



Clarke, Modet & Co

COLOMBIA

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SANTAFÉ DE BOGOTÁ, A.A. 92397

SUPERINDUSTRIA Y COMERCIO

Radicacion 00034584 00000001 Folios 111
Fecha (AMD) 2000-08-11 17 28 56
Tramite 002 PATENTES D 1 REGISTRO/ 329 COMPLEMEN
Dependencia 2020 DIVISION DE NUEVAS CREACIONES

SEÑORES
DIVISION DE NUEVAS CREACIONES
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
E S D

COD 08

REF SOLICITUD N° 00034584 /
PATENTE NOVEDOSO GEN DE LAWSONIA SSP Y USOS PARA EL MISMO I
SOLICITANTES AGRICULTURE VICTORIA SERVICES PTY LTD
PIG RESEARCH AND DEVELOPMENT CORPORATION
PFIZER PRODUCTS INC
FECHA DE LA SOLICITUD MAYO 12 DE 2 000

ASUNTO PRESENTACIÓN PRIORIDAD

DILIA RODRIGUEZ D ALEMAN, en mi condición de apoderada de la sociedad solicitante,
n esta oportunidad por el presente escrito me permito presentar

- Copia certificada de la prioridad provisional reivindicada de Estados Unidos
N° 60/134 022 de Mayo 13 de 1999
- Traducción al Español de la mencionada prioridad
- Por cuanto la prioridad reivindicada fue inicialmente presentada a nombre de
personas diferentes a los actuales solicitantes, presento en tiempo copias
auténticas y legalizadas de los respectivos documentos de cesión

Esta prioridad está siendo presentada dentro del término de 3 meses que
exige el Convenio de París, toda vez que la solicitud fué radicada el 12 de
Mayo de 2 000

Ruego a usted continuar con el presente trámite

Señor Jefe,

DILIA RODRIGUEZ D ALEMAN
LCD/srr
P- 335

103 P 335 100

TRADUCCIÓN OFICIAL No 00/167 de un documento escrito en inglés, el cual para su identificación se sella con el sello del traductor al tiempo con la presente Esta traducción ha sido preparada por Juan Pablo Piedrahita Aguilera, Traductor e Intérprete Oficial debidamente reconocido de conformidad con el Certificado de Idoneidad Profesional No 0045 expedido por la Universidad Nacional de Colombia (Se traduce un documento de Cesión de derechos, propiedad y participación sobre la Solicitud de Patente, otorgado por Michael PANACCIO y Detlef HASSE a favor de el ESTADO DE VICTORIA representado por su DEPARTAMENTO DE RECURSOS NATURALES Y DEL MEDIO AMBIENTE, escrito en inglés)

A TODOS A QUIENES ESTOS PRESENTES LLEGAREN

Yo, MARION HEATHCOTE, Notario Publico, debidamente inscrito y juramentado y ejerciendo en la ciudad de Sydney en el Estado de New South Wales de la Mancomunidad de Australia POR EL PRESENTE CERTIFICO que el documento adjunto es una copia fotografica autentica del documento original del cual es una copia del mismo tenor, he cuidadosamente confrontado y comparado dicha copia con dicho original y he encontrado que la misma concuerda con él

DOY FE Y EN TESTIMONIO DE LO CUAL Yo, el notario mencionado he suscrito mi nombre y he estampado mi sello Oficial en Sydney, hoy día Ocho de Mayo del Dos Mil

(Sello Notarial en Relieve)

(Firmado)

Notario Publico

Sydney, New South Wales

TRADUCCION OFICIAL No 00 / 167
JUAN PABLO PIEDRAHITA AGUILERA
TRADUCTOR E INTERPRETE OFICIAL
C IDONEIDAD 045
UNIVERSIDAD NACIONAL DE COLOMBIA

Australia

Sello a tinta Yo, Ranu Vatana, funcionario del Departamento de Relaciones Exteriores y Comercio, Australia, POR EL PRESENTE CERTIFICO que la firma de M Heathcote, la cual aparece en los documentos anteriores/adjuntos/al respaldo del presente es la firma auténtica de Marion Heathcote, Notario Publico residente en la ciudad de Sydney, en el estado de New South Wales, Australia y que el sello estampado es el Sello Oficial del mencionado Marion Heathcote Al certificarlo, ni Yo ni el Departamento avalamos ni hacemos declaración alguna en relación con la exactitud del contenido del documento

Dado bajo mi firma y sello del Departamento de Relaciones Exteriores y Comercio hoy dia 18 de Mayo del Dos Mil (2000)

(Sello a tinta del Departamento de Relaciones Exteriores y Comercio, Melbourne, Australia, [Firmado])

CESION

ENTRE

Los CEDENTES (i) Michael PANACCIO, ciudadano Australiano
de 122 Hill Road, North Balwyn, Victoria 3104, Australia, y
(ii) Detlef HASSE, ciudadano Australiano,
de 4 Scullin Court, Sunbury, Victoria 3429, Australia,

Y

El CESIONARIO

ESTADO DE VICTORIA representado por su DEPARTAMENTO DE
RECURSOS NATURALES Y DEL MEDIO AMBIENTE, de 8

TRADUCCION OFICIAL No 00 / 167
JUAN PABLO PIEDRAHITA AGUILERA
TRADUCTOR E INTERPRETE OFICIAL
C IDONEIDAD 045
UNIVERSIDAD NACIONAL DE COLOMBIA

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Nicholson Street, East Melbourne, Victoria 3002, Australia,

En relación con las invenciones divulgadas en las solicitudes de patente enumeradas en el Anexo adjunto al presente

POR CUANTO

1 Los CEDENTES son solicitantes conjuntos en relación con las solicitudes de patentes enumeradas en el Anexo,

2 El CESIONARIO era, en el momento en que fue hecha la invención, el empleador de los Cedentes, y el CESIONARIO desea adquirir participación en, para y bajo dicha invención y cualesquiera Certificados de Patente que de ella se obtengan,

3 Esta Cesión da fe de que, como contraprestación de UN DÓLAR (\$1 00), recibo del cual se reconoce por el presente, los CEDENTES por el presente venden, ceden, transfieren y traspasan al CESIONARIO sus derechos exclusivos de propiedad dominio tenencia, posesión, uso usufructo y goce de dichas invenciones y cualesquiera y todas las Solicitudes de Patente que reivindiquen el derecho de prioridad de cualesquiera y todas las Solicitudes de Patente que se enumeran en el Anexo y cualesquiera y todos los Certificados de Patente que se obtengan de cualesquiera y todas las Solicitudes de Patente mencionadas, y cualquier solicitud divisional, de continuación, de renovación, de reexpedición o sustituto de ellas para la totalidad de la vigencia o vigencias para las cuales se otorguen las mismas,

4 Los CEDENTES por el presente convienen en que no ha habido ni habrá cesión, venta, acuerdo o gravamen alguno que se haya celebrado o vaya a celebrarse, el cual haya entrado o entrare en conflicto con esta cesión y venta,

5 Los CEDENTES además se comprometen a suministrar a el CESIONARIO, cuando éste los solicite, con prontitud, todos los hechos pertinentes y documentos

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relacionados con las solicitudes de patentes, con las invenciones y con los
Certificados de Patente que puedan ser conocidos y a los cuales puedan tener
acceso los CEDENTES, y dara fe de los mismos en cuanto a cualquier interferencia o
litigio relacionados con los mismos, y suscribirá y entregara con prontitud a el
CESIONARIO o a sus representante legal cualesquiera y todos los documentos,
instrumentos o declaraciones juramentadas que se requieran para solicitar, obtener,
mantener y hacer cumplir aquellas solicitudes, aquellas invenciones y aquellos
Certificados de Patente que puedan ser necesanos o deseables para desarrollar los
propósitos del presente con cargo al CESIONARIO o al titular subsiguiente

EN TESTIMONIO DE LO CUAL las partes del presente han aqui suscrito esta
CESIÓN

Por y en nombre de los CEDENTES

(Firmado)

Nombre Michael PANACCIO

Testigo (Firmado)

Nombre Jim Parsons

Cargo Gerente de Desarrollo de Negocios

Con fecha hoy 11 de Octubre de 1999

(Firmado)

Nombre Detlef HASSE

Testigo (Firmado)

Nombre Meri Sinistaj

Cargo Científico Principal

Con fecha hoy 5 de Octubre de 1999

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104

Por y en nombre de los CESIONARIOS

ESTADO DE VICTORIA representado por su DEPARTAMENTO DE RECURSOS
NATURALES Y DEL MEDIO AMBIENTE

Por su funcionario autorizado cuya firma aparece al frente

(Firmado)

Nombre Bruce Kefford

Cargo Director Ejecutivo Agncultura

(Sello a Tinta Bruce Kefford Director Ejecutivo Industrias Prmanas)

Testigo (Firmado)

Nombre P Mackenzie

Cargo RPA

Con fecha hoy 17de Noviembre de 1999

P \OPER\MRO\MPDHDNRE ASS-27/9/99

ANEXO

País	Solicitud No	Título	Fecha de Presentación
Estados Unidos de America	USSN 60/134,022	Gen novedoso Lawsonia y sus usos I	Mayo 13, 1999
Estados Unidos de America	USSN 60/133,986	Gen novedoso Lawsonia y sus usos II	Mayo 13, 1999
Estados Unidos de América	(en blanco)	Gen novedoso Lawsonia y sus usos III	Mayo 13, 1999
Estados Unidos de América	USSN 60/133,973	Gen novedoso Lawsonia y sus usos IV	Mayo 13 1999
Estados Unidos de América	USSN 60/133,989	Gen novedoso Lawsonia y sus usos V	Mayo 13, 1999

P \OPER\MRO\MPDHDNRE ASS-27/9/99

TRADUCCION OFICIAL No 00 / 167

JUAN PABLO PIEDRAHITA AGUILERA

TRADUCTOR E INTERPRETE OFICIAL

C. IDONEIDAD 045

UNIVERSIDAD NACIONAL DE COLOMBIA

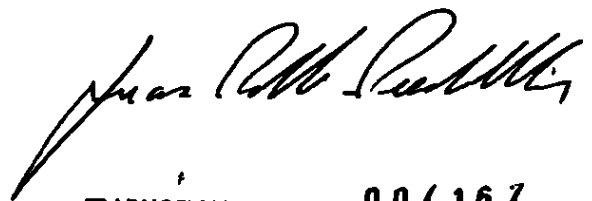
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En español, autenticación de la firma del funcionario del Departamento de Relaciones Exteriores y de Comercio de Melbourne, Australia No 120/00 de Junio 1, 2000 Pagados el Impuesto de Timbre y Derechos Consulares por US\$22 00)

Autenticación por el Ministerio de Relaciones Exteriores Numero 295657 de Julio 11 del 2000, suscrita por el Jefe de Legalizaciones

ES TRADUCCIÓN FIEL Y COMPLETA de la cual se deja copia en los archivos de esta oficina para su confrontación y a la cual me remito

Santafé de Bogotá, Agosto 3 del 2000



TRADUCCION OFICIAL No 00 / 167
JUAN PABLO PIEDRAHITA AGUILERA
TRADUCTOR E INTERPRETE OFICIAL
C IDONEIDAD 045
UNIVERSIDAD NACIONAL DE COLOMBIA

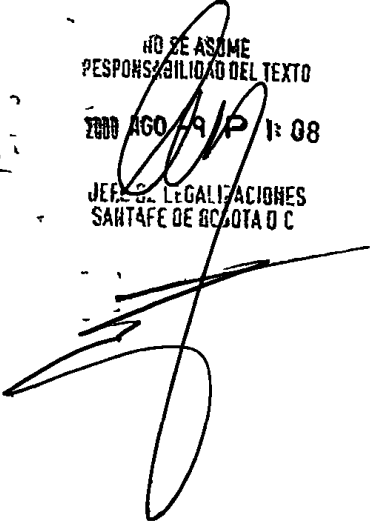
MINISTERIO DE RELACIONES
EXTERIORES

412560
Juan Pedro...
CUYA FIRMA APARECE EN EL
DOCUMENTO DESEMPEÑA
LAS FUNCIONES INDICADAS

NO SE ASUME
RESPONSABILIDAD DEL TEXTO

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JEF. DE LEGALIZACIONES
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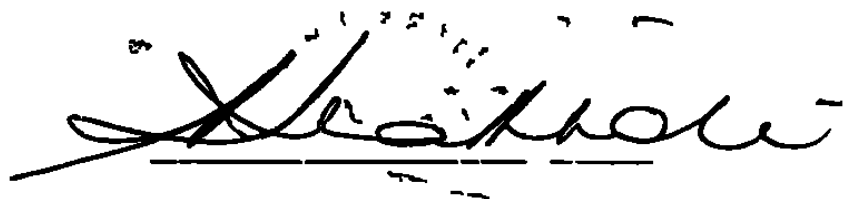
TO ALL WHOM THESE PRESENTS SHALL COME

I, MARION HEATHCOTE, Notary Public, duly admitted and sworn and practising in the City of Sydney in the state of New South Wales in the Commonwealth of Australia DO HEREBY CERTIFY that the document annexed is a true photographic copy of the original document of which it purports to be a copy, I having carefully collated and compared the said copy with the said original and found the same to agree therewith

IN FAITH AND TESTIMONY whereof I the said notary have subscribed my name and set and affixed my seal of Office at Sydney aforesaid this Eighth day of May Two thousand

I, RANU VATANA an officer
Of the Department of Foreign Affairs and Trade Australia,
DO HEREBY CERTIFY that this signature
M. HEATHCOTE
Appearing on the documents above/below/attached
Hereto/on the reverse hereto is the true signature of
MARION HEATHCOTE
Notary Public residing in the City of SYDNEY
in the State of NEW SOUTH WALES Australia and
That the seal impressed is the Official Seal of the said
MARION HEATHCOTE
In so certifying neither I nor the Department endorse nor
Make any statement as to the accuracy of the contents of
The document

Given under my hand and the seal of the Department of
Foreign Affairs and Trade this 18th day of MAY
Two Thousand and (2000)



Notary Public
Sydney, New South Wales
Australia



TRADUCCION OFICIAL No 00 / 167
JUAN PABLO PIEDRAHITA AGUILERA
TRADUCTOR E INTERPRETE OFICIAL
C. IDONEIDAD 045
UNIVERSIDAD NACIONAL DE COLOMBIA

EL CONSULADO DE COLOMBIA EN

SYDNEY 120/00 1 JUN 2000

Previa la confrontacion
correspondiente DA FE de que
la firma que aparece en este
documento es similar a la

REGISTRADA aqui por RANU VATANA,

OFICIAL DEL DEPARTAMENTO DE RELACIONES
EXTERIORES Y COMERCIO DE MELBOURNE, AUSTRALIA



J. Alzamor
JUAN ALFREDO ALZAMORA CAMPO
CONSUL GENERAL

PAGADOS IMPUESTO DE TIMBRE US\$7.00
DERECHOS CONSULARES US\$15.00

NO SE ASUME
RESPONSABILIDAD DEL TEXTO

MINISTERIO DE RELACIONES
EXTERIORES

295657
JUAN ALZAMORA
OFICIAL DE RELACIONES
EXTERIORES Y COMERCIO
DE MELBOURNE, AUSTRALIA

2000 JUN 10 11:21

OFICINA DE LEGALIZACIONES
DEPARTAMENTO DE RELACIONES
EXTERIORES Y COMERCIO

Juan Pablo Piedrahita Aguilera

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TRADUCCION OFICIAL No
JUAN PABLO PIEDRAHITA AGUILERA
TRADUCTOR E INTERPRETE OFICIAL

C. IDONEIDAD 045

UNIVERSIDAD NACIONAL DE COLOMBIA

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ASSIGNMENT

BETWEEN

The ASSIGNORS (i) Michael PANACCIO an Australian citizen
of 122 Hill Road North Balwyn Victoria 3104 Australia and

(ii) Detlef HASSE an Australian citizen
of 4 Scullin Court Sunbury Victoria 3429 Australia

AND

The ASSIGNEE STATE OF VICTORIA as represented by its DEPARTMENT OF
NATURAL RESOURCES AND ENVIRONMENT
of 8 Nicholson Street East Melbourne Victoria 3002 Australia

In respect of the inventions disclosed in the patent applications listed in the Schedule
affixed hereto

WHEREAS

1 The ASSIGNORS are co-applicants in respect of the patent applications listed
in the Schedule

2 The ASSIGNEE was at the time the invention was made the employer of the
Assignors and the ASSIGNEE is desirous of acquiring an interest in to and under said
invention and any Letters Patent to be obtained therefor;

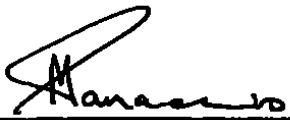
3 This Assignment Witnesses that, in consideration of ONE DOLLAR (\$1 00)
the receipt of which is hereby acknowledged the ASSIGNORS hereby sell, assign,
transfer and set over to the ASSIGNEE the exclusive rights titles and interests to have
and to hold use exercise and enjoy said inventions and any and all Patent Applications
which claim the benefit of priority from any or all of the Patent Applications listed in the
Schedule and any and all Letters Patent to be obtained on any and all of said Patent
Applications and any divisional application continuation application renewal reissue or
substitute thereof for the full term or terms for which the same may be granted

4 The ASSIGNORS hereby covenant that no assignment sale agreement or
encumbrance has been or will be made or entered into which would conflict with this
assignment and sale

5 The ASSIGNORS further covenant that the ASSIGNEE will upon request, be
provided promptly with all pertinent facts and documents relating to said applications
said inventions and said Letters Patent as may be known and accessible to the
ASSIGNORS, and will testify as to the same in any interference or litigation related
thereto and will promptly execute and deliver to the ASSIGNEE or its legal
representative any and all papers instruments or affidavits required to apply for obtain
maintain and enforce said applications said inventions and said Letters Patent which
may be necessary or desirable to carry out the purposes hereof at the cost of the
ASSIGNEE or subsequent titleholder

IN WITNESS WHEREOF the parties hereto have hereunto executed this Assignment

For and on behalf of the ASSIGNORS


Name Michael PANACCIO

Witness 
Name ~~Tim Parsons~~
Title Business Development Manager

DATED this 11th DAY of October, 1999


Name Detlef HASSE

Witness 
Name Meri Smiley
Title Senior Scientist

DATED this Fifth DAY of October, 1999

For and on behalf of the ASSIGNEE

STATE OF VICTORIA as represented by its
DEPARTMENT OF NATURAL RESOURCES AND ENVIRONMENT
by its authorized officer whose signature
appears opposite hereto

Witness 
Name P MACKENZIE
Title RPA


Name
Title Executive Director
BIOLOGICAL
BRUCE KEFFORD
Executive Director, Forest & Nature

DATED this 17th DAY of NOVEMBER, 1999

SCHEDULE

COUNTRY	APPLICATION No	TITLE	DATE FILED
United States of America	USSN 60/134,022	Novel Lawsonia gene and uses therefor I	May 13, 1999
United States of America	USSN 60/133,986	Novel Lawsonia gene and uses therefor II	May 13, 1999
United States of America		Novel Lawsonia gene and uses therefor III	May 13, 1999
United States of America	USSN 60/133,973	Novel Lawsonia gene and uses therefor IV	May 13, 1999
United States of America	USSN 60/133,989	Novel Lawsonia gene and uses therefor V	May 13, 1999

