



Clarke, Modet & Co.

COLOMBIA

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SANTAFÉ DE BOGOTÁ, A.A. 92397

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SUPERINTENDENCIA Y COMERCIO

Radicación : 00034580 00000001

Folios: 108

Fecha (AMD): 2004-05-11 17:30:02

Trámite : 002 PATENTES 3 1 REGISTRO/ 005 COMPLEMEN

Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

SEÑORES

DIVISION DE NUEVAS CREACIONES

SUPERINTENDENCIA DE INDUSTRIA Y CC

E.

S.

D.

COD:08

REF.: SOLICITUD N° 00034580 ✓

PATENTE: NOVEDOSO GEN DE LAWSONIA SSP. Y USOS V

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, en esta oportunidad por el presente escrito me permito presentar:

- Copia certificada de la prioridad provisional reivindicada de Estados Unidos N° 60/133.989 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

CCR/srr

P- 338

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
Hereon/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. 00/160
JUAN PABLO PIEDRAHITA AGUILERA
TRADUCTOR E INTERPRETE OFICIAL
C. IDONEIDAD 045
UNIVERSIDAD NACIONAL DE COLOMBIA

MINISTERIO DE RELACIONES
EXTERIORES

Toaquin
C 91420

NO VA FIRMAR APARECE EN EL
DOCUMENTO DESEMPEÑA
LAS FUNCIONES INDICADAS

NO SE ASUME
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2000 JUL 18 12:09

JEFE DE LEGALIZACIONES
SANTAFE DE BOGOTA D.C.

Juan Pablo Piedrahita Aguilera
TRADUCCION OFICIAL No. 001160
JUAN PABLO PIEDRAHITA AGUILERA

INTERPRETE OFICIAL

C. IDONEIDAD 045

UNIVERSIDAD NACIONAL DE COLOMBIA

EL CONSULADO DE COLOMBIA EN

SYDNEY 128/00 1/6/00

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. Alzamora

JOAQUIN ALFREDO ALZAMORA CAMERO
CONSUL GENERAL

PAGADOS: IMPUESTO DE TIMBRE: US\$ 2.00
DERECHOS CONSULARES: US\$ 15.00

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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;

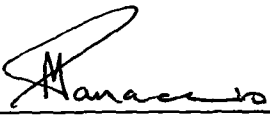
In respect of the inventions disclosed in the patent applications listed in the Schedule affixed hereto.

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:

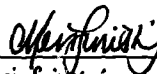
For and on behalf of the ASSIGNORS:


Name: Michael PANACCIO

Witness: 
Name: Jim Parsons
Title: BUSINESS DEVELOPMENT MANAGER

DATED this 11th DAY of October, 1999.

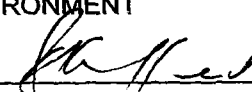

Name: Detlef HASSE

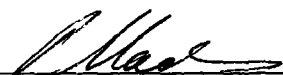
Witness: 
Name: Meri Smida
Title: Senior Scientist

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:


Name: _____
Title: Executive Director
Agri-Science
BRUCE KEFFORD
Executive Director - Primary Industries

Witness: 
Name: P. MACKENZIE
Title: RPA

DATED this 17th DAY of NOVEMBER, 1999.

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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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TRADUCCION OFICIAL No. **00 / 160**
JUAN PABLO PIEDRAHITA AGUILERA
TRADUCTOR E INTERPRETE OFICIAL
C. IDONEIDAD 045
UNIVERSIDAD NACIONAL DE COLOMBIA

1415

TRADUCCIÓN OFICIAL No. 00/160 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 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

Sydney, New South Wales

111 143

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:

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

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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 a 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 dará fe de los mismos en cuanto a cualquier interferencia o litigio relacionados con los mismos, y suscribirá y entregará 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 necesarios 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 aquí 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.

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 Agricultura

(Sello a Tinta: Bruce Kefford Director Ejecutivo Industrias Primarias)

Testigo: (Firmado)

Nombre: P Mackenzie

Cargo: RPA

Con fecha hoy 17 de Noviembre de 1999.

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ANEXO

País	Solicitud No.	Título	Fecha de Presentación
Estados Unidos de América	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 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

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En español, autenticación de la firma del funcionario del Departamento de Relaciones Exteriores y de Comercio de Melbourne, Australia No. 128/00 de Junio 1, 2000. Pagados el Impuesto de Timbre y Derechos Consulares por US\$22.00)
Autenticación por el Ministerio de Relaciones Exteriores Número 097428 de Julio 18 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.

NO SE ASUME
RESPONSABILIDAD DEL TEXTO
2000 AGO -9 P 1:07
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SANTAFÉ DE BOGOTÁ, C.

