheep**

**A Thesis presented in partial fulfilment of the  
requirements for the degree of**

**DOCTOR OF PHILOSOPHY**

**in  
Animal Science**

**at Massey University, Palmerston North,  
New Zealand**

**AYE KYAWT SOE**

**2008**

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## Abstract

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This study aimed to identify the origin and molecular nature of larval paralysis factor (LPF), an uncharacterised natural anthelmintic agent(s) known to be secreted by cells in the small intestine of nematode-parasite-immune sheep. The study first confirmed previous findings that gut mucus and small intestinal mucosal cell culture supernatants (CCSs) from parasite-immune sheep contain LPF. An *in vitro* larval migration inhibition (LMI) assay showed that LPF is active against numerous nematode parasite larvae of ruminants and adults of *Trichostrongylus colubriformis*. Lamina propria cells (LPCs) were a richer source of LPF than epithelial cells, and release of the factor was specifically triggered by parasite larval antigens.

A series of trials was performed to optimise LPF production *in vitro*. LPF production was highest when LPCs were selectively extracted from the first three metres of small intestine of resistant-line or hyperimmune parasitized sheep three days after oral challenge with *T. colubriformis* larvae. LPF release *in vitro* appeared to be related to the percentage of eosinophils present in cell cultures, but not with mucosal mast cells or globule leucocytes.

Subsequent *in vitro* experiments showed that mucus glycoproteins released from goblet cells enhanced and sustained the activity of LPF, which may point to a previously unrecognised effector link between goblet cell hyperplasia and mucosal immune responses to gut nematode parasites.

Sequential purification and molecular analysis of LPF showed that the active agent has a molecular mass less than 1kDa, it is polar and heat and enzyme resistant. Further purification using solid phase extraction and HPLC established that LPF did not bind either C18 or ion exchangers, but LPF activity in the flow through from these sorbents was retained on aminopropyl HPLC columns and eluted as a single active peak after flushing with acetonitrile/water mixture (70:30). This peak, analysed by LC-MS, comprised three main compounds of interest: a UV absorbing compound at 254nm with no MS data, and two non UV absorbing compounds at 254nm: one was a component of m/z 104 (ESI+ve) and the other was detected at m/z 113 (ESI-ve).

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## List of Abbreviations

AD	Arrested development
Ag	Antigen
ANOVA	Analysis of variance
<i>auto</i> MACS	Automated magnetic cell sorting
CCS	Cell culture supernatants
CD	Cluster of differentiation
COX <sub>1</sub>	Cyclo-oxygenase 1
COX <sub>2</sub>	Cyclo-oxygenase 2
CTMC	Connective tissue mast cells
DAD	Diode array detector
DMSO	Dimethylsulphoxide
EDTA	ethylenediaminetetraacetic acid
eEP/GO	enriched epithelial/goblet cell
EOF	Eosinophil factor
EP/GO	epithelial and goblet cell
EPA	Eosinophil potentiating activity
EPG	Eggs per gram
EPO	Eosinophil peroxidase
ES	Excretory secretory
ESI-MS	Electrospray ionisation-mass spectrometry
FcR	Fc receptor
FcRn	Fc-receptor of neonate
FCS	Foetal calf serum
FEC	Faecal egg count

## X

FITC	Fluorescein Isothiocyanate
FMLP	N-formyl-methionyl-leucyl-phenylalanine
GI	Gastrointestinal
GL	Globule leucocyte
GLF	Globule leucocyte factor
GM-CSF	Granulocyte macrophage colony stimulation factor
GO	Goblet cell
HID	High iron diamine
HBSS	Hank's balanced salt solution
HEPES	N-2-hydroxyethylpiperazine-N' -2-ethane
HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase
IEL	Intra-epithelial lymphocyte
IFNG	Interferon gamma gene
IFN- $\gamma$	Interferon gamma
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgG1	Immunoglobulin G1
IgG2	Immunoglobulin G2
IgM	Immunoglobulin M
Igs	Immunoglobulins
IL-1 – IL-13	Interleukin-1 - Interleukin - 13
L1	Larval stage 1
L3	Infective larval stage 3
L4	Larval stage 4

LCA	Leucocyte common antigen
LC-MS	Liquid chromatography- mass spectrometry
LMI	Larval migration inhibition
IFNG	interferon gamma gene
LP	Lamina propria
LPC	Lamina propria cell
LPA	Lymphocyte proliferation assay
LPF	Larval paralysis factor
LPL	Lamina propria lymphocyte
LR	Lactation rise
LT	Leukotriene
LTB <sub>4</sub>	Leukotriene B4
LTC <sub>4</sub>	Leukotriene C4
LTD <sub>4</sub>	Leukotriene D4
LTE <sub>4</sub>	Leukotriene E4
m/z	Mass/z (z = charge of the ion in the mass spectrometer)
MHC I	Major Histocompatibility Complex I
MHC II	Major Histocompatibility Complex II
MMC	Mucosal mast cell
MMCF	Mucosal mast cell factor
MMD	Monocyte-macrophage-dendritic cell
MMDF	Monocyte-macrophage-dendritic cell factor
MQ	Milli-Q
MS-MS	Tandem mass spectrometry
MWD	Molecular weight distribution
P1	Population 1

P2	Population 2
PAF	Platelet activating factor
PAMPs	Pathogen-associated-molecular-patterns
PAS	Periodic acid-Schiff
PBS	Phosphate buffered saline
PG	Prostaglandin
PGD2	Prostaglandin D2
PGE1	Prostaglandin E1
PGE2	Prostaglandin E2
PGF2	Prostaglandin F2
PGI2	Prostaglandin I2
Pk1	Peak 1
PPR	Periparturient rise
QTL	Quantitative trait locus
RE	Rapid expulsion
rIL	Recombinant interleukin
RMCP I	Rat mast cell proteinase I
RMCP II	Rat mast cell proteinase II
S.D.	Standard deviation
SAX	Strong anion exchange
SCF	Stem cell factor
SCX	Strong cation exchange
SMCP	Sheep mast cell proteinase
SPE	Solid phase extraction
SRS-A	Slow releasing substances of anaphylaxis
TcL3	<i>Trichostrongylus colubriformis</i> larval stage 3

TCR	T cell receptor
TGF- $\beta$	Transforming growth factor- $\beta$
Th1	T helper cell 1
Th2	T helper cell 2
TLC	Thin layer chromatography
TLR	Toll-like-receptor
TMB	3,3,5,5-Tetramethylbenzidine
TNF- $\alpha$	Tumour necrosis factor- $\alpha$
UV	Ultraviolet
v/v	Volume by volume
VIP	Vasoactive intestinal peptide

Location	Nematodes
Abomasum	<i>Haemonchus contortus</i> *
	<i>Teladorsagia circumcincta</i> *; <i>Ostertagia trifurcata</i> *;
	<i>Trichostrongylus axei</i> *
Small intestine	<i>Cooperia curticei</i> *
	<i>Nematodirus filicollis</i> *
	<i>Nematodirus spathiger</i> *
	<i>Trichostrongylus colubriformis</i> *
	<i>Trichostrongylus vitrinus</i> *
Large intestine	<i>Chabertia ovina</i>
	<i>Oesophagostomum venulosum</i> ; <i>Oesophagostomum columbianum</i> ;
	<i>Trichuris ovis</i> .

\* Considered as major important species.

Table 1.1 Economically important Trichostrongylid nematodes and their location in the gastrointestinal tract of sheep (Vlassoff & McKenna, 1994).