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TRADUCCION OFICIAL No 00/167
JUAN PABLO PIEDRAHITA AGUILERA
TRADUCTOR E INTERPRETE OFICIAL
C IDONEIDAD 045
UNIVERSIDAD NACIONAL DE COLOMBIA

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TRADUCCIÓN OFICIAL No 00/168 de un documento escrito en inglés, el cual para su identificación se sella con el sello del traductor al tiempo con la presente. Esta traducción ha sido preparada por Juan Pablo Piedrahita Aguilera, Traductor e Intérprete Oficial debidamente reconocido de conformidad con el Certificado de Idoneidad Profesional No 0045 expedido por la Universidad Nacional de Colombia (Se traduce un documento de Cesión de derechos, propiedad y participación sobre la Solicitud de Patente, otorgado por el ESTADO DE VICTORIA representado por su DEPARTAMENTO DE RECURSOS NATURALES Y DEL MEDIO AMBIENTE a favor de AGRICULTURE VICTORIA SERVICES PTY LTD y PIG RESEARCH AND DEVELOPMENT CORPORATION, escrito en inglés)

A TODOS A QUIENES ESTOS PRESENTES LLEGAREN

Yo, MARION HEATHCOTE, Notario Público, debidamente inscrito y juramentado y ejerciendo en la ciudad de Sydney en el Estado de New South Wales de la Mancomunidad de Australia POR EL PRESENTE CERTIFICO que el documento adjunto es una copia fotográfica auténtica del documento original del cual es una copia del mismo tenor, he cuidadosamente confrontado y comparado dicha copia con dicho original y he encontrado que la misma concuerda con él

DOY FE Y EN TESTIMONIO DE LO CUAL Yo, el notario mencionado he suscrito mi nombre y he estampado mi sello Oficial en Sydney, hoy día Ocho de Mayo del Dos Mil

(Sello Notarial en Relieve)

(Firmado)

Notario Público

TRADUCCION OFICIAL No 00/168
JUAN PABLO PIEDRAHITA AGUILERA
TRADUCTOR E INTERPRETE OFICIAL
C. IDONEIDAD 045
UNIVERSIDAD NACIONAL DE COLOMBIA

Sydney, New South Wales

Australia

Sello a tinta Yo, Ranu Vatana, funcionario del Departamento de Relaciones Exteriores y Comercio, Australia, POR EL PRESENTE CERTIFICO que la firma de M Heathcote, la cual aparece en los documentos anteriores/adjuntos/al respaldo del presente es la firma auténtica de Marion Heathcote, Notario Público residente en la ciudad de Sydney, en el estado de New South Wales, Australia y que el sello estampado es el Sello Oficial del mencionado Marion Heathcote Al certificarlo, ni Yo ni el Departamento avalamos ni hacemos declaración alguna en relación con la exactitud del contenido del documento

Dado bajo mi firma y sello del Departamento de Relaciones Exteriores y Comercio hoy día 18 de Mayo del Dos Mil (2000)

(Sello a tinta del Departamento de Relaciones Exteriores y Comercio, Melbourne, Australia, [Firmado])

CESION

ENTRE

El CEDENTE ESTADO DE VICTORIA representado por su DEPARTAMENTO DE RECURSOS NATURALES Y DEL MEDIO AMBIENTE, de 8 Nicholson Street, East Melbourne, Victoria 3002, Australia,

Y

Los CESIONARIOS

- (i) AGRICULTURE VICTORIA SERVICES PTY LTD de 475 Mickleham Road, Attwood, Victoria 3049, Australia, y
- (ii) PIG RESEARCH AND DEVELOPMENT CORPORATION,

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112

de 3rd Floor, Industry House, Brisbane Avenue, Australian
Capital Territory 2600, Australia,

En relación con las invenciones divulgadas en las solicitudes de patente enumeradas
en el Anexo adjunto al presente

POR CUANTO

1 El CEDENTE es el empleador y el cesionario de los inventores en relación con
las invenciones descritas en las solicitudes de patentes enumeradas en el Anexo,

2 Los CESIONARIOS desean adquirir participacion en, para y bajo dicha
invencion y cualesquiera Certificados de Patente que de ella se obtengan,

3 Esta Cesión da fe de que, como contraprestación de UN DÓLAR (\$1 00),
recibo del cual se reconoce por el presente, el CEDENTE por el presente vende,
cede, transfiere y traspasa a los CESIONARIOS sus derechos exclusivos de
propiedad dominio, tenencia, posesión, uso, usufructo y goce de dichas invenciones y
cualesquiera y todas las Solicitudes de Patente que reivindiquen el derecho de
propiedad de cualesquiera y todas las Solicitudes de Patente que se enumeran en el
Anexo y cualesquiera y todos los Certificados de Patente que se obtengan de
cualesquiera y todas las Solicitudes de Patente mencionadas, y cualquier solicitud
divisional, de continuacion, de renovación, de reexpedición o sustituto de ellas para la
totalidad de la vigencia o vigencias para las cuales se otorguen las mismas,

4 El CEDENTE por el presente conviene en que no ha habido ni habra cesión,
venta, acuerdo o gravamen alguno que se haya celebrado a vaya a celebrarse, el
cual haya entrado o entrare en conflicto con esta cesión y venta,

5 El CEDENTE además se compromete a suministrar a los CESIONARIOS,
cuando éstos los soliciten, con prontitud, todos los hechos pertinentes y documentos

116 113

relacionados con las solicitudes de patentes, con las invenciones y con los
Certificados de Patente que puedan ser conocidos y a los cuales pueda tener acceso
el CEDENTE, y dara fe de los mismos en cuanto a cualquier interferencia o litigio
relacionados con los mismos, y suscribirá y entregará con prontitud a los
CESIONARIOS o a sus representantes legales cualesquiera y todos los documentos,
instrumentos o declaraciones juramentadas que se requieran para solicitar, obtener,
mantener y hacer cumplir aquellas solicitudes, aquellas invenciones y aquellos
Certificados de Patente que puedan ser necesanos o deseables para desarrollar los
propositos del presente con cargo a Agnculture Victoria Services Pty Ltd o al titular
subsiguiente con sujeción a cualquier Contrato en relación con ellos entre Agriculture
Victoria Services Pty Ltd y el titular subsiguiente

EN TESTIMONIO DE LO CUAL las partes del presente han aquí suscrito esta
CESIÓN

Por y en nombre del CEDENTE

ESTADO DE VICTORIA representado por su DEPARTAMENTO DE RECURSOS
NATURALES Y DEL MEDIO AMBIENTE

Por su funcionario autorizado cuya firma aparece al frente

(Firmado)

Nombre Bruce Kefford

Cargo Director Ejecutivo Industrias Primarias

Testigo (Firmado)

Nombre P Mackenzie

Cargo RPA

Con fecha hoy 19 de Noviembre de 1999

1/2 TRADUCCION OFICIAL No 00 / 168
2 JUAN PABLO PIEDRAHITA AGUILERA
TRADUCTOR E INTERPRETE OFICIAL
C IDONEIDAD 045
UNIVERSIDAD NACIONAL DE COLOMBIA

Por y en nombre de los CESIONARIOS

AGRICULTURE VICTORIA SERVICES PTY LTD

Por su funcionario autorizado cuya firma aparece al frente

(Firmado)

Nombre MR Bruce Lang

Cargo Secretario de la Compañía

Testigo (Firmado)

Nombre Adrian Trioli

Cargo Gerente - Propiedad Intelectual y Contratos

Con fecha hoy 19 de Noviembre de 1999

PIG RESEARCH AND DEVELOPMENT CORPORATION

Por su funcionario autorizado cuya firma aparece al frente

(Firmado)

Nombre Dr Anthony Peacock

Cargo Director Ejecutivo

Pig Research and Development Corporation

Testigo (Firmado)

Nombre Sally O'Brien

Cargo Asistente Ejecutivo

Con fecha hoy 23 de Noviembre de 1999

P \ O P E R I M R O \ D N R E A V S A S S - 27 / 9 / 99

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ANEXO

País	Solicitud No	Título	Fecha de Presentación
Estados Unidos de America	USSN 60/134,022	Gen novedoso Lawsonia y sus usos I	Mayo 13, 1999
Estados Unidos de América	USSN 60/133,986	Gen novedoso Lawsonia y sus usos II	Mayo 13, 1999
Estados Unidos de America	(en blanco)	Gen novedoso Lawsonia y sus usos III	Mayo 13, 1999
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Estados Unidos de América	USSN 60/133,989	Gen novedoso Lawsonia y sus usos V	Mayo 13, 1999

P \ O P E R I M R O I D N R E A V S A S S - 27/9/99

En español, autenticación de la firma del funcionario del Departamento de Relaciones Exteriores y de Comercio de Melbourne, Australia No 121/00 de Junio 1, 2000
Pagados el Impuesto de Timbre y Derechos Consulares por US\$22 00)

Autenticación por el Ministerio de Relaciones Exteriores Numero 295656 de Julio 11 del 2000, suscrita por el Jefe de Legalizaciones

ES TRADUCCIÓN FIEL Y COMPLETA de la cual se deja copia en los archivos de esta oficina para su confrontación y a la cual me remito
Santafé de Bogotá, Agosto 3 del 2000


TRADUCCION OFICIAL No 00 / 168
JUAN PABLO PIEDRAHITA AGUILERA
TRADUCTOR E INTERPRETE OFICIAL
C IDONEIDAD 045
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MINISTERIO DE RELACIONES
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ASSIGNMENT

BETWEEN

The ASSIGNOR STATE OF VICTORIA as represented by its DEPARTMENT OF
 NATURAL RESOURCES AND ENVIRONMENT
 of 8 Nicholson Street, East Melbourne Victoria 3002 Australia

AND

The ASSIGNEES (i) AGRICULTURE VICTORIA SERVICES PTY LTD
 of 475 Mickleham Road Attwood Victoria 3049 Australia, and

 (ii) PIG RESEARCH AND DEVELOPMENT CORPORATION
 of 3rd Floor Industry House, Brisbane Avenue Australian Capital
 Territory 2600 Australia,

In respect of the inventions disclosed in the patent applications listed in the Schedule affixed hereto

WHEREAS

1 The ASSIGNOR is the employer and assignee of inventors in respect of the inventions described in the patent applications listed in the Schedule

2 The ASSIGNEES are desirous of acquiring an interest in to and under said invention and any Letters Patent to be obtained therefor,

3 This Assignment Witnesses that in consideration of ONE DOLLAR (\$1 00) the receipt of which is hereby acknowledged the ASSIGNOR hereby sells assigns transfers and sets over to the ASSIGNEES their exclusive right title and interest to have and to hold use exercise and enjoy said inventions and any and all Patent Applications which claim the benefit of priority from any or all of the Patent Applications listed in the Schedule and any and all Letters Patent to be obtained on any and all of said Patent Applications and any divisional application continuation application renewal reissue or substitute thereof for the full term or terms for which the same may be granted

4 The ASSIGNOR hereby covenants that no assignment sale agreement or encumbrance has been or will be made or entered into which would conflict with this assignment and sale

5 The ASSIGNOR further covenants that the ASSIGNEES will upon their request be provided promptly with all pertinent facts and documents relating to said applications, said inventions and said Letters Patent as may be known and accessible to the ASSIGNOR and will testify as to the same in any interference or litigation related thereto and will promptly execute and deliver to the ASSIGNEES or their legal representative any and all papers, instruments or affidavits required to apply for obtain maintain and enforce said applications, said inventions and said Letters Patent which may be necessary or desirable to carry out the purposes hereof at the cost of Agriculture Victoria Services Pty Ltd or subsequent titleholder as subject to any Agreement thereto

between Agriculture Victoria Services Pty Ltd and said subsequent titleholder

IN WITNESS WHEREOF the parties hereto have hereunto executed this Assignment

For and on behalf of the ASSIGNOR

STATE OF VICTORIA as represented by its
DEPARTMENT OF NATURAL RESOURCES AND ENVIRONMENT
by its authorized officer whose signature
appears opposite hereto

Witness

Name

Title

[Signature]
P. MACKENZIE
RPA

Name

Title

[Signature]
BRIAN LEFFORD
Pig Research Industries

DATED this

19th

DAY of

NOVEMBER

1999

For and on behalf of the ASSIGNEES

AGRICULTURE VICTORIA SERVICES PTY LTD
by its authorized officer whose signature
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MANAGER - INTELLECTUAL PROPERTY AND CONTRACTS

Name

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MR BRUCE LANG
COMPANY SECRETARY

DATED this

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NOVEMBER

1999

PIG RESEARCH AND DEVELOPMENT CORPORATION
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[Signature]
Sally O'Brien
Executive Assistant

Name

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Dr Anthony Peacock
Executive Director
Pig Research and Development Corporation

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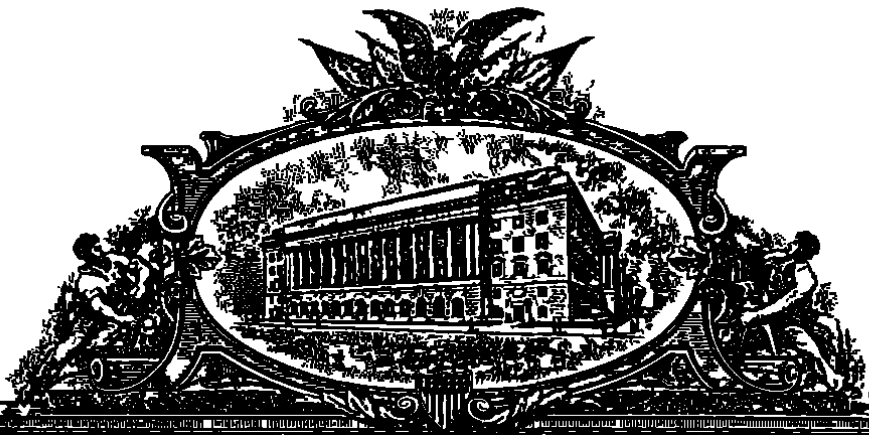
SCHEDULE

COUNTRY	APPLICATION No	TITLE	DATE FILED
United States of America	USSN 60/134 022	Novel Lawsonia gene and uses therefor I	May 13, 1999
United States of America	USSN 60/133,986	Novel Lawsonia gene and uses therefor II	May 13, 1999
United States of America		Novel Lawsonia gene and uses therefor III	May 13, 1999
United States of America	USSN 60/133,973	Novel Lawsonia gene and uses therefor IV	May 13, 1999
United States of America	USSN 60/133,989	Novel Lawsonia gene and uses therefor V	May 13, 1999

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FILING DATE UNDER 35 USC 111**

APPLICATION NUMBER 60/134,022

FILING DATE May 13, 1999



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PROVISIONAL APPLICATION FOR PATENT
COVER SHEET

Case No DAVIE70.001PRF
Date May 13, 1999
Page 1

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D C. 20231

ATTENTION PROVISIONAL PATENT APPLICATION

Sir

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR § 1.53(c)

For NOVEL LAWSONIA GENE AND USES THEREFOR I

Name of First Inventor Michael Panaccio
Residence Address 112 Hill road, North Balwyn, Victoria, 3104, Australia
Citizenship Australian

Name of Second Inventor Everett Lee Rosey
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Name of Fourth Inventor Robert Gerard Ankenbauer
Residence Address 104 Castle Hill Road, Pawcatuck, Connecticut 06379
Citizenship USA

Enclosed are

- (X) Application in 71 pages with 4 pages of sequence listing
- (X) 1 sheet of drawings
- (X) A check in the amount of \$150 to cover the filing fee is enclosed.
- (X) A return prepaid postcard.
- (X) The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Account No 11-1410 A duplicate copy of this sheet is enclosed.

Was this invention made by an agency of the United States Government or under a contract with an agency of the United States Government?

- (X) No
- () Yes The name of the U S Government agency and the Government contract number are

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PROVISIONAL APPLICATION FOR PATENT
COVER SHEET

Case No DAVIE70.001PRF
Date May 13 1999
Page 2

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Respectfully submitted,



Daniel E Altman
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TRADUCCION OFICIAL No 00452
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NOVEL *LAWSONIA SPP* GENE AND USES THEREFOR I

FIELD OF THE INVENTION

5 The present invention relates generally to therapeutic compositions for the treatment and/or prophylaxis of intestinal disease conditions in animals and birds caused or exacerbated by *Lawsonia intracellularis* or similar or otherwise related microorganism. In particular, the present invention provides a novel gene derived from *Lawsonia intracellularis* which encodes an immunogenic hemolysin peptide polypeptide or
10 Furthermore, the hemolysin polypeptide described herein or a peptide homologue, analogue or derivative thereof is particularly useful as an antigen in vaccine preparation for conferring humoral immunity against *Lawsonia intracellularis* and other pathogens in animal hosts. The present invention is also directed to methods for the treatment and/or prophylaxis of such intestinal disease conditions and to
15 diagnostic agents and procedures for detecting *Lawsonia intracellularis* or similar or otherwise related microorganisms.

GENERAL

Bibliographic details of the publications numerically referred to in this specification are
20 collected at the end of the description. All patents, patent applications and publications cited herein are incorporated by reference in their entirety.

Reference hereinafter to "*Lawsonia intracellularis*" or its abbreviation "*L. intracellularis*" includes all microorganisms similar to or otherwise related to this microorganism as
25 described by Stills (1991) or Jones et al (1997) or Lawson et al (1993) or McOrist et al.

As used herein, the word "*tlhA*" or the term "*tlhA* gene" shall be taken to refer to the gene encoding the hemolysin polypeptide of the present invention. It shall also be
30 understood that the term "*tlhA* polypeptide" refers to the hemolysin polypeptide of the

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Definition

As used herein the term 'derived from' shall be taken to indicate that a specified product in particular a macromolecule such as a peptide polypeptide protein, gene or nucleic acid molecule antibody molecule Ig fraction or other macromolecule or a biological sample comprising said macromolecule may be obtained from a particular source organism tissue organ or cell albeit not necessarily directly from that source organism tissue organ or cell

Throughout this specification unless the context requires otherwise the word comprise or variations such as comprises or 'comprising' will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements but not the exclusion of any other step or element or integer or group of elements or integers

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps features compositions and compounds referred to or indicated in this specification individually or collectively and any and all combinations or any two or more of said steps features compositions and compounds

The present invention is not to be limited in scope by the specific embodiments described herein which are intended for the purposes of exemplification only. Functionally equivalent products compositions and methods are clearly within the scope of the invention as described herein

BACKGROUND OF THE INVENTION

The meat producing sector of the agricultural industry is dependant upon the health

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of its livestock and there is a need to maintain disease-free livestock for human consumption. The industry is subject to rapid economic downturn in response to disease conditions adversely affecting livestock and the quality of meat products derived therefrom including those diseases which may potentially be transmitted to humans. It is important therefore to have well defined treatments and prophylactic and diagnostic procedures available to deal with infections or potential infections in livestock animals and humans.