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412556
Juan P. Piedrahita
CUB. FIDEL. MARELLI
DOCUMENTO DESEMPLEA
LAS FUENTES INDICADAS



TRADUCCION OFICIAL No. 00/160
JUAN PABLO PIEDRAHITA AGUILERA
TRADUCTOR E INTERPRETE OFICIAL
C. IDONEIDAD 045
UNIVERSIDAD NACIONAL DE COLOMBIA

PA 244616



P 338

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THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

May 08, 2000

**THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM
THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK
OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT
APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A
FILING DATE UNDER 35 USC 111.**

APPLICATION NUMBER: 60/133,989

FILING DATE: May 13, 1999



**By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS**

H. Phillips
H. PHILLIPS

Certifying Officer

TRADUCCION OFICIAL No. CO/159
JUAN PABLO PIEDRAHITA AGUILERA

TRADUCTOR E INTERPRETE OFICIAL

C. IDONEIDAD 045

UNIVERSIDAD NACIONAL DE COLOMBIA

May 08, 2000

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TRADUCCION OFICIAL No. 00/154
JUAN PABLO PIEDRAHITA AGUILERA
TRADUCTOR E INTERPRETE OFICIAL
C. IDONEIDAD 045
UNIVERSIDAD NACIONAL DE COLOMBIA

PROVISIONAL APPLICATION FOR PATENT
COVER SHEET

Case No. DAVIE74.001PRF

Date: May 13, 1999

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05/13/99
JCS95 U.S. PTO

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 V

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
Residence Address: 457 Route 164, Preston, Connecticut 06365
Citizenship: USA

Name of Third Inventor: Detlef Hasse
Residence Address: 4 Scullin Court, Sunbury, Victoria, 3429, Australia
Citizenship: Australian

Name of Fourth Inventor: Catherine Wright
Residence Address: 49/422 Cardigan Street, Carlton, Victoria, 3053, Australia
Citizenship: Australian

Name of Fifth Inventor: Robert Gerard Ankenbauer
Residence Address: 104 Castle Hill Road, Pawcatuck, Connecticut 06379
Citizenship: USA

Enclosed are:

- (X) Application in 73 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?

KNOBBE, MARTENS, OLSON & BEAR, LLP
620 NEWPORT CENTER DR. 16TH FLOOR NEWPORT BEACH, CA 92660
(949) 760-0404 FAX (949) 760-9502

TRADUCCION OFICIAL No. 00/134
JUAN PABLO PIEDRAHITA AGUILERA
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C. IDONEIDAD 045
UNIVERSIDAD NACIONAL DE COLOMBIA

JCS41 U.S. PTO
60/133989
05/13/99

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PROVISIONAL APPLICATION FOR PATENT
COVER SHEET

Case No. DAVIE74.001PRF

Date: May 13, 1999

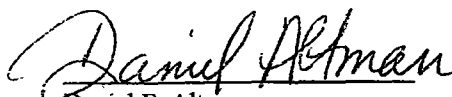
Page 2

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121
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- (X) No.
- () Yes. The name of the U.S. Government agency and the Government contract number are:
- (X) Please send correspondence to:

Daniel E. Altman
Knobbe, Martens, Olson & Bear, LLP
620 Newport Center Dr., 16th Floor
Newport Beach, CA 92660

Respectfully submitted,


Daniel E. Altman
Registration No. 34,115

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TRADUCCION OFICIAL No. 601154
JUAN PABLO PIEDRAHITA AGUILERA
TRADUCTOR E INTERPRETE OFICIAL
C. IDONEIDAD 045
UNIVERSIDAD NACIONAL DE COLOMBIA

NOVEL LAWSONIA SPP. GENE AND USES THEREFOR V

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 peptide, polypeptide or protein. The

10 polypeptide described herein, designated as sodC, 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 related 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 diagnostic agents and

15 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.* (1995)

As used herein, the word "sodC", or the term "sodC gene", shall be taken to refer to the gene encoding the SodC polypeptide of the present invention.

30

As used herein the term "derived from" shall be taken to indicate that a specified

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product, in particular a macromolecule such as a peptide, polypeptide, protein, gene
nucleic acid molecule, antibody molecule, Ig fraction, or other macromolecule, or
biological sample comprising said macromolecule, may be obtained from a particular
source, organism, tissue, organ or cell, albeit not necessarily directly from that source,
5 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
10 or integers 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
15 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.

20 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.

25

BACKGROUND OF THE INVENTION

The meat-producing sector of the agricultural industry is dependant upon the health
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
30 adverse conditions adversely affecting livestock and the quality of meat products
derived therefrom, including those diseases which may potentially be transmitted to

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- 3 -

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.