Meat products derived from porcine and avian species are significant commercial products in the agriculture industry. In particular pigs form a major component of the meat industry. However pigs are sensitive to a wide spectrum of intestinal diseases collectively referred to as porcine proliferative enteropathy (PPE). These diseases have previously been known as intestinal adenomatosis complex (Barker and van Drunen 1985) porcine intestinal adenomatosis (PIA) necrotic enteritis (Rowland and Lawson 1976) proliferative haemorrhagic enteropathy (Love and Love 1977) regional ileitis (Jonsson and Martinsson 1976) haemorrhagic bowel syndrome (O'Neil 1970) porcine proliferative enteritis and *Campylobacter* spp - induced enteritis (Straw 1990).

There are two main forms of PPE - a non-haemorrhagic form represented by intestinal adenomatosis which frequently causes growth retardation and mild diarrhoea and a haemorrhagic form which is often fatal represented by proliferative haemorrhagic enteropathy (PHE) where the distal small intestine lumen becomes engorged with blood. PPE has been reported in a number of animal species including pigs (McOrist *et al* 1993) hamsters (Stills 1991) ferrets (Fox *et al* 1989) guinea pigs (Elwell *et al* 1990) and birds (Hendeb and Fox 1990) as well as avian species (Mason *et al* 1998).

The causative organism of PPE is a *Campylobacter*-like organism referred to herein as "*Lawsonia intracellulans*" (McOrist *et al* 1995). The organism has also been previously referred to as *Symbioblast intracellulans* (Stills 1991). PPE like diseases in pigs may also be caused by other pathogens such as various species of

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Campylobacter (Gebhart *et al* 1983)

Lawsonia intracellularis is an intracellular possibly obligate intracellular bacterium. It can only be cultured *in vitro* with tissue culture cells (Jones *et al* 1997, Lawson *et al* 1993, McOrist *et al* 1995, International Patent Application No. PCT/US96/09576). *L. intracellularis* is located in the cytoplasm of the villus cells and intestinal crypt cells of infected animals. Pigs suffering from PPE are characterised by irregularities in the villus cells and intestinal crypt structure with epithelial cell dysplasia wherein crypt abscesses form as the villi and intestinal crypts become branched and fill with inflammatory cells.

A significant cost component associated with the pig industry especially in terms of stock losses, medication costs, reduced growth rates of pigs and increased feed costs. PPE also contributes to downstream indirect costs in for example additional labour costs and environmental costs in dealing with antibiotic residue contamination and in control measures to prevent the organism from being passed on or carried to other animals or humans.

Current control strategies for PPE rely on the use of antibacterials. However such a strategy is considered to only be short to medium term especially since governmental regulatory pressures tend to discourage animal husbandry practices which involve the use of prophylactic antibiotics. There is a need therefore to develop effective, safe and low cost alternatives to the use of antibiotics and in particular, to develop vaccine preparations capable of conferring protective immunity against *Lawsonia intracellularis* infection in livestock animals.

The most effective vaccine preparations are generally comprised of a highly antigenic component such as a peptide, polypeptide, protein or other macromolecule which is derived from the pathogenic organism against which the vaccine is directed wherein said antigenic component produces little or no contraindications when administered to a susceptible host animal and produces little or no antigenic cross-reactivity with

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desirable organisms such as non pathogenic organisms that are a part of the normal flora of the intestinal tract or other tissues of said host animal. In summary, an effective vaccine preparation must be immunogenic, specific and safe.

- 5 Accordingly, there is a need to identify highly immunogenic antigens produced by the bacterium *Lawsonia intracellularis*.

International Patent Application No. PCT/AU96/00767 describes several *L. intracellularis* partial genetic sequences and partial polypeptides encoded thereby.
 10 However, there is a need to further identify polypeptide immunogens produced by the bacterium *L. intracellularis* and immunogenic peptides derived therefrom, including those immunogens which are genus- or species-specific for use in improved vaccine compositions. The presently-described invention provides such immunogens.

15 SUMMARY OF THE INVENTION

One aspect of the present invention is directed to an isolated or recombinant immunogenic polypeptide which comprises, mimics or cross-reacts with a B-cell or T-cell epitope of *Lawsonia spp*. Preferably, the isolated or recombinant immunogenic polypeptide is selected from the group consisting of the following:

- 20 (i) a peptide, oligopeptide or polypeptide which comprises an amino acid sequence which has at least about 70% sequence identity overall to the amino acid sequence set forth in SEQ ID NO. 1;
 (ii) a peptide, oligopeptide or polypeptide which comprises an amino acid sequence having at least about 50% sequence identity to about amino acid
 25 residues 1 to 50 of SEQ ID NO. 1; or
 (iii) a homologue, analogue or derivative of (i) or (ii) which mimics a B-cell or T-cell epitope of *Lawsonia spp*.

In a preferred embodiment, the polypeptide comprises or consists essentially of the
 30 amino sequence of SEQ ID NO. 1 or about amino acids 1 to 50 thereof.

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A further aspect of the present invention provides a vaccine composition for the prophylaxis or treatment of infection in an animal such as a pig or bird by *L. intracellularis* or a similar or otherwise related microorganism said vaccine composition comprising an immunologically effective amount of an immunogenic component which comprises an isolated or recombinant polypeptide having at least about 70% overall sequence identity to the amino acid sequence set forth in SEQ ID NO 1 or at least about 50% overall sequence identity to amino acid residues 1 to 50 of SEQ ID NO 1 or an immunogenic homologue analogue or derivative thereof which is immunologically cross-reactive with *Lawsonia intracellularis* and one or more carriers diluents and/or adjuvants suitable for veterinary or pharmaceutical use

In a preferred embodiment the polypeptide of the vaccine composition comprises or consists essentially of the amino acid sequence of SEQ ID NO 1 or about amino acids 1 to 50 thereof

A further aspect of the invention extends to an immunologically interactive molecule such as an antibody or antibody fragment which is capable of binding to the immunogenic polypeptide of the invention

A further aspect of the invention provides a method of diagnosing infection of an animal by *Lawsonia intracellularis* or a related microorganism said method comprising the steps of contacting a biological sample derived from said animal with an immunologically interactive molecule of the present invention for a time and under conditions sufficient for a complex such as an antigen antibody complex to form and detecting said complex formation

A further aspect of the present invention contemplates a method of determining whether or not an animal has suffered from a past infection or is currently infected, by *Lawsonia intracellularis* or a related microorganism said method comprising contacting a tissue or fluid sample such as blood or serum derived from said animal with the immunogenic polypeptide of the invention for a time and under conditions

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sufficient for a complex such as an antigen antibody complex to form and then detecting said complex formation

Another aspect of the present invention provides an isolated nucleic acid molecule which comprises a sequence of nucleotides that encodes or is complementary to a nucleic acid molecule that encodes a peptide oligopeptide or polypeptide selected from the following

- (i) a peptide oligopeptide or polypeptide which comprises an amino acid sequence which has at least about 70% overall sequence identity to the amino acid sequence set forth in SEQ ID NO 1
- (ii) a peptide oligopeptide or polypeptide which comprises an amino acid sequence having at least about 50% overall sequence identity to amino acid residues 1 to 50 of SEQ ID NO 1 or
- (iii) a homologue analogue or derivative of (i) or (ii) which mimics a B-cell or T-cell epitope of *Lawsonia spp*

In a preferred embodiment the isolated nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO 2 or at least that portion of SEQ ID NO 2 encoding amino acid residues 1 to 50 of SEQ ID NO 1 or a degenerate variant thereof or has at least about 70% sequence identity to all or a part thereof

Another further aspect of the invention provides a diagnostic method of detecting *Lawsonia intracellulans* or related microorganism in a biological sample derived from

- (a) subject said method comprising the steps of hybridising one or more nucleotide or oligonucleotide probes or primers derived from the nucleotide sequence set forth in SEQ ID NO 2 or a complementary nucleotide sequence thereof or a homologue analogue or derivative thereof to said sample and then detecting said hybridisation using a detection means. The detection means according to this aspect of the invention is any nucleic acid-based hybridisation or amplification reaction

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A further aspect of the invention provides an isolated probe or primer derived from SEQ ID NO 2 or a complementary nucleotide sequence thereto

3rd EF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation comparing the deduced amino acid sequence of *Lawsonia intracellularis* (L int) hemolysin to the amino acid sequences of hemolysin polypeptides derived from *Serpulina (Treponema) hyodysenteriae* (S hyo) *Mycobacterium tuberculosis* (M tub) *Aerifex aeolicus* (A aeo) *Borrelia burgdorferi* (B bur), *Helicobacter pylori* (H pyl) *Synechocystis* sp (Synec) and *Bacillus subtilis* (B sub). Gaps have been introduced to optimise the alignment. Residues that are identical in all eight sequences appear in boldface. The *L intracellularis* hemolysin sequence (SEQ ID NO 1) was deduced by translation of the nucleotide sequence of the *hlyA* gene set forth in SEQ ID NO 2

DETAILED DESCRIPTION OF THE INVENTION

In proceeding up to the present invention the inventors sought to identify immunogenic proteins of *Lawsonia intracellularis* for use in vaccines for the prophylaxis and treatment of PPE in animals including pigs and birds

Accordingly, one aspect of the present invention is directed to an isolated or recombinant immunogenic polypeptide which comprises, mimics or cross-reacts with an epitope of *Lawsonia* spp

Epitopes of *Lawsonia* spp may be B cell epitopes or T-cell epitopes. It is well-known that antibody-binding sites (B-cell epitopes) involve linear as well as conformational epitopes (van Regenmortel 1992). B-cell epitopes are predominantly conformational. In contrast T-cells recognize predominantly linear epitope sequences in combination with MHC class II molecules

The precise identification and careful selection of epitopes of *Lawsonia* spp facilitates

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the development of diagnostic reagents and vaccine compositions for the effective treatment or prophylaxis of *Lawsonia* infections. Epitope identification and characterization (i.e. determination of the molecular weight, amino acid sequence and structure of epitopes of *Lawsonia* spp.) may be performed using art-recognised techniques. For the detection of conformational epitopes, degrading and denaturing of the epitope molecule must be avoided in order to conserve the three-dimensional structure, because the antigen-antibody reaction will be diminished if the secondary structure of the epitope is altered significantly. In practice, the characterisation and identification of non-conformational epitopes is easier, because any immunoreactive epitopes are contained within a single peptide fragment or single amino acid sequence which is capable of being purified under a range of conditions.

Both non-conformational and conformational epitopes may be identified by virtue of their ability to bind detectable amounts of antibodies (such as IgM or IgG) from sera of animals immunised against or infected with *Lawsonia* spp. and in particular *L. intracellularis*, or an isolated polypeptide derived therefrom or alternatively by virtue of their ability to bind detectable amounts of antibodies in a purified Ig fraction derived from such sera. The antibodies may be derived from or contained within pools of polyclonal sera or may be monoclonal antibodies. Antibody fragments or recombinant antibodies such as those expressed on the surface of a bacteriophage or virus particle such as in a phage display library may also be employed.

Identification of T-cell epitopes is performed by analysing the ability of the epitope peptides to induce the proliferation of peripheral blood lymphocytes or T-cell clones. The identification of T-cell epitopes is accomplished using a variety of methods as known in the art, including the use of whole and fragmented native or recombinant antigenic proteins as well as the more commonly employed "overlapping peptide" method. In the latter method, overlapping peptides which span the entire sequence of a polypeptide derived from *Lawsonia* spp. are synthesized and tested for their capacity to stimulate T-cell cytotoxic or proliferative responses *in vitro*.

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Structure determination of both conformational non-linear and non-conformational linear epitopes may be performed by nuclear magnetic resonance spectroscopy (NMR) or X-ray crystallographic analysis. The determination of epitopes using X-ray techniques requires the protein-antibody complex to be crystallized whereas NMR analysis of the complex in a liquid state. NMR measures the amount of amino acids as well as the neighbourhood of protons of different amino acid residues, wherein the alternating effect of two protons along the carbon backbone is characteristic of a particular epitope.

1. A successful method to recognize non-conformational linear epitopes is the immunoblot and in particular the Western blot. Peptides may be generated from a complete *Lawsonia spp.* polypeptide by digestion with site-specific proteases such as trypsin or chymotrypsin and the peptides generated thereby can be separated using standard electrophoretic or chromatographic procedures. For example, after electrophoresis according to molecular weight using SDS/PAGE (SDS/polyacrylamide gel electrophoresis) and/or according to isoelectric point using IEF (isoelectric focusing) or alternatively by two-dimensional electrophoresis, the peptides can be transferred to immobilizing nylon or nitrocellulose membranes and incubated with sera against the intact polypeptides. Peptides that comprise immunogenic regions (i.e. B-cell or T-cell epitopes) are bound by the antibodies in the sera and the bound antibodies may be detected using secondary antibodies such as anti-IgG antibodies that have been labelled radioactively or enzymatically. The epitopes may then be characterised by purification based upon their size, charge or ability to bind specifically to antibodies against the intact polypeptide using one or more techniques such as size-exclusion chromatography, ion-exchange chromatography, affinity chromatography or ELISA amongst others. After purification of the epitope, only one band or spot should be detectable with gel electrophoresis. The N-terminal or total sequencing of the peptide offers the possibility to compare the peptide with known proteins in databases.

2. Several computer-driven algorithms have now been devised to search for T-cell

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epitopes in proteins (Margalit *et al* 1987 Vajda and C DeLisi 1990 Altuvia *et al* 1995 Parker *et al* 1994 De Groot *et al* 1995 Gabriel *et al* 1995 Meister *et al* 1995) These algorithms search the amino acid sequence of a given protein for characteristics believed to be common to immunogenic peptides locating regions that are likely to induce a cellular immune response *in vitro*. Computer-driven algorithms can identify regions of a *Lawsonia spp* polypeptide that contain epitopes and are less variable among different isolates. Alternatively computer-driven algorithms can rapidly identify each isolate's more variable proteins that should be included in a multivalent vaccine

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The AMPHI algorithm (Margalit *et al* 1987) which is based on the periodicity of T cell epitopes has been widely used for the prediction of T-cell antigenic sites from sequence information alone. Essentially AMPHI describes a common structural pattern of MHC binding motifs since MHC binding motifs (i.e. patterns of amino acids that appear to be common to most of the peptides that bind to a specific MHC molecule) appear to exhibit the same periodicity as an alpha helix. Identification of T-cell epitopes by locating MHC binding motifs in an amino acid sequence provides an alternative means of identifying immunogenic epitopes in diagnostic assays

20 The EpiMer algorithm (Meister *et al* 1995 Gabriel *et al* 1995 De Groot *et al* 1995) locates clustered MHC binding motifs in amino acid sequences of proteins based on the correlation between MHC binding motif-dense regions and peptides that may have the capacity to bind to a variety of MHC molecules (promiscuous or multi-determinant binders) and to stimulate an immune response in these various MHC contexts as well (promiscuous or multi-determinant epitopes). The EpiMer algorithm uses a library of MHC binding motifs for multiple class I and class II HLA alleles to predict antigenic sites within a protein that have the potential to induce an immune response in subjects with a variety of genetic backgrounds. EpiMer locates matches to each MHC-binding motif within the primary sequence of a given protein antigen. The relative density of these motif matches is determined along the length of the antigen, allowing in the generation of a motif-density histogram. Finally the algorithm identifies

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protein regions in this histogram with a motif match density above an algorithm-defined motif density value and produces a list of subsequences representing these filtered or motif-rich regions. The regions selected by EpiMer may be more likely to act as multi-determinant binding peptides than randomly chosen peptides from the same antigen due to their concentration of MHC-binding motif matches. The selection of regions that are MHC binding motif-dense increases the likelihood that the predicted peptide is a valid motif and furthermore that the reiteration of identical motifs may contribute to peptide binding.

10 Additional MHC binding motif-based algorithms have been described by Parker *et al* (1994) and Altuvia *et al* (1995). In these algorithms binding to a given MHC molecule is predicted by a linear function of the residues at each position, based on empirically defined parameters and in the case of the Altuvia *et al* (1995) algorithm known crystallographic structures may also be taken into consideration.

1 recombinant methods offer the opportunity to obtain well characterized epitopes of high purity for the production of diagnostic reagents and epitope-specific vaccine formulations (Mohapatra *et al* 1995). Based upon the amino acid sequence of a linear epitope and identification of the corresponding nucleotide sequence encoding same, 20 polymerase chain reaction (PCR) may be performed to amplify the epitope-encoding sequence from cDNA. After cloning and expression in a suitable vector/host system a large amount of epitopes of high purity can be extracted. Accordingly, the present invention clearly extends to both isolated non-recombinant polypeptides and recombinant polypeptides in an impure or isolated form.

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The term polypeptide as used herein shall be taken to refer to any polymer consisting of amino acids linked by covalent bonds and includes within its scope full-length proteins and parts or fragments thereof such as, for example, oligopeptides and short peptide sequences consisting of at least about 5 amino acid residues, preferably at least about 10 amino acid residues, more preferably at least about 12 amino acid residues, and even more preferably at least about 15 amino acid residues. Also

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included within the scope of the definition of a "polypeptide" are amino acid sequence variants, containing one or more preferably conservative amino acid substitutions, deletions or insertions which do not alter at least one essential property of said polypeptide such as for example its immunogenicity use as a diagnostic reagent or effectiveness as a peptide vaccine against *Lawsonia spp* amongst others. Accordingly a polypeptide may be isolated from a source in nature or chemically synthesized. Furthermore a polypeptide may be derived from a full-length protein by chemical or enzymatic cleavage using reagents such as CNBr trypsin or chymotrypsin amongst others.

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Conservative amino acid substitutions are well-known in the art. For example one or more amino acid residues of a native hemolysin polypeptide of the invention can be substituted conservatively with an amino acid residue of similar charge size or polarity with the resulting polypeptide retaining an ability to function in a vaccine or as a diagnostic reagent as described herein. Rules for making such substitutions include those described by Dayhof (1978). More specifically conservative amino acid substitutions are those that generally take place within a family of amino acids that are similar in their side chains. Genetically-encoded amino acids are generally divided into four groups (1) acidic=aspartate glutamate (2) basic=lysine arginine and histidine (3) non polar=alanine valine leucine isoleucine proline phenylalanine methionine and tryptophan and (4) uncharged polar= glycine asparagine, glutamine, asparagine, serine, threonine and tyrosine. Phenylalanine, tyrosine and tryptophan are also jointly classified as aromatic amino acids. One or more replacements within any particular group such as for example the substitution of leucine for isoleucine or valine or alternatively the substitution of aspartate for glutamate or threonine for serine or of any other amino acid residue with a structurally-related amino acid residue will generally have an insignificant effect on the function of the resulting polypeptide.

The present invention is not limited by the source of the subject immunogen and includes isolated and recombinant polypeptides which are derived from a

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natural or a non natural occurring source

The term recombinant polypeptide as used herein shall be taken to refer to a polypeptide which is produced *in vitro* or in a host cell by the expression of a genetic sequence encoding said polypeptide which genetic sequence is under the control of a promoter wherein a genetic manipulation has been performed in order to achieve said expression. Accordingly the term "recombinant polypeptide" clearly encompasses polypeptides produced by the expression of genetic sequences contained in viral vectors, plasmids or cosmids that have been introduced into prokaryotic or eukaryotic cells, tissues or organs. Genetic manipulations which may be used in this context will be known to those skilled in the art and include, but are not limited to, nucleic acid isolation, restriction endonuclease digestion, exonuclease digestion, end-filling using the Klenow fragment of *E. coli* DNA polymerase I or T4 DNA polymerase enzymes, blunt-ending of DNA molecules using T4 DNA polymerase, Exonuclease enzymes, site-directed mutagenesis, ligation and amplification reactions. As will be known to those skilled in the art, additional techniques such as nucleic acid sequencing and nucleotide sequence analysis may also be utilised in the production of recombinant polypeptides. In confirming the identity of a nucleic acid molecule encoding a desired recombinant polypeptide and a genetic construct, a nucleic acid molecule

Wherein the polypeptide of the present invention is a recombinant polypeptide, it may be produced in and, if desirable, isolated from a recombinant viral vector or host cell expression system. As will be known to those skilled in the relevant art, a cell for production of a recombinant polypeptide is selected on the basis of several parameters including the genetic constructs used to express the polypeptide under consideration, as well as the stability and activity of said polypeptide. It will also be known to those skilled in the art that the stability or activity of a recombinant polypeptide may be determined at least in part by post translational modifications to the polypeptide such as, for example, glycosylation, acylation or phosphorylation reactions, and that these may vary between cell lines used to produce the recombinant

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polypeptide

Accordingly in a more particularly preferred embodiment the present invention extends to a recombinant polypeptide or a derivative, homologue or analogue thereof as present in a virus particle or as produced in prokaryotic or eukaryotic host cell or virus or cell culture thereof

The present invention also extends to a recombinant polypeptide according to any of the foregoing embodiments which is produced in a bacterial cell belonging to the genus *Lawsonia* in particular a cell of *L. intracellularis* or a culture thereof

The term "isolated polypeptide" refers to a polypeptide of the present invention which has been purified to some extent preferably to at least about 20% by weight of protein preferably to at least about 50% by weight of protein more preferably to at least about 60% by weight of protein still more preferably to at least about 70% by weight of protein and even more preferably to at least about 80% by weight of protein from its natural source or in the case of non-naturally-occurring polypeptides from the culture medium or cellular environment in which it was produced. Purification may be performed to improve the immunogenicity of the polypeptide of the present invention or to improve the specificity of the immune response against that polypeptide or to remove toxic or undesirable contaminants therefrom. The necessary or required degree of purity of an isolated polypeptide will vary depending upon the purpose for which the polypeptide is intended and for many applications it will be sufficient for the polypeptide preparation to contain no contaminants which would reduce the immunogenicity of the polypeptide when administered to a host animal in particular a porcine or avian animal being immunized against PPE or alternatively which would inhibit immuno-specific binding in an immunoassay for the diagnosis of PPE or a causative agent thereof

The purity of an isolated polypeptide of the present invention may be determined by those skilled in the art including the degree of homogeneity of

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protein preparation as assessed by SDS/polyacrylamide gel electrophoresis 2-
isoelectric electrophoresis or amino acid composition analysis or sequence
analysis.

substantially free of nonspecific proteins as assessed by SDS/polyacrylamide gel electrophoresis 2-dimensional electrophoresis or amino acid composition analysis or sequence analysis

10 The polypeptide of the present invention can be purified for use as a component of a vaccine composition by any one or a combination of methods known to those of ordinary skill in the art including for example reverse phase chromatography HPLC ion exchange chromatography and affinity chromatography among others

1. The preferred embodiment the isolated or recombinant polypeptide of the invention possesses hemolysin activity or is derived from a polypeptide which possesses hemolysin activity such as for example *L. intracellularis* hemolysin or alternatively is immunologically cross reactive with the *L. intracellularis* hemolysin polypeptide of the invention as determined by standard immunoassay such as RIA or ELISA.

As used herein the term 'hemolysin activity' shall be taken to mean hemolysin enzyme activity as determined by any means known to those skilled in the art such as for example by the lysis of red blood cells as described by Basaraba *et al* (1998)

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In a further preferred embodiment the isolated or recombinant polypeptide of the invention is derived from *Lawsonia spp* and more preferably the subject polypeptide derived from *Lawsonia intracellulans*

thereof may comprise any combination of the following

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1. POLYPEPTIDES

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which polypeptide preferably possesses hemolysin activity

To improve the immunogenicity of a subject polypeptide of the present invention one or more amino acids not corresponding to the original protein sequence can be added to the amino or carboxyl terminus of the polypeptide. Such extra amino acids are useful for coupling the polypeptide to another peptide or polypeptide to a large carrier protein or to a solid support. Amino acids that are useful for these purposes include but are not limited to tyrosine, lysine, glutamic acid, aspartic acid, cysteine and derivatives thereof. Additional protein modification techniques can be used such as e.g. NH_2 acetylation or COOH -terminal amidation to provide additional means for coupling the polypeptide to another polypeptide, protein or peptide molecule or to a solid support. Procedures for coupling polypeptides to each other or to carrier proteins or solid supports are well known in the art. Polypeptides containing the above-mentioned extra amino acid residues at either the carboxyl- or amino-termini are either uncoupled or coupled to a carrier or solid support are consequently within the scope of the present invention.

The polypeptide can be immobilised to a polymeric carrier or support.

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In an alternative embodiment the immunogenicity of a polypeptide of the present invention may be improved using molecular biology techniques to produce a fusion protein containing one or more polypeptides of the present invention fused to a carrier molecule such as a highly immunogenic protein. For example a fusion protein containing a polypeptide of the present invention fused to the highly immunogenic B subunit of cholera toxin can be used to increase the immune response to the polypeptide. The present invention also contemplates fusion proteins comprising a polypeptide of the present invention fused to a carrier molecule such as an interleukin, fused to the subject polypeptide of the present invention and genes encoding same.

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Preferably the polypeptide of the present invention or a derivative, homologue or

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analogue thereof when administered to a mammal induces an immune response in said mammal. More preferably the polypeptide of the present invention when administered to a mammal in particular a porcine animal (e.g. a pig) induces a protective immune response against *Lawsonia spp.* and preferably against *L. intracellularis* therein. As used herein the phrase induction of a protective immune response, and the like refers to the ability of the administered polypeptide of the present invention to prevent or detectably slow the onset, development or progression of symptoms associated with *Lawsonia* infection and preferably to prevent or detectably slow the onset, development or progression of symptoms associated with PPE in pigs.

Preferably the immunogenic polypeptide of the invention comprises an amino acid sequence which is substantially the same as the amino acid sequence set forth in SEQ ID NO 1 or is at least about 70% identical overall to SEQ ID NO 1 or is at least about 70% identical to at least about 8 or more contiguous amino acids of SEQ ID NO 1. In a preferred embodiment the immunogenic polypeptide of the present invention comprises at least a portion of the amino acid sequence of SEQ ID NO 1 or the amino acid sequence encoded by the hemolysin-encoding nucleotide sequence present in pALK12 (ATCC 207195) or about the first fifty amino acids thereof.

For the purposes of nomenclature the amino acid sequence set forth in SEQ ID NO 1 represents the hemolysin polypeptide encoded by the *Lawsonia intracellularis* *hlyA* gene the nucleotide sequence of which is set forth in SEQ ID NO 2.

Preferably the percentage amino acid sequence identity to SEQ ID NO 1 is at least about 80% more preferably at least about 85% even more preferably at least about 90% and still even more preferably at least about 95% similar to SEQ ID NO 1.

In determining whether or not two amino acid sequences fall within these percentage limits those skilled in the art will be aware that it is necessary to conduct a side-by-side comparison or multiple alignment of sequences. In such comparisons or

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alignments differences will arise in the positioning of non-identical residues depending upon the algorithm used to perform the alignment. In the present context,

the percentage sequence identity or similarity between two or more amino acid sequences shall be taken to refer to the number of identical and similar residues respectively between said sequences as determined using any standard algorithm known to those skilled in the art. For example, amino acid sequence identities or similarities may be calculated using the GAP programme of the Computer Genetics Group Inc. University Research Park, Madison, Wisconsin, United States of America (Devereaux *et al* 1984). The GAP programme utilizes the algorithm of Needleman and Wunsch (1970) to maximise the number of identical/similar residues and to minimise the number and/or length of sequence gaps in the alignment. Alternatively or in addition where more than two amino acid sequences are being compared the ClustalW programme of Thompson *et al* (1994) can be used.

15 The present invention further encompasses homologues, analogues and derivatives of a polypeptide comprising the amino acid sequence set forth in SEQ ID NO. 1.

Homologues of a polypeptide are those polypeptides which contain amino acid substitutions, deletions and/or additions relative to the polypeptide without altering one or more of its properties such as its immunogenicity, biological activity or catalytic activity. In such molecules amino acids can be replaced by other amino acids having similar properties such as, for example, hydrophobicity, hydrophilicity, hydrophobic moment, antigenicity, propensity to form or break α -helical structures or β -sheet structures, and so on.

Substitutional variants are those in which at least one residue in the sequence has been removed and a different residue inserted in its place. Amino acid substitutions are typically of single residues but may be clustered depending upon functional constraints placed upon the polypeptide. Insertions will usually be of the order of about 1-20 residues and deletions will range from about 1-20 residues. Typically amino acid substitutions will comprise conservative amino acid

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substitutions such as those described *supra*

In insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein. Insertions can
5 comprise amino-terminal and/or carboxyl terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally insertions within the amino acid sequence will be smaller than amino or carboxyl terminal fusions of the order of about 1 to 4 residues.

(f) Deletional variants are characterized by the removal of one or more amino acids from the sequence.

Amino acid variants of the polypeptide of the present invention may readily be made using peptide synthetic techniques well known in the art such as solid phase peptide
15 synthesis and the like or by recombinant DNA manipulations. The manipulation of sequences to produce variant proteins which manifest as substitutional, insertional or deletional variants are well known in the art. For example techniques for making substitution mutations at predetermined sites in DNA having known sequence are well known to those skilled in the art such as by M13 mutagenesis or
20 other site-directed mutagenesis protocol.

Analogues are defined as peptides, oligopeptides and polypeptides which are functionally equivalent to the peptides of the present invention but which contain certain non naturally occurring or modified amino acid residues as will be known to
25 those skilled in the art. Accordingly, an "analogue" as defined herein need not comprise an amino acid sequence which is similar to the amino acid sequence set forth herein such as for example peptides oligopeptides and polypeptides which are derived from computational predictions or empirical data revealing the secondary tertiary or quaternary structure of the hemolysin polypeptide of the present invention which therefore do not comprise the same primary amino acid sequence of said hemolysin polypeptide yet nevertheless mimic or cross react with B-cell or T-cell

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epitope of *Lawsonia intracellularis* spp and preferably mimic or cross-react with B-cell or T-cell epitope of *Lawsonia intracellularis*

For example mimotopes (polypeptide analogues that cross-react with a B-cell or T-cell epitope of the *Lawsonia* polypeptide of the invention but, however comprise a different amino acid sequence to said epitope) may be identified by screening random amino acid sequences in peptide libraries with antibodies that bind to a desired T-cell or B-cell epitope. As with techniques for the identification of B-cell or T-cell epitopes as described *supra* the antibodies used to identify such mimotopes may be polyclonal or monoclonal or recombinant antibodies in crude or purified form. Mimotopes of a T-cell epitope may then be assayed further for their ability to stimulate T-cell cytotoxic or proliferative responses *in vitro*. Mimotopes are particularly useful as analogues of non-linear (or conformational) epitopes of the polypeptide of the present invention because conformational epitopes are generally formed from non-contiguous regions of the polypeptide. The mimotopes provide immunogenic equivalents thereof in the form of a single peptide molecule.

Additionally the use of polypeptide analogues can result in polypeptides with increased immunogenic and/or antigenic activity that are less sensitive to enzymatic degradation and which are more selective. A suitable proline analogue is 2-aminocyclopentane carboxylic acid (β AC²c) which has been shown to increase the immunogenic activity of a native polypeptide more than 20 times (Mierke *et al*, 1990; Portoghese *et al* 1990; Goodman *et al* 1987).

Derivatives of a polypeptide described herein are those peptides, oligopeptides and polypeptides which comprise at least about five contiguous amino acid residues of the amino acid sequence set forth in SEQ ID NO 1. A "derivative" may further comprise additional naturally-occurring altered glycosylated acylated or non-naturally occurring amino acid residues compared to the amino acid sequence set forth in SEQ ID NO 1. Alternatively or in addition a derivative may comprise one or more non-amino acid substituents such as for example a reporter molecule or other ligand covalently or

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... bound to the amino acid sequence such as for example a reporter molecule which is bound thereto to facilitate its detection

Other examples of recombinant or synthetic mutants and derivatives of the peptide immunogens of the present invention include those incorporating single or multiple substitutions deletions and/or additions therein, such as carbohydrates lipids and/or proteins or polypeptides. Naturally occurring or altered glycosylated or acylated forms of the subject peptides are particularly contemplated by the present invention. Additionally homopolymers or heteropolymers comprising one or more copies of the subject peptide listed in SEQ ID NO. 1 or one or more derivatives homologues or analogues hereof are within the scope of the invention.

Preferably homologues analogues and derivatives of the polypeptide of the invention are defined hereinafter as the ability of said polypeptide or a derivative homologue or analogue thereof to elicit B cell and/or T cell responses in the host in response to immunization.

Preferred homologues analogues and derivatives of the amino acid sequence set forth in SEQ ID NO. 1 include those amino acid variants that function as B cell or T cell epitopes of said amino acid sequence which are capable of mediating an immune response such as for example mimotopes of the immunogenic polypeptide described herein which have been produced by synthetic means such as by Fmoc chemistry. The only requirement of such molecules is that they cross-react immunologically with a polypeptide which comprises the amino acid sequence set forth in SEQ ID NO. 1 or the first 50 amino acid residues thereof or a derivative thereof which comprises at least a contiguous amino acids in length of SEQ ID NO. 1.

... apparent to those skilled in the art, such homologues analogues and derivatives of the polypeptide of the invention molecules will be useful to prepare antibodies that cross-react with antibodies against said polypeptide and/or to elicit a protective immune response of similar specificity to that elicited by said polypeptide.

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Such molecules will also be useful in diagnostic and other applications that are immunological in nature such as for example diagnostics which utilise one or more immunoassay formats (eg ELISA RIA and the like)

5 Accordingly the immunogen of the present invention or a derivative homologue or analogue thereof is useful in vaccine compositions that protect an individual against infection by *L. intracellularis* and/or as an antigen to elicit polyclonal or monoclonal antibody production and/or in the detection of antibodies against *L. intracellularis* in infected animals particularly in porcine and avian animals

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The present inventors have also shown that the N-terminal region of SEQ ID NO 1 is particularly unique as compared to other immunogenic amino acid sequences including those of the hemolysin molecules of other animal pathogens. Accordingly peptides and polypeptides which comprise such unique epitope regions of SEQ ID NO 1 will have improved specificity compared to other regions of the *Lawsonia spp* hemolysin molecule. The particular advantages of such peptides will be immediately apparent to those skilled in the production of vaccine compositions where specificity against a pathogen of interest is an important consideration

20 In particular the present inventors have shown that amino acids 1 to 50 of the *L. intracellularis* hemolysin polypeptide as set forth in SEQ ID NO 1 is not highly conserved compared to the corresponding region of other hemolysin polypeptides being only about 50% identical to the amino acid sequences in the N-terminal region of the *Mycobacterium tuberculosis* and *Serpula hyodysenteriae* hemolysin polypeptides. Accordingly this region of the *L. intracellularis* hemolysin polypeptide is a promising antigenic peptide for the formulation of *Lawsonia*-specific vaccines and diagnostics for the specific detection of *Lawsonia spp* in biological samples

Accordingly in an alternative embodiment the present invention provides an isolated or recombinant immunogenic polypeptide or a derivative homologue or analogue thereof which comprises mimics or cross-reacts with a B-cell or T-cell epitope of a

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Lawsonia *sop* wherein said polypeptide comprises a sequence of amino acids which has at least about 50% sequence identity to about amino acid residue 1 to about amino acid residue 50 of the *L. intracellularis* hemolysin polypeptide as set forth in SEQ ID NO 1. Preferably the percentage sequence identity to amino acids 1 to 50 of SEQ ID NO 1 is at least about 60%, more preferably at least about 70% even more preferably at least about 80% and still even more preferably at least about 90%. In a particularly preferred embodiment the subject polypeptide will comprise a sequence of amino acids from about amino acid 1 to about amino acid 50 of SEQ ID NO 1.

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A second aspect of the present invention provides a vaccine composition for the prophylaxis or treatment of infection in a mammal or bird by *L. intracellularis* or similar pathogenic microorganism said vaccine composition comprising an effective amount of an immunogenic component which comprises an isolated or recombinant polypeptide having at least about 70% sequence identity overall to the amino acid sequence set forth in SEQ ID NO 1 or at least about 50% sequence identity overall to about amino acid residue 1 to about amino acid residue 50 of SEQ ID NO 1, or an immunogenic homologue, analogue or derivative thereof which is immunologically cross-reactive with *Lawsonia intracellularis* and one or more carriers, diluents or adjuvants suitable for veterinary or pharmaceutical use.

As used herein the term 'immunogenic component' refers to a peptide, polypeptide or protein encoded by DNA from, or derived from *L. intracellularis* or a related microorganism thereof which is capable of inducing a protective immune response in an animal, in particular a porcine or avian animal, whether or not said peptide, polypeptide or protein is in an isolated or recombinant form. Accordingly the vaccine composition clearly encompasses those vaccine compositions which comprise killed or non-pathogenic isolates or forms of *L. intracellularis* or related microorganisms thereof which comprise or express said peptide, polypeptide or protein.

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By "protective immune response" is meant that the immunogenic component elicits an immune response in the animal to which the vaccine composition is administered at the humoral and/or cellular level which is sufficient to prevent infection by *Lawsonia intracellularis* or a related microorganism thereto and/or which is sufficient to

5 detectably reduce one or more symptoms or conditions or to detectably slow the onset of one or more symptoms or conditions, associated with infection by *Lawsonia intracellularis* or a related microorganism thereto in an animal host as compared to a control infected animal. The term "effective amount" of an immunogenic component present in the vaccine composition refers to that amount of said immunogenic

10 component that is capable of inducing a protective immune response after a single complete dose has been administered or after several divided doses have been administered.

Preferably the polypeptide component of the subject vaccine composition comprises

15 an amino acid sequence which is both immunogenic and specific by virtue of its immunological cross-reactivity with the causative agent of PPE *Lawsonia intracellularis*. In this regard it will be apparent from the preceding description that such polypeptide components may comprise an amino acid sequence derived from SEQ ID NO 1 or a homologue, analogue or derivative of the amino acid sequence set

20 forth in SEQ ID NO 1 such as for example a mimotope of said sequence.

The immunogenic polypeptide or immunogenic homologue, analogue or derivative may be a naturally-occurring peptide, oligopeptide or polypeptide in isolated or recombinant form according to any of the embodiments described *supra* or exemplified

25 herein. Preferably the immunogenic polypeptide or immunogenic homologue, analogue or derivative is derived from *Lawsonia spp.* in particular *L. intracellularis* or a microorganism that is related thereto.

Preferably the immunogenic component has undergone at least one purification step

30 or at least partial concentration from a cell culture comprising *L. intracellularis* or a related microorganism thereto or from a lysed preparation of *L. intracellularis* cells or

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related microorganism or from another culture in which the immunogenic component is recombinantly expressed. The purity of such a component which has the requisite immunogenic properties is preferably at least about 20% by weight of protein in a particular preparation, more preferably at least about 50% even more preferably at least about 60% still more preferably at least about 70% and even more preferably at least about 80% or greater.

The immunogenic component of the vaccine of the present invention can comprise a single peptide, polypeptide or protein or a range or combination of different peptides or polypeptides or proteins covering different or similar epitopes. In addition or alternatively a single polypeptide can be provided with multiple epitopes. The latter is referred to as a polyvalent vaccine. A multiple epitope includes two or more epitopes located within a peptide or polypeptide molecule.

The formulation of vaccines is generally known in the art and reference can conveniently be made to Remington's Pharmaceutical Sciences 17th ed., Mack Publishing Co. Easton, Pennsylvania, USA.

A particularly useful form of the vaccine is a recombinant vaccine produced for example in a vaccine vector such as but not limited to a cell transfected with a vaccinia virus vector or a bacterial cell capable of expressing the immunogenic component.

The present invention clearly extends to recombinant vaccine compositions in which the immunogenic component at least is contained within killed vaccine vectors and for example by heat, formalin or other chemical treatment, electric shock or low pressure forces. According to this embodiment the immunogenic component of the vaccine is generally synthesized in a live vaccine vector which is killed prior to administration to an animal.

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Furthermore the vaccine vector expressing the immunogenic component may be non-

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pathogenic or attenuated. Within the scope of this embodiment are cells that have been transected with non-pathogenic or attenuated viruses encoding the immunogenic component of the vaccine and non-pathogenic or attenuated cells that directly express the immunogenic component.

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Attenuated or non-pathogenic host cells include those cells which are not harmful to an animal to which the subject vaccine is administered. As will be known to those skilled in the art, live vaccines can comprise an attenuated virus vector encoding the immunogenic component or a host cell comprising same which is capable of replicating in an animal to which it is administered and using host cell machinery to express the immunogenic component, albeit producing no adverse side-effects.

Such vaccine vectors may colonise the gut or other organ of the vaccinated animal. Such live vaccine vectors are efficacious by virtue of their ability to continually express the immunogenic component in the host animal for a time and at a level sufficient to confer protective immunity against a pathogen which expresses an immunogenic equivalent of said immunogenic component. The present invention clearly encompasses the use of such attenuated or non-pathogenic vectors and live vaccine preparations.

20 The vaccine vector may be a virus, bacterial cell or a eukaryotic cell such as an avian, porcine or other mammalian cell or a yeast cell or a cell line such as COS, VERO, HeLa, mouse C127, Chinese hamster ovary (CHO), WI-38, baby hamster kidney (BHK) or MDCK cell lines. Suitable prokaryotic cells include *Mycobacterium spp*, *Corynebacterium spp*, *Salmonella spp*, *Escherichia coli*, *Bacillus spp* and *Pseudomonas spp* amongst others. Bacterial strains which are suitable for the present purpose are well-known in the relevant art (Ausubel *et al* 1987, Sambrook *et al* 1989).

Such cells and cell lines are capable of expression of a genetic sequence encoding a peptide, polypeptide or protein of the present invention from *L. intracellulans* in a manner effective to induce a protective immune response in the animal. For example

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a non pathogenic bacterium could be prepared containing a recombinant sequence capable of encoding a peptide, polypeptide or protein from *L. intracellularis*. The recombinant sequence would be in the form of an expression vector under the control of a constitutive or inducible promoter. The bacterium would then be permitted to colonise suitable locations in a pig's gut and would be permitted to grow and produce the recombinant peptide, polypeptide or protein in amount sufficient to induce a protective immune response against *L. intracellularis*.

In a further alternative embodiment the vaccine can be a DNA or RNA vaccine comprising a DNA or RNA molecule encoding a peptide, polypeptide or protein of the immunogen. The vaccine is injected into muscular tissue or other suitable tissue in a pig under conditions sufficient to permit transient expression of said DNA or RNA to produce an amount of peptide, polypeptide or protein effective to induce a protective immune response. In a preferred embodiment, the DNA vaccine is in the form of a plasmid in which the DNA is operably connected with a promoter region capable of expressing the nucleotide sequence encoding the immunogen in cells of the immunized animal.

In the production of a recombinant vaccine except for a DNA vaccine described herein it is therefore necessary to express the immunogenic component in a suitable vector system. For the present purpose the immunogenic component can be expressed by

(i) placing an isolated nucleic acid molecule in an expressible format, said nucleic acid molecule comprising the coding region of the nucleotide sequence described in SEQ ID NO 2 or a protein-encoding homologue, analogue or derivative of SEQ ID NO 2 selected from the group consisting of

(a) nucleotide sequences that have at least about 70% sequence identity to SEQ ID NO 2

(b) nucleotide sequences that hybridise under at least low stringency hybridisation, preferably under at least moderate stringency conditions and even more preferably under high stringency conditions to the

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complement of SEQ ID NO 2 and

(c) nucleotide sequences that encode the amino acid sequence set forth in SEQ ID NO 1 or a homologue analogue or derivative thereof including for example a mimotope of the amino acid set forth in SEQ ID NO 1

(ii) introducing the isolated nucleic acid molecule of (i) in an expressible format into a suitable vaccine vector and

(iii) incubating or growing the vaccine vector for a time and under conditions sufficient for expression of the immunogenic component encoded by said nucleic acid molecule to occur

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For the purposes of defining the level of stringency a low stringency is defined herein as being a hybridisation and/or a wash carried out in 6xSSC buffer 0.1% (w/v) SDS at 28°C. A moderate stringency is defined herein as being a hybridisation and/or washing carried out in 2xSSC buffer 0.1% (w/v) SDS at a temperature in the range 45°C to 65°C. A high stringency is defined herein as being a hybridisation and/or wash carried out in 0.1xSSC buffer 0.1% (w/v) SDS at a temperature of at least 65°C.

Generally the stringency is increased by reducing the concentration of SSC buffer, or by increasing the concentration of SDS and/or increasing the temperature of the hybridisation and/or wash. Those skilled in the art will be aware that the conditions for hybridisation and/or wash may vary depending upon the nature of the hybridisation membrane or the type of hybridisation probe used. Conditions for hybridisations and washes are well understood by one normally skilled in the art. For the purposes of the invention the parameters affecting hybridisation between nucleic acid molecules of reference is found in pages 2.10.8 to 2.10.16 of Ausubel *et al* (1987) which is herein incorporated by reference.

As used herein a nucleic acid molecule in an expressible format is a protein-encoding region of a nucleic acid molecule placed in operable connection with a promoter or other regulatory sequence capable of regulating expression in the vaccine

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vector system

Reference herein to a 'promoter' is to be taken in its broadest context and includes transcriptional regulatory sequences of a classical genomic gene including the TATA box which is required for accurate transcription initiation with or without a CCAAT box sequence and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli or in a tissue-specific manner. In the present context, the term 'promoter' is also used to describe a recombinant, synthetic or fusion promoter or derivative which confers, activates or enhances the expression of a nucleic acid molecule to which it is operably connected and which encodes the immunogenic polypeptide. Preferred promoters can contain additional copies of one or more specific regulatory elements to further enhance expression and/or to alter the spatial expression and/or temporal expression of the said nucleic acid molecule.

- 15 Placing a nucleic acid molecule under the regulatory control of i.e. in operable connection with a promoter sequence means positioning the said molecule such that expression is controlled by the promoter sequence. Promoters are generally but not necessarily positioned 5' (upstream) to the genes that they control. In the
- 20 construction of heterologous promoter/structural gene combinations it is generally preferred to position the promoter at a distance from the gene transcription start site that is approximately the same as the distance between that promoter and the gene it controls in its natural setting, i.e. the gene from which the promoter is derived. Furthermore, the regulatory elements comprising a promoter are usually positioned
- 25 within 2 kb of the start site of transcription of the gene. As is known in the art, some variation in this distance can be accommodated without loss of promoter function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting, i.e. the genes from which it is derived. Again, as is
- 30 known in the art, some variation in this distance can also occur.

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about 10 μ g to about 5 mg and most preferably from about 50 μ g to about 1 mg equivalent of the immunogenic component in a volume of about 1ml to about 5 ml. For DNA vaccines, a preferred amount is from about 0.1 μ g/ml to about 5 mg/ml in a volume of about 1 to about 5 ml. The DNA can be present in "naked" form or it can be administered together with an agent facilitating cellular uptake (e.g. in liposomes or cationic lipids). The important feature is to administer sufficient immunogen to induce a protective immune response. The above amounts can be administered as stated or calculated per kilogram of body weight. Dosage regime can be adjusted to provide the optimum therapeutic response. For example, several divided doses can be administered or the dose can be proportionally reduced as indicated by the exigencies of the therapeutic situation. Booster administration may also be required.

The vaccine of the present invention can further comprise one or more additional immunomodulatory components such as, for example, an adjuvant or cytokine molecule amongst others that is capable of increasing the immune response against the immunogenic component. Non-limiting examples of adjuvants that can be used in the vaccine of the present invention include the RIBI adjuvant system (Ribi Inc., Hamilton, MT, USA), alum, mineral gels such as aluminium hydroxide gel, oil-in-water emulsions, water-in-oil emulsions such as, for example, Block co-polymer (CytRx, Atlanta, GA, USA), QS 21 (Cambridge Biotech Inc., Cambridge, MA, USA), SAF-M (Chiron, Emeryville, CA, USA), AMPHIGEN[®] adjuvant, Freund's complete adjuvant, Freund's incomplete adjuvant, and Saponin, QuilA or other saponin fraction, monophosphoryl lipid A, and Avridine lipid-amine adjuvant. Other immunomodulatory agents that can be included in the vaccine include, for example, one or more cytokines such as interferon and/or interleukin, or other known cytokines. Non-ionic surfactants such as, for example, polyoxyethylene oleyl ether and n-hexadecyl polyethylene ether may also be included in the vaccines of the present invention.

The vaccine composition can be administered in a convenient manner such as by oral, intramuscular, subcutaneous, intranasal, intradermal or suppository routes or by implantation (e.g., using slow release

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technology) Depending on the route of administration the immunogenic component may be required to be coated in a material to protect it from the action of enzymes, acids and other natural conditions which may inactivate it such as those in the digestive tract

The vaccine composition may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof. Under ordinary conditions of storage and use these preparations can contain a preservative to prevent the growth of microorganisms. Alternatively the vaccine composition can be stored in lyophilised form to be rehydrated with an appropriate vehicle or carrier prior to use.

Pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be fluid to the extent that easy syringeability exists, unless the pharmaceutical form is a solid or semi-solid such as when slow release technology is employed. In any event it must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms.

The carrier may be a solvent or dispersion medium containing, for example, water or for example, glycerol, propylene glycol and liquid polyethylene glycol and the like) suitable mixtures thereof and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents such as, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases it will be preferable to include isotonic agents such as, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption such as, for example, aluminum

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monostearate and gelatin

sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above as required followed by filter-sterilization. Generally dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients enumerated from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

The present invention extends to vaccine compositions which confer protection against infection by one or more isolates or sub-types of *L. intracellularis* including those that belong to the same serovar or serogroup as *Lawsonia intracellularis*. The vaccine composition preferably also confers protection against infection by other species of the genus *Lawsonia* or other microorganisms related thereto as determined at the nucleotide biochemical structural, physiological and/or immunointeractive level the only requirement being that said other species or other microorganism expresses a polypeptide which is immunologically cross-reactive to the polypeptide of the invention described herein. For example, such related microorganisms may comprise genomic DNA which is at least about 70% identical overall to the genomic DNA of *Lawsonia intracellularis* as determined using standard genomic DNA hybridisation and analysis techniques.

The terms 'serogroup' and 'serovar' relate to a classification of microorganisms which is based upon serological typing data in particular data obtained using agglutination assays such as the microscopic agglutination test (MAT). Those skilled in the art will be aware that serovar and serogroup antigens are a mosaic on the cell surface and as a consequence there will be no strict delineation between bacteria belonging to a

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serovar and/or serogroup. Moreover, organisms which belong to different species may be classified into the same serovar or serogroup because they are indistinguishable by antigenic determination. As used herein, the term 'serovar' means one or more strains which are antigenically identical with respect to antigenic determinants produced by one or more loci. Quantitatively, serovars may be differentiated from one another by cross-agglutination absorption techniques. As used herein, the term 'serogroup' refers to a group of *Lawsonia spp.* whose members cross-agglutinate with shared group antigens and do not cross-agglutinate with the members of other groups and, as a consequence, the members of a serogroup have more or less close antigenic relations with one another by simple cross-agglutination.

The present invention thus clearly extends to vaccine compositions for the treatment and/or prophylaxis of animals, in particular, vaccine compositions for the treatment and/or prophylaxis of porcine and/or avian species against any bacterium belonging to the same serovar or serogroup as *Lawsonia intracellularis*. Preferably, such organisms will express a polypeptide having an amino acid sequence identity of at least about 70% overall with respect to SEQ ID NO. 1 and/or at least about 50% with respect to amino acids 1 to 50 of SEQ ID NO. 1.

20 The present invention extends further to vaccine compositions capable of conferring immunity against a genetic variant of *Lawsonia intracellularis*, the only requirement being that said variant expresses a polypeptide having an overall amino acid sequence identity of at least about 70% with respect to SEQ ID NO. 1 and/or at least about 50% with respect to amino acids 1 to 50 of SEQ ID NO. 1 or a homologue or derivative thereof which is immunologically cross-reactive thereto.

25 Genetic variants of *L. intracellularis* can be developed by mutation, recombination, conjugation or transformation of *L. intracellularis* or may occur naturally. It will be known to a person skilled in the art how to produce such variants.

In a particularly preferred embodiment, the vaccine composition of the invention is adapted for or suitable for the prophylaxis and/or treatment of infection in a porcine

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porcine animal by *L. intracellularis*

Those skilled in the art will recognise the general applicability of the invention in vaccinating animals other than porcine and avian animals against *L. intracellularis* and/or related microorganisms. In the general application of the vaccine or the present invention, the only prerequisite is that the animal on which protection is conferred is susceptible of being infected with *Lawsonia intracellularis* and/or a related microorganism and that in the case of a related microorganism to *L. intracellularis* said related microorganism expresses a B-cell or T-cell epitope which mimics or cross-reacts with the polypeptide component of the vaccine composition described herein. Animals which may be protected by the vaccine of the present invention include, but are not limited to, humans, primates, companion animals (e.g. cats, dogs), livestock animals (e.g. pigs, sheep, cattle, horses, donkeys, goats), laboratory test animals (e.g. mice, rats, guinea pigs, rabbits) and captive wild animals (e.g. kangaroos, foxes, deer). The present invention also extends to the vaccination of birds such as poultry, farmed birds and caged birds.

The present invention further extends to combination vaccines comprising an effective amount of a first immunogenic component comprising the polypeptide of the present invention combined with an effective amount of a second immunogenic component comprising one or more other antigens capable of protecting a porcine animal, or bird, against either *Lawsonia spp.* or another pathogen that infects and causes disease in said animal. In a preferred embodiment, the second immunogenic component is selected from the group consisting of the *L. intracellularis* autolysin, OmpH, FigE and SodC polypeptides and homologues, analogues or derivatives thereof, in particular immunogenic variants or derivatives thereof, and nucleic acid molecules encoding same.

The isolated or recombinant polypeptide of the invention, or an immunologically-valent homologue, analogue or derivative thereof, is also useful for the preparation of immunologically interactive molecules which are useful in the diagnosis of infection.

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As used herein the term "immunologically interactive molecule" includes antibodies and antibody derivatives and functional equivalents such as a Fab or a SCAB (single-chain antibody), any of which optionally can be conjugated to an enzyme, radioactive isotope, fluorescent tag amongst others. The only requirement of such immunologically interactive molecules is that they are capable of binding specifically to the immunogenic polypeptide of the present invention as hereinbefore described.

Accordingly a further aspect of the invention extends to an immunologically interactive molecule which is capable of binding to any one or more of the following

- (i) a peptide oligopeptide or polypeptide which comprises an amino acid sequence which has at least about 70% sequence identity overall to the amino acid sequence set forth in SEQ ID NO 1
- (ii) a peptide oligopeptide or polypeptide which comprises an amino acid sequence having at least about 50% overall sequence identity to amino acid residues 1 to 50 of SEQ ID NO 1 or
- (iii) a homologue analogue or derivative of (i) or (ii) which mimics a B-cell or T-cell epitope thereof

In a preferred embodiment the immunologically interactive molecule is an antibody that binds specifically to a polypeptide consisting of the amino acid of SEQ ID NO 1 or to the first fifty amino acids thereof

Conventional methods can be used to prepare the immunologically interactive molecules. For example, by using a polypeptide of the present invention, polyclonal antisera or monoclonal antibodies can be made using standard methods. For example, an animal (e.g., a mouse, hamster or rabbit) can be immunized with an immunogenic form of the polypeptide of the present invention which elicits an antibody response in the animal. Techniques for conferring immunogenicity on a polypeptide include

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conjugation to carriers or other techniques well known in the art. For example, the polypeptide can be administered in the presence of adjuvant or can be coupled to a carrier molecule as known in the art that enhances the immunogenicity of the polypeptide. The progress of immunization can be monitored by detection of antibody. Standard ELISA or other immunoassay can be used with the immunogen as antigen to assess the levels of antibodies. Following immunization, antisera can be obtained and, for example, IgG molecules corresponding to the polyclonal antibodies can be isolated from the antisera.

- 10 To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from an animal immunised with a peptide of the present invention and fused with myeloma cells by standard somatic cell fusion procedures, thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art and include, for example, the hybridoma technique originally developed by Kohler and Milstein (1975), as well as other techniques such as the human B-cell hybridoma technique (Kozbor et al., 1983), the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985), and screening of combinatorial antibody libraries (Huse et al., 1989). Hybridoma cells can be isolated and screened empirically for production of antibodies that are specifically reactive with the polypeptide, and monoclonal antibodies isolated therefrom.

As with all immunogenic compositions for eliciting antibodies, the immunogenically effective amounts of the peptides of the invention must be determined empirically. Factors to be considered include the immunogenicity of the native peptide, whether or not the peptide will be complexed with or covalently attached to an adjuvant or carrier protein or other carrier, the route of administration for the composition, i.e., intravenous, intramuscular, subcutaneous, etc., and the number of immunizing doses to be administered. Such factors are known in the vaccine art and it is well within the skill of immunologists to make such determinations without undue experimentation.

The term "antibody" as used herein is intended to include fragments thereof which are

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specifically reactive with a peptide that mimics or cross-reacts with a B cell or T cell epitope of the *Lawsonia intracellularis* hemolysin polypeptide set forth in SEQ ID NO 1. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example F(ab)₂ fragments can be generated by treating antibody with pepsin. The resulting F(ab)₂ fragment can be treated to reduce disulfide bridges to produce Fab fragments.

It is within the scope of this invention to include any secondary antibodies (monoclonal polyclonal or fragments of antibodies) including anti-idiotypic antibodies directed to the first mentioned antibodies discussed above. Both the first and second antibodies can be used in detection assays or a first antibody can be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of a peptide which mimics or cross-reacts with a B cell or T cell epitope of the *Lawsonia intracellularis* hemolysin polypeptide set forth in SEQ ID NO 1 as hereinbefore described.

The antibodies described herein are useful for determining B cell or T cell epitopes of an amino acid sequence set forth in SEQ ID NO 1 such as, for example, by testing the ability of synthetic peptides to cross-react immunologically with said amino acid sequence or to elicit the production of antibodies which cross-react with said amino acid sequence. Using methods described herein polyclonal antibodies monoclonal antibodies or chimeric monoclonal antibodies can also be raised to peptides which mimic or cross-react with a B cell or T cell epitope of the *Lawsonia intracellularis* hemolysin polypeptide set forth in SEQ ID NO 1.

More particularly the polyclonal monoclonal or chimeric monoclonal antibodies can be used to detect the peptides of the invention and/or any homologues analogues or derivatives thereof in various biological materials. For example they can be used in an immunoassay or histochemical test. In other words, the antibodies can be used to test for binding to a polypeptide of the invention or to a homologue

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technician how to modify or optimise such assays to perform this embodiment of the present invention and all such modifications and optimisations are encompassed by the present invention.

in one alternative embodiment the present invention contemplates a method of identifying whether or not an animal has suffered from a past infection or is currently infected with *Lawsonia intracellularis* or a related microorganism thereto said method comprising contacting blood or serum derived from said animal with the immunogenic polypeptide of the invention for a time and under conditions sufficient for an antigen antibody complex to form and detecting said complex formation This embodiment differs from the embodiment described *supra* in that it relies upon the detection of circulating antibodies against *Lawsonia intracellularis* or related organism in the animals blood or serum which are present as a consequence of a past or present infection by this pathogen However it will be apparent to those skilled in the art that the principle of the assay format is the same As with other embodiments of the invention referred to *supra* conventional immunoassays can be used Persons skilled in the art will readily be capable of varying known immunoassay formats to perform the method of the invention This embodiment of the invention can also utilise derivatives of blood and serum which comprise immunologically interactive molecules, for example partially-purified IgG or IgM fractions and buffy coat samples amongst others The preparation of such fractions will also be known to those skilled in the art

A further aspect of the present invention provides an isolated nucleic acid molecule which comprises a sequence of nucleotides which encodes or is complementary to a nucleic acid molecule which encodes a peptide oligopeptide or polypeptide selected from the following

- (i) a peptide oligopeptide or polypeptide which comprises an amino acid sequence which has at least about 70% sequence identity overall to the amino acid sequence set forth in SEQ ID NO. 1
- 30 (ii) a peptide oligopeptide or polypeptide which comprises an amino acid sequence having at least about 50% sequence identity overall to about amino

- 30 (ii) a peptide oligopeptide or polypeptide which comprises an amino acid
 35 having at least about 50% sequence identity overall to about amino

about residue 1 to about amino acid residue 50 of SEQ ID NO 1 or

(iii) a homologue analogue or derivative of (i) or (ii) which mimics a B-cell epitope of *Lawsonia spp*

5 In a preferred embodiment the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides which encodes or is complementary to a nucleic acid molecule which encodes a polypeptide immunogen which comprises mimics or cross-reacts with a B cell or T cell epitope of the *Lawsonia intracellularis* hemolysin polypeptide set forth in SEQ ID NO 1

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In a particularly preferred embodiment the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides encoding the *L. intracellularis* hemolysin polypeptide having an amino acid sequence set forth in SEQ ID NO 1 or encoding about the first fifty amino acid residues thereof

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It is within the scope of the invention to encompass polymeric forms of the polypeptide described herein such as aggregates of the amino acid sequence set forth in SEQ ID NO 1 or a homologue analogue or derivative thereof or alternatively as polypeptides comprising repeats of the amino acid sequence set forth in SEQ ID NO 1 or a homologue analogue or derivative thereof. The present invention extends further to nucleic acid molecules encoding such polymeric forms

Alternatively or in addition the isolated nucleic acid molecule of the invention further comprises a sequence of nucleotides which has at least about 70% overall sequence identity to the nucleotide sequence set forth in SEQ ID NO 2 or to a complementary nucleotide sequence thereof. More preferably the percentage sequence identity to SEQ ID NO 2 or to a complementary nucleotide sequence thereof is at least about 80%. More preferably the percentage sequence identity is at least about 90%. Yet still more preferably the percentage sequence identity is at least about 95%

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In a preferred embodiment the nucleic acid molecule comprises the nucleotide

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sequence set forth in SEQ ID NO 2 or the hemolysin-encoding nucleotide sequence present in strain K12 (ATCC 207195), or a degenerate variant thereof and complements thereof

- 5 In determining whether or not two nucleotide sequences fall within these percentage limits those skilled in the art will be aware that it is necessary to conduct a side-by-side comparison or multiple alignment of sequences. In such comparisons or alignments differences may arise in the positioning of non-identical residues depending upon the algorithm used to perform the alignment. In the present context
- 10 reference to a percentage identity between two or more nucleotide sequences shall be taken to refer to the number of identical residues between said sequences as determined using any standard algorithm known to those skilled in the art. For example nucleotide sequences may be aligned and their identity calculated using the Needleman-Wunsch algorithm or other appropriate programme of the Computer Genetics
- 15 Group Inc. University Research Park Madison Wisconsin United States of America

- Alternatively or in addition the isolated nucleic acid molecule of the invention is capable of hybridising under at least low stringency conditions to the nucleotide
- 20 sequence set forth in SEQ ID NO 2 or to a complementary nucleotide sequence thereto or to a nucleic acid fragment comprising at least about 20 contiguous nucleotides in length derived from the sequence set forth in SEQ ID NO 2 or to a complementary nucleotide sequence thereto

- 25 Preferably said nucleic acid molecule is capable of hybridising under at least moderate stringency conditions and even more preferably under high stringency conditions

The present invention clearly encompasses genetic constructs comprising the subject nucleic acid in an expressible form suitable for the preparation of a recombinant immunogenic polypeptide of the present invention such as for use in

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recombinant univalent or polyvalent recombinant vaccines

in some cases the nucleic acid molecule will be operably connected to a promoter sequence which can thereby regulate expression of said nucleic acid molecule in a prokaryotic or eukaryotic cell as described *supra*

The genetic construct optionally further comprises a terminator sequence. The term 'terminator' refers to a DNA sequence at the end of a transcriptional unit which signals termination of transcription. A "terminator" is a nucleotide sequence generally located within the 3'-non-translated region of a gene or mRNA comprising a polyadenylation signal to facilitate the post-transcriptional addition of a polyadenylate sequence to the end of a primary mRNA transcript. Terminator sequences may be isolated from the genomic sequences of bacteria, fungi, viruses, animals and/or plants. Terminators active in animal cells are known and described in the literature.

In a preferred embodiment, the genetic construct can be a cloning or expression vector as known in the art, such as a plasmid, cosmid or phage, comprising a nucleic acid molecule of the present invention and host cells transformed or transfected therewith. In a non-limiting embodiment, the vector is plasmid pALK12 (ATCC Accession No. 207195).

The genetic constructs of the present invention are particularly useful for producing the immunogenic component of the vaccine composition described herein or for use as a DNA vaccine.

A range of genetic diagnostic assays to detect infection of an animal by *Lawsonia* or a related microorganism can be employed using the nucleic acid molecule described herein such as, for example, assays based upon the polymerase chain reaction (PCR) and nucleic acid hybridisation. All such assays are contemplated in the present invention.

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According to a further aspect of the invention provides a diagnostic method of detecting *Lawsonia intracellularis* or related microorganism in a biological sample derived from an animal subject, said method comprising the steps of hybridising one or more probes or primers derived from the nucleotide sequence set forth in SEQ ID NO 2 or a complementary nucleotide sequence thereto or a homologue analogue or derivative thereof to a DNA or RNA molecule present in said sample and then detecting said hybridisation using a detection means

As used herein the term probe refers to a nucleic acid molecule which is derived from the nucleotide sequence set forth in SEQ ID NO 2 and which is capable of being used in the detection thereof. Probes may comprise DNA (single-stranded or double-stranded) or RNA (i.e. riboprobes) or analogues thereof

The term 'primer' refers to a probe as hereinbefore defined which is further capable of amplifying a nucleotide sequence from *Lawsonia intracellularis* or a related microorganism thereto in a PCR

Preferred probes and primers include fragments of the nucleotide sequence set forth in SEQ ID NO 2 and synthetic single-stranded DNA or RNA molecules of at least about 15 nucleotides in length derived from the sequence set forth in SEQ ID NO 2 or a complementary nucleotide sequence thereto

Preferably probes and primers according to this embodiment will comprise at least about 20 contiguous nucleotides derived from SEQ ID NO 2 or a complementary sequence thereto even more preferably at least about 25 contiguous nucleotides still even more preferably at least about 50 contiguous nucleotides and even more preferably at least about 100 nucleotides to about 500 nucleotides derived from the sequence set forth in SEQ ID NO 2 or a complement thereof. Probes and primers comprising the full-length of SEQ ID NO 2 or a complementary nucleotide sequence thereto are also encompassed by the present invention

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For present purpose "homologues" of a nucleotide sequence shall be taken to refer to an isolated nucleic acid molecule which encodes a polypeptide that is functionally equivalent to the polypeptide encoded by the nucleic acid molecule of the present invention or to a polypeptide which is a homologue, analogue or derivative of SEQ ID NO. 1 notwithstanding the occurrence within said sequence of one or more nucleotide substitutions, insertions, deletions or rearrangements.

Analogues of a nucleotide sequence set forth herein shall be taken to refer to an isolated nucleic acid molecule which encodes a functionally-equivalent polypeptide to the polypeptide encoded by the nucleic acid molecule of the present invention or a homologue, analogue or derivative of a polypeptide having the amino acid sequence of SEQ ID NO. 1 notwithstanding the occurrence of any non-nucleotide constituents not normally present in said isolated nucleic acid molecule such as, for example, carbohydrates, radiochemicals including radio nucleotides, reporter molecules such as, but not limited to, biotin, DIG, alkaline phosphatase or horseradish peroxidase amongst others.

Derivatives of a nucleotide sequence set forth herein shall be taken to refer to any isolated nucleic acid molecule which contains at least about 50% nucleotide sequence identity to 15 or more contiguous nucleotides present in the nucleotide sequence set forth in SEQ ID NO. 2 or a complementary nucleotide sequence thereto. Generally, the nucleotide sequence of the present invention may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or insertions. Nucleotide insertional derivatives of the nucleotide sequence of the present invention include 5' and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides or nucleotide analogues. Insertional nucleotide sequence variants are those in which one or more nucleotides or nucleotide analogues are inserted at a predetermined site in the nucleotide sequence of said sequence. Although random insertion is also possible with suitable screening of the resulting product being performed. Deletional nucleotide sequence variants are characterised by the removal of one or more nucleotides from the nucleotide sequence.

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Substitutional nucleotide sequence variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide or nucleotide analogue inserted in its place. In a preferred embodiment, such substitutions are selected based on the degeneracy of the genetic code, as known in the art, with the resulting substitutional variant encoding the amino acid sequence of SEQ ID NO. 1 or at least about the first 50 amino acids thereof.

Probes or primers can comprise inosine adenine guanine thymidine cytosine or
 exact residues or functional analogues or derivatives thereof that are capable of being
 incorporated into a polynucleotide molecule provided that the resulting probe or
 primer is capable of hybridising under at least low stringency conditions to SEQ ID
 NO. 1 or to a complementary nucleotide sequence thereof or is at least about 70%
 identical to SEQ ID NO. 2 or to a complementary nucleotide sequence thereof

A biological sample according to this aspect of the invention includes any organ tissue, cell or exudate which contains or is likely to contain *Lawsonia intracellularis* or a nucleic acid derived therefrom. A biological sample can be prepared in a suitable solution such as, for example, an extraction buffer or suspension buffer. The present invention extends to the testing of biological solutions thus prepared, the only requirement being that said solution at least comprises a biological sample as described herein.

The diagnostic assay of the present invention is useful for the detection of *Lawsonia intracellularis* or a microorganism which is related thereto which expresses the 2^a hemolysin polypeptide of the present invention or a hemolysin-like polypeptide

The present invention clearly contemplates diagnostic assays which are capable of genus-specific and species-specific detection. Accordingly, in one embodiment a probe or primer or a homologue, analogue or derivative thereof comprises DNA capable of being used to detect multiple *Lawsonia spp.* In an alternative embodiment, the probe or primer or a homologue, analogue or derivative thereof comprises DNA

capable of being used to distinguish *Lawsonia intracellularis* from related microorganisms

Less-highly conserved regions within SEQ ID NO 2 such as those encoding about 5 amino acid residues 1 to 50 of the *Lawsonia intracellularis* hemolysin polypeptide are particularly useful as species-specific probes and/or primers for the detection of *L. intracellularis* and very closely related species

Furthermore the diagnostic assays described herein can be adapted to a genus-specific or species-specific assay by varying the stringency of the hybridisation step. Accordingly a low stringency hybridisation can be used to detect several different species of *Lawsonia* in one or more biological samples being assayed while a high stringency hybridisation can be used to distinguish *Lawsonia intracellularis* from such other species

The detection means according to this aspect of the invention may be any nucleic acid-based detection means such as for example nucleic acid hybridisation techniques or paper chromatography hybridisation assay (PACHA) or an amplification reaction such as PCR or nucleic acid sequence-based amplification (NASBA) system

20 The invention further encompasses the use of different assay formats of said nucleic acid-based detection means including restriction fragment length polymorphism (RFLP) amplified fragment length polymorphism (AFLP) single strand chain polymorphism (SSCP) amplification and mismatch detection (AMD) interspersed repetitive sequence polymerase chain reaction (IRS-PCR) inverse polymerase chain reaction (PCP) *in situ* polymerase chain reaction and reverse transcription polymerase chain reaction (RT-PCR) amongst others

The detection means is a nucleic acid hybridisation technique the probe can be labelled with a reporter molecule capable of producing an identifiable signal (e.g. 30 a radioisotope such as ^{32}P or ^{35}S or a biotinylated molecule) According to this embodiment those skilled in the art will be aware that the detection of said reporter

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molecule provides for identification of the probe and that following the hybridisation reaction the detection of the corresponding nucleotide sequences in the biological sample is facilitated. Additional probes can be used to confirm the assay results obtained using a single probe.

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A variation of the nucleic acid hybridisation technique contemplated by the present invention is the paper chromatography hybridisation assay (PACHA) described by Reinhartz *et al* (1993) and equivalents thereof wherein a target nucleic acid molecule is labelled with a reporter molecule such as biotin applied to one end of a
10 nitrocellulose or nylon membrane filter strip and subjected to chromatography under the action of capillary or other forces (e.g. an electric field) for a time and under conditions sufficient to promote migration of said target nucleic acid along the length of the strip to a zone at which a DNA probe is immobilised thereto such as for example in the middle region. According to this detection format labelled target
15 nucleic acid comprising the *Lawsonia spp* nucleotide sequences complementary to the probe will hybridise thereto and become immobilised in that region of the membrane in which the probe is bound. Non-complementary sequences to the probe will diffuse past the site at which the probe is bound. The target nucleic acid may comprise a crude or partially-pure extract of DNA or RNA or alternatively an amplified
20 or purified DNA. Additional variations of this detection means which utilise the nucleotide sequences described herein are clearly encompassed by the present invention.

Wherein the detection means is a RFLP nucleic acid derived from the biological
25 sample in particular DNA is digested with one or more restriction endonuclease enzymes and the digested DNA is subjected to electrophoresis, transferred to a solid support such as for example a nylon or nitrocellulose membrane and hybridised to a probe optionally labelled with a reporter molecule as hereinbefore defined. According to this embodiment a specific pattern of DNA fragments is displayed on the
30 support wherein said pattern is preferably specific for a particular *Lawsonia species* to enable the user to distinguish between different species of the bacterium.

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Wherein the detection means is an amplification reaction such as for example a polymerase chain reaction or a nucleic acid sequence-based amplification (NASBA) system or a variant thereof one or more nucleic acid primer molecules of at least 15 contiguous nucleotides in length derivable from SEQ ID NO 2 or its complementary nucleotide sequence or a homologue analogue or derivative thereof is hybridised to nucleic acid derived from a biological sample and nucleic acid copies of the hemolysin-encoding genetic sequences in said sample or a part or fragment thereof are enzymically-amplified

10 Those skilled in the art will be aware that there must be a sufficiently high percentage of nucleotide sequence identity between the primers and the sequences in the biological sample template molecule to which they hybridise (i.e. the "template molecule"). As stated previously the stringency conditions can be selected to promote hybridisation

15 Preferably each primer is at least about 95% identical to a region of SEQ ID NO 2 or its complementary nucleotide sequence in the template molecule to which it hybridises

20 Those skilled in the art will also be aware that in one format PCR provides for the hybridisation of non-complementary primers to different strands of the template molecule such that the hybridised primers are positioned to facilitate the 5' - 3' synthesis of nucleic acid in the intervening region under the control of a thermostable DNA polymerase enzyme. As a consequence PCR provides an advantage over other
25 detection means in so far as the nucleotide sequence in the region between the hybridised primers may be unknown and unrelated to any known nucleotide sequence

In an alternative embodiment wherein the detection means is AFLP the primers are selected such that when nucleic acid derived from the biological sample in particular
30 DNA is amplified different length amplification products are produced from different *Lawsonia spp*. The amplification products can be subjected to electrophoresis

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transferred to a solid support such as for example a nylon or nitrocellulose membrane and hybridised to a probe optionally labelled with a reporter molecule as hereinbefore described. According to this embodiment a specific pattern of amplified DNA fragments is displayed on the support said pattern optionally specific for a particular *Lawsonia* spp. to enable the user to distinguish between different species of the bacterium in much the same way as for RFLP analysis.

The technique of AMD facilitates not only the detection of *Lawsonia* spp. DNA in a biological sample but also the determination of nucleotide sequence variants which differ from the primers and probes used in the assay format. Wherein the detection means is AMD the probe is end-labelled with a suitable reporter molecule and mixed with an excess of the amplified template molecule. The mixtures are subsequently denatured and allowed to renature to form nucleic acid probe template hybrid molecules or hybrids such that any nucleotide sequence variation between the probe and the template molecule to which it is hybridised will disrupt base-pairing in the hybrids. These regions of mismatch are sensitive to specific chemical modification using hydroxylamine (mismatched cytosine residues) or osmium tetroxide (mismatched thymidine residues) allowing subsequent cleavage of the modified site using piperidine. The cleaved nucleic acid may be analysed using denaturing polyacrylamide gel electrophoresis followed by standard nucleic acid hybridisation as described *supra* to detect the *Lawsonia*-derived nucleotide sequences. Those skilled in the art will be aware of the means of end-labelling a genetic probe according to the performance of the invention described in this embodiment.

According to this embodiment the use of a single end-labelled probe allows unambiguous localisation of the sequence variation. The distance between the point(s) of sequence variation and the end-label is represented by the size of the cleavage product.

In an alternative embodiment of AMD the probe is labelled at both ends with a reporter molecule to facilitate the simultaneous analysis of both DNA strands.

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Wherein the detection means is RT PCR the nucleic acid sample comprises an RNA molecule which is a transcription product of *Lawsonia*-derived DNA or a homologue analogue or derivative thereof As a consequence this assay format is particularly useful when it is desirable to determine expression of one or more *Lawsonia* genes

- 4 According to this embodiment the RNA sample is reverse-transcribed to produce the complementary single-stranded DNA which is subsequently amplified using standard procedures

Variations of the embodiments described herein are described in detail by McPherson
10 et al (1991)

The present invention clearly extends to the use of any and all detection means referred to *supra* for the purposes of diagnosing *Lawsonia spp* and in particular *Lawsonia intracellularis* infection in animal

- 15 The amplification reaction detection means described *supra* can be further coupled to a classical hybridisation reaction detection means to further enhance sensitivity and specificity of the inventive method such as by hybridising the amplified DNA with a probe which is different from any of the primers used in the amplification reaction

- 2 Similarly the hybridisation reaction detection means described *supra* can be further coupled to a second hybridisation step employing a probe which is different from the probe used in the first hybridisation reaction

- Another aspect of the invention provides an isolated probe or primer derived from SEQ ID NO 2 or a complementary nucleotide sequence thereto

The present invention is further described by the following non-limiting examples

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EXAMPLE 1 SOURCES OF PIG TISSUE

Infected Pig Intestines

Sections of grossly thickened ilea were taken from pigs naturally or experimentally affected by PPE. The presence of *L. intracellularis* bacteria in the ilea was confirmed using immunofluorescent staining with specific monoclonal antibodies (McOrist *et al* 1977). An example of a suitable antibody is monoclonal antibody IG4 available from the University of Edinburgh, UK.

EXAMPLE 2

ISOLATION OF *LAWSONIA INTRACELLULARIS* BACTERIA FROM THE INFECTED PIG ILEUM

Lawsonia intracellularis bacteria were extracted directly from lesions of PPE in pigs by filtration and further purified over a Percoll (Pharmacia, Uppsala, Sweden) gradient as follows. Infected ilea were collected from pigs and the presence of *L. intracellularis* was confirmed histologically before storage at -80°C. Sections of ileum were thawed and approximately 8g of infected mucosa were scraped from the intestinal wall. The mucosa was homogenised with 40 ml sterile phosphate buffered saline (PBS) on half speed for 10 seconds using a Sorvall omnimixer. This suspension was centrifuged at 2000 xg for 4 minutes. The supernatant was discarded and the cell pellet was resuspended in 40 ml PBS and re-centrifuged. This washing step was repeated twice. The cell pellet was then resuspended in 20 ml PBS and homogenised at full speed for one minute to release *L. intracellularis* bacteria.

The mixture was centrifuged at 1000 xg for 4 minutes giving a pellet containing a mixture of homogenised epithelial cells and intestinal bacteria. The supernatant was filtered using filters with pore sizes of 3 µm, 1.2 µm and 0.8 µm (Millipore Corporation, MA, USA). The filtrate was centrifuged at 8000 xg for 30 minutes, giving a small pellet of *L. intracellularis* bacteria. The *L. intracellularis* bacteria were further purified using a 45% self-forming Percoll gradient as follows. 20 ml of the bacterial preparation was mixed by inversion into 30 ml of a 45% self-forming Percoll (Pharmacia LKB, Uppsala, Sweden) gradient (45% v/v of Percoll 150

mm NaCl). The gradients were centrifuged in a Sorvall centrifuge using the SS34 rotor at 20 000 rpm for 30 minutes at 4°C. Usually a number of bands form within the gradient. The band (usually located approx 10-20 mm from the base of the tube) containing the *L. intracellularis* bacteria was collected and the volume made up to 16 ml with PBS. The solution was then centrifuged for 15 minutes at 8000 rpm. The resultant pellet was washed with PBS before being resuspended in a final volume of approximately one ml.

EXAMPLE 3

1. PURIFICATION OF *LAWSONIA INTRACELLULARIS* GENOMIC DNA

Genomic DNA was extracted from percoll-gradient purified *Lawsonia intracellularis* bacteria recovered from infected pig ilea scrapings (Example 2) by the methods described by Anderson *et al* (1984) and Sambrook *et al* (1989).

1.2

Briefly the *L. intracellularis* cells were pelleted by centrifugation at 14 000 x g at 4°C. The cells were resuspended in 10 ml of TE buffer (1 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) and centrifuged as before. The pellet was then resuspended in 4 ml TE buffer containing 4 mg/ml lysozyme (Sigma Chemical Co.) and incubated at 37°C for 20 min. SDS and proteinase K (Promega WI, USA) were added to final concentrations of 1% (w/v) and 200 µg/ml respectively and incubation was continued at 45°C for 4 hours. The lysate was then extracted with an equal volume of phenol, 1:1, and chloroform respectively and the nucleic acids were recovered from the supernatant by ethanol precipitation. The pellet was gently dissolved in TE, treated with RnaseA (Promega WI, USA) at 37°C for 30 min and then digested with proteinase K in the presence of 0.5% (w/v) SDS for 1 h at 50°C. After another round of phenol:chloroform (1:1) and ethanol precipitation the purified DNA was dissolved in TE. The DNA was then stored at 4°C.

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EXAMPLE 4

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IMMUNOSCREENING OF A *L. INTRACELLULARIS* LIBRARY USING EXPERIMENTAL SERA FROM VACCINATED PIGS

The genomic DNA from Example 3 was partially digested with the restriction
endonuclease *Sau3A* (Promega) and ligated into Lambda ZAP Express (Stratagene
CA USA). The lambda library was plated on a lawn of *E. coli* XLI-Blue cells at a
density of 1 000 phage forming units (pfu) per 150 mm L-broth agar plate. The library
was screened using the method described in the Protoblot Technical Manual
(Stratagene, CA USA). The filters were blocked in blocking buffer (10 mM Tris-HCl pH
7.5, 150 mM NaCl, 0.05% Tween 20 and 5% blotto) prior to screening with sera from
the pigs Y12 and/or 395. The pigs Y12 and 395 had previously been immunised with
formalin-killed *L. intracellularis* and heat-killed *L. intracellularis*, respectively as
described in International Patent Application No. PCT/AU96/00767. Positive plaques
identified in the primary screen were picked, replated at a lower density and
rescreened with either or both sera until an individual positive plaque was identified.
Plasmid DNA from the positive lambda phage clone was isolated by *in vivo* excision
as recommended by the manufacturer (Stratagene, CA USA). This clone contained
part of the *tlpA* gene of *L. intracellularis*.

EXAMPLE 5

ANALYSIS OF *L. INTRACELLULARIS* EXPRESSING PHAGE CLONES

Plasmid DNA from positive λ ZAP Express phage clones was isolated by *in vivo*
excision by the conditions recommended by the manufacturer (Stratagene).

Plasmid DNA for restriction analysis was extracted by alkaline-lysis as described by
Sambrook et al. (1989) and for automated sequencing using the High Pure Plasmid
Kit as recommended by the manufacturer (Boehringer Mannheim, Mannheim,
Germany).

The nucleotide sequence (320 bp) of the 3' region of the *tlpA* gene of *Lawsonia*

5.1.3. The Vectorsette System enables the amplification of specific DNA fragments where the sequence of only one primer is known. There are three basic steps as follows:

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IDENTIFICATION OF *L. INTRACELLULARIS* COMPONENTS

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identified from Example 4 and 5 was identified using BLAST algorithms (Gish and Altschul, 1993) to search GenBANK. The nucleotide sequence set forth herein as SEQ ID NO. 2 and its corresponding deduced amino acid sequence set forth as SEQ ID NO. 1 have some degree of sequence similarity to hemolysin-encoding genes and peptides respectively that are derived from other pathogenic microorganisms.

Similarities of the *Lawsonia intracellularis* hemolysin polypeptide are apparent from a comparison of the amino acid sequence of this polypeptide to those from other microorganisms (Figure 1).

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EXAMPLE 7 PREPARATION OF BIOLOGICAL MATERIAL FOR DEPOSIT - AMPLIFICATION OF *tlyA*

Template DNA was purified from pig intestinal mucosa isolated from the ileum of pigs
... *L. intracellularis* DNA purification from intestinal mucosa was performed
according to the method of Nollau *et al* (1996) Due to the presence of contaminating
polysaccharides and other material the DNA content of the samples was not
10 quantified and samples were used empirically in PCR amplifications

The PCR amplifications consisted of 1 μ l DNA isolated from infected pig intestinal
mucosa 1 μ M each of the forward (RA168 5' AAATAATAAGATGAG 3' SEQ ID
NO 3) and reverse primers (RA169 5' ATAGAATACAAATTATAATAAG 3' SEQ ID
1 NO 4) 7.5 units KlenTaqI polymerase (Ab Peptides Inc. St Louis Missouri) 0.075
units μ l polymerase (Stratagene Cloning Systems La Jolla California), 1x PC2
KlenTaqI buffer and 0.2 mM dNTPs in a 50 μ l volume PCR was carried out in 4
steps (i) 94°C for 5 min (ii) 94°C for 1 min 58°C for 30 seconds 72°C for 2 min x
33 cycles (iii) 72°C for 10 min (iv) hold at 4°C

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PCR fragment comprising the *tlyA* gene of *L. intracellularis* was subcloned into
 μ CR2.1 TOPO (Invitrogen Corp Carlsbad CA) and designated pALK12

DEPOSIT OF BIOLOGICAL MATERIAL

25 The plasmid pALK12 was deposited with the American Type Culture Collection
(ATCC) at 10801 University Boulevard Manassas VA 20110 USA on 8th April 1999
and was assigned ATCC Accession No 207195

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WE CLAIM

1. An isolated or recombinant immunogenic polypeptide which comprises residues or cross reacts with a B-cell or T-cell epitope of a *Lawsonia spp* hemolysin polypeptide

2. The isolated or recombinant immunogenic polypeptide of claim 1 capable of eliciting the production of antibodies against *Lawsonia spp* when administered to an avian or porcine animal

3. The isolated or recombinant immunogenic polypeptide of claim 1 capable of conferring a protective immune response against *Lawsonia spp* when administered to an avian or porcine animal

4. The isolated or recombinant immunogenic polypeptide of claim 2 wherein the *sonia spp* is *L. intracellularis*

5. The isolated or recombinant immunogenic polypeptide of claim 3 wherein the *Lawsonia spp* is *intracellularis*

6. An isolated or recombinant immunogenic polypeptide selected from the following

- (i) a peptide oligopeptide or polypeptide which comprises an amino acid sequence which has at least about 70% sequence identity overall to the amino acid sequence set forth in SEQ ID NO 1
- (ii) a peptide oligopeptide or polypeptide which comprises an amino acid sequence having at least about 50% sequence identity overall to amino acid residues 1 to 50 of SEQ ID NO 1 or
- (iii) a homologue analogue or derivative of (i) or (ii) which mimics a B-cell epitope of a *Lawsonia spp* hemolysin polypeptide

7. The isolated or recombinant immunogenic polypeptide of claim 6 capable of

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For the production of antibodies against *Lawsonia spp* in a porcine or avian animal

A recombinant immunogenic polypeptide of claim 7 capable of inducing a protective immune response against *Lawsonia spp* in a porcine or avian animal

9 The isolated or recombinant immunogenic polypeptide of claim 8 capable of inducing humoral immunity against *Lawsonia spp* in a porcine or avian animal

10 The isolated or recombinant immunogenic polypeptide of claim 9 capable of inducing humoral immunity against *Lawsonia spp* in a porcine animal

11 The isolated or recombinant immunogenic polypeptide of claim 8 wherein the *Lawsonia spp* is *intracellularis*

12 The isolated or recombinant immunogenic polypeptide of claim 10 wherein the *Lawsonia spp* is *intracellularis*

13 The isolated or recombinant immunogenic polypeptide of claim 6 that comprises the amino acid sequence set forth in SEQ ID NO 1 or the amino acid sequence encoded by the hemolysin-encoding nucleotide sequence of pALK12 (ATCC 207195) and is capable of eliciting the production of antibodies against *Lawsonia intracellularis* when administered to an avian or porcine animal

14 The isolated or recombinant immunogenic polypeptide of claim 13 that consists of the amino acid sequence of SEQ ID NO 1 or the amino acid sequence encoded by the hemolysin-encoding nucleotide sequence of pALK12 (ATCC 207195)

15 The isolated or recombinant immunogenic polypeptide of claim 13 or 14 capable of inducing a protective immune response against *Lawsonia intracellularis* in a porcine animal

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for animal

16 The isolated or recombinant immunogenic polypeptide of claim 15 capable of inducing a protective immune response against *Lawsonia intracellularis* in a porcine animal

17 The isolated or recombinant immunogenic polypeptide of claim 6 that comprises amino acid residues about 1 to about 50 of SEQ ID NO 1 and is capable of eliciting the production of antibodies against *Lawsonia intracellularis* when administered to an avian or porcine animal

18 The isolated or recombinant immunogenic polypeptide of claim 17 that consists essentially of about amino acid 1 to about amino acid 50 of SEQ ID NO 1

19 The isolated or recombinant immunogenic polypeptide of claim 17 capable of inducing a protective immune response against *Lawsonia intracellularis* in a porcine or avian animal

20 The isolated or recombinant immunogenic polypeptide of claim 19 capable of inducing a protective immune response against *Lawsonia intracellularis* in a porcine animal

21 A vaccine composition for the prophylaxis or treatment of infection of an animal by *Lawsonia spp* said vaccine composition comprising an effective amount of an immunogenic component which comprises an isolated or recombinant polypeptide having at least about 70% sequence identity overall to the amino acid sequence set forth in SEQ ID NO 1 or at least about 50% sequence identity overall to amino acid residues 1 to 50 of SEQ ID NO 1 or an immunogenic homologue, analogue or derivative which is immunologically cross-reactive with *Lawsonia intracellularis* and one or more carriers diluents or adjuvants suitable for veterinary or pharmaceutical use

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The vaccine composition according to claim 21 wherein the *Lawsonia spp* is *L. intracellulans*

The vaccine composition according to claim 22 wherein the immunogenic component comprises an isolated or recombinant polypeptide that comprises the amino acid sequence set forth in SEQ ID NO 1 or the amino acid sequence encoded by the hemolysin-encoding nucleotide sequence of pALK12 (ATCC 207195)

24 The vaccine composition of claim 23 wherein the immunogenic component consists essentially of the amino acid sequence of SEQ ID NO 1

25 The vaccine composition according to claim 22 wherein the immunogenic component comprises an isolated or recombinant polypeptide that comprises about amino acid residue 1 to about amino acid residue 50 of SEQ ID NO 1

26 The vaccine composition of claim 25 wherein the immunogenic component comprises about amino acid 1 to about amino acid 50 of SEQ ID NO 1

27 A combination vaccine composition for the prophylaxis or treatment of infection of an animal by *Lawsonia spp* said vaccine composition comprising

(i) a first immunogenic component which comprises an isolated or recombinant polypeptide having at least about 70% sequence identity overall to the amino acid sequence set forth in SEQ ID NO 1 or at least about 50% sequence identity overall to amino acid residues 1 to 50 of SEQ ID NO 1 or an immunogenic homologue analogue or derivative thereof which is immunologically cross-reactive with *Lawsonia intracellulans*

(ii) a second immunogenic component comprising an antigenic *L. intracellulans* peptide polypeptide or protein and

(iii) one or more carriers diluents or adjuvants suitable for veterinary or pharmaceutical use

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28 A vaccine vector that comprises in an expressible form an isolated nucleic acid molecule having a nucleotide sequence that encodes an isolated or recombinant polypeptide which comprises the amino acid sequence set forth in SEQ ID NO 1 such that said immunogenic polypeptide is expressible at a level sufficient to confer immunity against *Lawsonia spp* when administered to a porcine or avian animal

29 The vaccine vector of claim 28 wherein the immunogenic polypeptide is expressed using the steps of

- (i) placing an isolated nucleic acid molecule which comprises the nucleotide sequence set forth in SEQ ID NO 2 or degenerate variant a homologue analogue or derivative thereof which has at least about 70% sequence identity thereto in operable connection with a promoter sequence
- (ii) introducing the isolated nucleic acid molecule and promoter sequence of step (a) into the vaccine vector and
- (iii) culturing growing or propagating the vaccine vector for a time and under conditions sufficient for expression of the immunogenic polypeptide encoded by said nucleic acid molecule to occur

30 The vaccine vector of claim 28 wherein the *Lawsonia spp* is *L. intracellularis*

31 A polyclonal or monoclonal antibody molecule that is capable of binding specifically to a hemolysin polypeptide or a derivative of a hemolysin polypeptide that is derived from *Lawsonia spp* and has at least about 70% sequence identity overall to the amino acid sequence set forth in SEQ ID NO 1

32 The antibody molecule of claim 31 wherein the hemolysin polypeptide or derivative thereof comprises the amino acid sequence set forth in SEQ ID NO 1

33 The antibody molecule of claim 31 wherein the hemolysin polypeptide or derivative thereof comprises about amino acid 1 to about amino acid 50 of SEQ ID NO 1

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3. A method of diagnosing infection of a porcine or avian animal by *Lawsonia intracellularis* or a microorganism that is immunologically cross-reactive thereto said method comprising the steps of contacting a biological sample derived from said animal with the antibody molecule of claim 31 for a time and under conditions sufficient for an antigen antibody complex to form and then detecting said complex formation

35 The method of claim 34 wherein the biological sample comprises whole serum
 40 lymph nodes ileum caecum small intestine large intestine faeces or a rectal swab
 45 derived from a porcine animal

35 A method of identifying whether or not a porcine or avian animal has suffered
from a past infection or is currently infected with *Lawsonia intracellularis* or a
microorganism that is immunologically cross-reactive thereto said method comprising
contacting a blood or serum derived from said animal with the immunogenic polypeptide
of claim 1 for a time and under conditions sufficient for an antigen antibody complex
to form and then detecting said complex formation

37 An isolated nucleic acid molecule which comprises a sequence of nucleotides that encodes or is complementary to a nucleic acid molecule that encodes a peptide oligopeptide or polypeptide selected from the following

- (i) a peptide oligopeptide or polypeptide which comprises an amino acid sequence which has at least about 70% sequence identity overall to the amino acid sequence set forth in SEQ ID NO. 1

1. a peptide oligopeptide or polypeptide which comprises an amino acid sequence which comprises an amino having at least about 50% sequence identity overall to amino acid residue 1 to about amino acid residue 50 of SEQ ID NO. 1 or

a homologue, analogue or derivative of (i) or (ii) which mimics a B cell or T-cell epitope of *Lawsonia* spp

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38 The isolated nucleic acid molecule of claim 37 wherein the peptide oligopeptide sequence comprises the amino acid sequence set forth in SEQ ID NO 1 or the amino acid sequence encoded by the hemolysin-encoding nucleotide sequence of SEQ ID NO 1 (ATCC 207195) or about amino acid residue 1 to about amino acid residue 50 thereof or a B-cell epitope or T-cell epitope thereof

39 The isolated nucleic acid molecule of claim 38 comprising the nucleotide sequence set forth in SEQ ID NO 2 or a complementary nucleotide sequence thereto or a degenerate variant thereof

40 The isolated nucleic acid molecule of claim 39 consisting essentially of the nucleotide sequence of SEQ ID NO 2 or a degenerate variant thereof

41 The isolated nucleic acid molecule of claim 38 comprising that portion of the nucleotide sequence of SEQ ID NO 2 or a degenerate variant thereof which encodes about amino acid residue 1 to about amino acid residue 50 of SEQ ID NO 2

42 The isolated nucleic acid molecule of claim 41 consisting essentially of that portion of the nucleotide sequence of SEQ ID NO 2 or a degenerate variant thereof which encodes about amino acid residue 1 to about amino acid residue 50 of SEQ ID NO 2

43 A method of detecting *Lawsonia intracellularis* or related microorganism in a biological sample derived from a porcine or avian animal subject said method comprising the steps of hybridising one or more probes or primers derived from the nucleotide sequence set forth in SEQ ID NO 2 or a complementary nucleotide sequence thereto to said sample and then detecting said hybridisation using a detection means

44 The method of claim 43 wherein the biological sample comprises whole serum, duodenum, ileum, caecum, small intestine, large intestine, faeces or a rectal swab

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dered for use in animal

45 The method of claim 44 wherein the detection means comprises any nucleic acid based hybridisation or amplification reaction

46 A probe or primer having at least about 15 contiguous nucleotides in length derived from SEQ ID NO 2 or a complementary nucleotide sequence thereto

47 A plasmid designated pALK12 (ATCC Accession No 207195)

48 The combination vaccine composition according to claim 27 wherein the second immunogenic component comprises an antigenic *L. intracellularis* peptide polypeptide or protein selected from the group consisting of SodC OmpH FlgE and autolysin

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S hpo	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
M tub	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
A aeo	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
B buc	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
R pyl	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
Synec	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
B sub	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100

FIGURE 1

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NUMERO DE SOLICITUD 60/134,022

FECHA DE SOLICITUD Mayo 13 1999

Por autondad del

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Página 1

(Al margen codigos de barras jc662 U S PTO, 05/13/99, jc541 U S PTO
60/134022, 05/13/99)

(Sello al margen en 2 páginas 60134022 051399)

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Esta es una peticion para presentar una Solicitud Provisional de Patente bajo 37 CFR

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TRADUCTOR E INTERPRETE OFICIAL
C. IDONEIDAD 045
UNIVERSIDAD NACIONAL DE COLOMBIA

2007 203

§ 1 53 (c)

Para GEN NOVEDOSO LAWSONIA Y SUS USOS I

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Adjuntos se encuentran

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contrato con una agencia del Gobierno de los Estados Unidos?

(X) No

() Si El nombre de la agencia del Gobierno de los Estados Unidos y el numero del contrato son (en blanco)

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Pagina 2

(X) Favor enviar la correspondencia a

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Presentado respetuosamente,

(Firmado)

Daniel E Altman

Registro No 34,115

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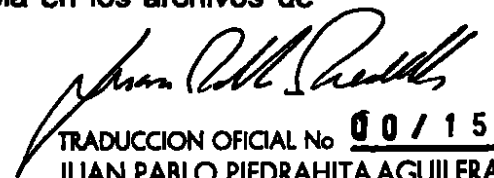
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620 NEWPORT CENTER DR 16TH FLOOR NEWPORT BEACH, CA 92660

(949) 760-0404 FAX (949) 760-9502

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Santafé de Bogotá, Agosto 3 del 2000


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REIVINDICACIONES

Habiendo así especialmente descrito y determinado la naturaleza de la presente invención y la forma como la misma ha de ser llevada a la práctica se declara reivindicar como de propiedad y derecho exclusivo

1 Un polipeptido inmunogénico recombinante o aislado que comprende imitaciones o reactivos en forma cruzada con un epítopo de células B o células T de un polipeptido de hemolisina de *Lawsonia* spp

2 El polipéptido inmunogénico recombinante o aislado de acuerdo con la reivindicación 1 capaz de producir la producción de anticuerpos contra *Lawsonia* spp cuando se administra a un ave o puerco

3 El polipeptido inmunogénico recombinante o aislado de acuerdo con la reivindicación 1 capaz de conferir una respuesta inmune protectora contra *Lawsonia* spp cuando se administra a un ave o puerco

4 El polipeptido inmunogénico recombinante o aislado de acuerdo con la reivindicación 2 en donde la *Lawsonia* spp es *L. intracellularis*

5 El polipeptido inmunogénico recombinante o aislado de acuerdo con la reivindicación 3 en donde la *Lawsonia* spp es *L. intracellularis*

6 Un polipeptido inmunogénico recombinante o aislado seleccionado entre lo que sigue

(i) un péptido oligopeptido o polipeptido que comprende una secuencia de aminoácidos que tiene por lo menos aproximadamente 70 % de identidad de secuencia total con la secuencia de aminoácidos expuesta en SEC ID NO 1

(ii) un péptido oligopeptido o polipeptido que comprende una secuencia de aminoácidos que tiene por lo menos aproximadamente 50 % de identidad de secuencia con aproximadamente los residuos aminoácidos 1 a 50 de SEC ID NO 1, o

(iii) un homólogo análogo o derivado de (i) o (ii) que imita a un epítopo de células B o células C de un polipeptido de hemolisina de *Lawsonia* spp

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7 El polipeptido inmunogeno recombinante o aislado de acuerdo con la reivindicacion 6 capaz de causar la produccion de anticuerpos contra *Lawsonia* spp en un ave o puerco

8 El polipeptido inmunogeno recombinante o aislado de acuerdo con la reivindicacion 7 capaz de conferir una respuesta inmune protectora contra *Lawsonia* spp en un ave o puerco

9 El polipeptido inmunogeno recombinante o aislado de acuerdo con la reivindicacion 8 capaz de inducir inmunidad humoral contra *Lawsonia* spp en un ave o puerco

10 El polipeptido inmunogeno recombinante o aislado de acuerdo con la reivindicacion 9 capaz de inducir inmunidad humoral contra *Lawsonia* spp en un puerco

11 El polipeptido inmunogeno recombinante o aislado de acuerdo con la reivindicacion 8 en donde la *Lawsonia* spp es *L. intracellularis*

12 El polipeptido inmunogeno recombinante o aislado de acuerdo con la reivindicacion 10 en donde la *Lawsonia* spp es *L. intracellularis*

13 El polipeptido inmunogeno recombinante o aislado de acuerdo con la reivindicacion 6 que comprende la secuencia de aminoacidos expuesta en SEC ID NO 1 o la secuencia de aminoacidos codificada por la secuencia de nucleotidos que codifica a la hemolisina de pALK12 (ATCC 207195) y es capaz de inducir la produccion de anticuerpos contra *Lawsonia intracellularis* cuando se administra a un ave o puerco

14 El polipeptido inmunogeno recombinante o aislado de acuerdo con la reivindicacion 13 que consiste esencialmente en la secuencia de aminoacidos de SEC ID NO 1 o la secuencia de aminoacidos codificada por la secuencia de nucleotidos que codifica a la hemolisina de pALK (ATCC 207195)


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15 El polipeptido inmunogeno recombinante o aislado de acuerdo con la reivindicacion 13 o 14 capaz de inducir una respuesta inmune protectora contra *Lawsonia intracellularis* en un ave o puerco

16 El polipeptido inmunogeno recombinante o aislado de acuerdo con la reivindicacion 15 capaz de inducir una respuesta inmune protectora contra *Lawsonia intracellularis* en un animal porcino

17 El polipeptido inmunogeno recombinante o aislado de acuerdo con la reivindicacion 6 que comprende los residuos aminoacidos aproximadamente 1 a aproximadamente 50 de SEC ID NO 1 y es capaz de inducir la produccion de anticuerpos contra *Lawsonia intracellularis* cuando se administra a un ave o animal porcino

18 El polipeptido inmunogeno recombinante o aislado de acuerdo con la reivindicacion 17 que consiste esencialmente en aproximadamente el aminoacido 1 hasta aproximadamente el aminoacido 50 de SEC ID NO 1

19 El polipeptido inmunogeno recombinante o aislado de acuerdo con la reivindicacion 17 capaz de inducir una respuesta inmune protectora contra *Lawsonia intracellularis* en un animal porcino o ave

20 El polipeptido inmunogeno recombinante o aislado de acuerdo con la reivindicacion 19 capaz de inducir una respuesta inmune protectora contra *Lawsonia intracellularis* en un animal porcino

21 Una composicion para vacunas para la profilaxis o el tratamiento de infeccion de un animal por *Lawsonia* spp, comprendiendo dicha composicion de vacuna una cantidad efectiva de un componente inmunogeno el cual comprende un polipeptido aislado o recombinante que tiene por lo menos aproximadamente 70 % de identidad de secuencia total con la secuencia de aminoacidos expuesta en SEC ID NO 1, o por lo menos aproximadamente 50 % de identidad de secuencia total con los residuos aminoacidos 1 a 50 de SEC ID NO 1 o un

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homologo analogo o su derivado inmunogeno que es inmunologicamente reactivo en forma cruzada con *Lawsonia intracellularis* y uno o mas portadores diluyentes o adyuvantes adecuados para uso veterinario o farmaceutico

22 La composicion para vacunas de acuerdo con la reivindicacion 21 en donde la *Lawsonia spp* es *L. intracellularis*

23 La composicion para vacunas de acuerdo con la reivindicacion 22 en donde el componente inmunogeno comprende un polipeptido aislado o recombinante que comprende la secuencia de aminoacidos expuesta en SEC ID NO 1 o la secuencia de aminoacidos codificada por la secuencia de nucleotidos que codifica a la hemolisina de pALK12 (ATCC 207195)

24 La composicion para vacunas de acuerdo con la reivindicacion 23 en donde el componente inmunogeno consiste esencialmente en la secuencia de aminoacidos de SEC ID NO 1

25 La composicion para vacunas de acuerdo con la reivindicacion 22 en donde el componente inmunogeno comprende un polipeptido aislado o recombinante que comprende aproximadamente al residuo aminoacido 1 has a aproximadamente el residuo aminoacido 50 de SEC ID NO 1

26 La composicion para vacunas de acuerdo con la reivindicacion 25 en donde el componente inmunogeno consiste esencialmente en aproximadamente el aminoacido 1 hasta aproximadamente el aminoacido 50 de SEC ID NO 1

27 Una composicion para vacunas de combinacion para la profilaxis o el tratamiento de la infeccion de un animal por *Lawsonia spp* comprendiendo dicha composicion para vacunas

(i) un primer componente inmunogeno que comprende un polipeptido aislado o recombinante que tiene por lo menos aproximadamente 70 % de identidad de secuencia total con la secuencia de aminoacidos expuesta en SEC ID NO 1 o por lo menos aproximadamente 50 % de identidad de secuencia total

con los residuos aminoácidos 1 a 50 de SEC ID NO 1 o un homólogo análogo o derivado inmunogénico de la misma que es inmunológicamente reactiva en forma cruzada con *Lawsonia intracellularis*

(ii) un segundo componente inmunogénico que comprende un péptido polipeptídico o proteína de *L. intracellularis* antígeno y

(iii) uno o más portadores diluyentes o adyuvantes adecuados para uso veterinario o farmacéutico

28 Un vector para vacunas que comprende en una forma expresable, una molécula de ácido nucleico aislada que tiene una secuencia de nucleótidos que codifica un polipeptido inmunogénico recombinante o aislado que comprende la secuencia de aminoácidos expuesta en SEC ID NO 1 de modo que dicho polipeptido inmunogénico es expresable a un nivel suficiente para conferir inmunidad contra *Lawsonia* spp cuando se administra a un animal porcino o ave

29 El vector para vacunas de acuerdo con la reivindicación 28 en donde el polipeptido inmunogénico se expresa utilizando los pasos de

(i) colocar una molécula de ácido nucleico aislada que comprende la secuencia de nucleótidos expuesta en SEC ID NO 2 o una variante degenerada un homólogo análogo o derivado de la misma que tiene por lo menos aproximadamente 70 % de identidad de secuencia a la misma en conexión operable con una secuencia promotora

(ii) introducir la molécula de ácido nucleico aislada y secuencia promotora del paso (a) en el vector para vacunas y

(iii) incubar, cultivar o propagar el vector para vacunas durante un tiempo y bajo condiciones suficientes para que se produzca la expresión del polipeptido inmunogénico codificado por dicha molécula de ácido nucleico

30 El vector para vacunas de acuerdo con la reivindicación 28 en donde la *Lawsonia* spp es *L. intracellularis*

31 Una molecula de anticuerpo policlonal o monoclonal que es capaz de unirse especificamente a un polipeptido de hemolisina o un derivado de un polipeptido de hemolisina que es derivado de *Lawsonia* spp y tiene por lo menos aproximadamente 70 % de identidad de secuencia total a la secuencia de aminoacidos expuesta en SEC ID NO 1

32 La molecula de anticuerpo de acuerdo con la reivindicacion 31 en donde el polipeptido de hemolisina o su derivado comprende la secuencia de aminoacido expuesta en SEC ID NO 1

33 La molecula de anticuerpo de acuerdo con la reivindicacion 31 en donde el polipeptido de hemolisina o su derivado comprende aproximadamente al aminoacido 1 hasta aproximadamente el aminoacido 50 de SEC ID NO 1

34 Un metodo para el diagnostico de la infeccion de un animal porcino o ave por *Lawsonia intracellularis* o un microorganismo que es inmunologicamente reactivo en forma cruzada con la misma comprendiendo dicho metodo los pasos de poner en contacto una muestra biologica derivada de dicho animal con la molecula de anticuerpo de la reivindicacion 31 durante un tiempo y bajo condiciones suficientes para que se forme un complejo antígeno anticuerpo y luego detectando dicha formacion de complejo

35 El metodo de acuerdo con la reivindicacion 34 en donde la muestra biologica comprende suero entero nodulos linfaticos hilon intestino ciego intestino delgado, intestino grueso, materia fecal o una torunda rectal derivado de un animal porcino

36 Un metodo para identificar si un animal porcino o ave ha sufrido o no de una infeccion pasada o esta actualmente infectado con *Lawsonia intracellularis* o un microorganismo que es inmunologicamente reactivo en forma cruzada con la misma comprendiendo dicho metodo poner en contacto sangre o suero derivado de dicho animal con el polipeptido inmunogeno de acuerdo con la

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reivindicacion 1 durante un tiempo y bajo condiciones suficientes para que se forme un complejo antígeno anticuerpo y luego detectar dicha formacion de complejo

37 Una molecula de acido nucleico aislada que comprende una secuencia de nucleotidos que codifica o es complementaria a una molecula de acido nucleico que codifica un peptido oligopeptido o polipeptido seleccionado entre lo siguiente

(i) un peptido oligopeptido o polipeptido que comprende una secuencia de aminoacidos que tiene por lo menos aproximadamente 70 % de identidad de secuencia total con la secuencia de aminoacidos expuesta en SEC ID NO 1

(ii) un peptido oligopeptido o polipeptido que comprende una secuencia de aminoacidos que comprende un amino que tiene por lo menos aproximadamente 50 % de identidad de secuencia total con el residuo aminoacido 1 hasta aproximadamente el residuo aminoacido 50 de SEC ID NO 1 o

(iii) un homologo, analogo o derivado de (i) o (ii) que imita a un epitope de celulas B o celulas C de *Larmonia* ssp

38 La molecula de acido nucleico aislada de acuerdo con la reivindicacion 37, en donde el peptido oligopeptido o polipeptido comprende la secuencia de aminoacidos expuesta en SEC ID NO 1 o la secuencia de aminoacidos codificada por la secuencia de nucleotidos que codifica a la hemolisina de pALK12 (ATCC 207195) o aproximadamente el residuo aminoacido 1 hasta aproximadamente el residuo aminoacido 50 de la misma o su epitope de celulas B o el epitope de celulas T

39 La molecula de acido nucleico aislada de acuerdo con la reivindicacion 38 que comprende la secuencia de nucleotidos expuesta en SEC ID

NO 2 o una secuencia de nucleótidos complementaria a la misma o su variante degenerada

40 La molécula de ácido nucleico aislada de acuerdo con la reivindicación 39 que consiste esencialmente en la secuencia de nucleótidos de SEC ID NO 2 o su variante degenerada

41 La molécula de ácido nucleico aislada de acuerdo con la reivindicación 38 que comprende esa porción de la secuencia de nucleótidos de SEC ID NO 2 o su variante degenerada que codifica aproximadamente el residuo aminoácido 1 hasta aproximadamente el residuo aminoácido 50 de SEC ID NO 2

42 La molécula de ácido nucleico aislada de acuerdo con la reivindicación 11 que consiste esencialmente en esa porción de la secuencia de nucleótidos de SEC ID NO 2 o su variante degenerada que codifica aproximadamente el residuo aminoácido 1 hasta aproximadamente el residuo aminoácido 50 de SEC ID NO 2

43 Un método para detectar la presencia intracelularis o microorganismo relacionado en una muestra biológica derivada de un sujeto animal porcino o ave, comprendiendo dicho método los pasos de hibridar una o más sondas o cebadores derivados de la secuencia de nucleótidos expuesta en SEC ID NO 2 o su secuencia de nucleótidos complementaria a dicha muestra y luego detectar dicha hibridación utilizando un medio de detección

44 El método de acuerdo con la reivindicación 43 en donde la muestra biológica comprende suero entero, nodulos linfáticos, hígado, intestino ciego, intestino delgado, intestino grueso, materia fecal o una torunda rectal derivado de un animal porcino

45 El metodo de acuerdo con la reivindicacion 44 en donde el medio de deteccion comprende cualquier hibridacion basada en acido nucleico o reaccion de amplificacion

46 Una sonda o cebador que tiene por lo menos aproximadamente 15 nucleotidos contiguos de longitud derivados de SEC ID NO 2 o su secuencia de nucleotidos complementaria

47 Un plasmido designado pALK12 (No de Acceso del ATCC 207195)

48 La composicion para vacunas de combinacion de acuerdo con la reivindicacion 27 en donde el segundo componente inmunogeno comprende un peptido polipeptido o proteina de *L. intracellularis* antigeno seleccionado entre el grupo que consiste en SodC OmpH FlgE y autolisina

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