- 5 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
- 10 Drumel, 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).

- 15 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
- 20 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*, 1981), rabbits (Schodeb 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
- 25 as "*Lawsonia intracellularis*" (McOrist *et al*, 1995). The organism has also been previously referred to as *Ileal symbiont intracellularis* (Stills, 1991). PPE-like diseases in pigs may also be caused by other pathogens such as various species of *Campylobacter* (Gebhart *et al*, 1983).

- 30 *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*

33 McOnist et al, 1995: International Patent Application No. PCT/US96/09576).
1 *Lawsonia 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
5 abscesses form as the villi and intestinal crypts become branched and fill with
inflammatory cells.

PPE is a significant cost component associated with the pig industry, especially in
terms of stock losses, medication costs, reduced growth rates of pigs and increased
10 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.

15 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
20 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
25 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
desirable organisms, such as non-pathogenic organisms that are a part of the normal
30 flora of the intestinal tract or other tissues of said host animal. In summary, an
effective vaccine preparation must be immunogenic, specific and safe.

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. 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.

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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 the SodC polypeptide derived from *Lawsonia spp.* Preferably, the isolated or recombinant immunogenic polypeptide is selected from the group consisting 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;
- 20 (ii) a peptide, oligopeptide or polypeptide which comprises an amino acid sequence having at least about 50% sequence identity to about amino acid residues 1 to about 42 of SEQ ID NO:1,
- (iii) a peptide, oligopeptide or polypeptide which comprises at least about 5 contiguous amino acids of the amino acid sequence defined by residues 1 to
- 25 about 42 of SEQ ID NO:1, or
- (iv) a homologue, analogue or derivative of (i) or (ii) or (iii), which mimics a B-cell or T-cell epitope of *Lawsonia spp.*

In a preferred embodiment, the polypeptide comprises or consists essentially of the amino acid sequence of SEQ ID NO:1, or about amino acids 1 to 42 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 about 42 of SEQ ID NO 1 or comprising at least 5 contiguous amino acids in the region of positions 1 to about 42 of SEQ ID NO:1 or an immunogenic homologue, analogue or derivative thereof which is immunologically cross-reactive with *L. 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 42 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 then 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 sufficient for a complex, such as an antigen:antibody complex, to form, and then detecting said complex formation

A further 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 42 of SEQ ID NO:1; or
- (iii) a peptide, oligopeptide or polypeptide which comprises at least about 5 contiguous amino acids of the amino acid sequence defined by residues 1 to about 42 of SEQ ID NO:1; or
- (iv) a homologue, analogue or derivative of (i) or (ii) or (iii), 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 42 of SEQ ID NO: 1 or a degenerate variant thereof, has at least about 50% sequence identity to all or a part thereof.

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 polynucleotide 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

10 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of a deduced amino acid sequence alignment of various bacterial [Cu, Zn]-superoxide dismutase (SOD) polypeptides. Amino acid sequences of SodC from *Lawsonia intracellularis* (L.int), *Escherichia coli* (E.coli), *Salmonella typhimurium* (S typhi), *Photobacterium leiognathi* (P.leio), *Haemophilus*
 15 *parainfluenzae* (H para), *Brucella abortus* (B.abo), *Actinobacillus pleuropneumoniae* (A pleu) and *Legionella pneumophila* (L.pneu) are shown aligned with each other. Gaps have been introduced to optimise the alignment. Positions containing identical amino acid with respect to the SodC sequence of *L. intracellularis* are shaded. Residues identical in all sequences appear in boldface. Leader peptide sequences are
 20 shown at the top of the Figure in the first grouping. The separation point of the leader peptide from the mature [Cu, Zn]-SodC polypeptide for *L. intracellularis* is proposed between Ala26 and Cys27. Amino acids that are recognised as critical for the function of bovine SOD (not shown) are each marked with an asterisk. The *L. intracellularis* SodC sequence (SEQ ID NO. 1) was deduced by translation of the nucleotide
 25 sequence of the SodC gene obtained from clone p98LI50.

DETAILED DESCRIPTION OF THE INVENTION

In work leading up to the present invention, the inventors sought to identify immunogenic proteins of *Lawsonia intracellularis* for use in vaccines for the
 30 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 a B-cell or T-cell epitope of the SodC polypeptide derived from *Lawsonia spp.*

- 5 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.

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A precise identification and careful selection of epitopes of *Lawsonia spp.* facilitates 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 isolation of linear non-conformational epitopes is easier, because any immunoreactive regions are contained within a single peptide fragment or single amino acid sequence which is capable of being purified under a range of conditions.

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- 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

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particle, such as in a phage display library, may also be employed.

The determination 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 protein, 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*.

Structure determination of both conformational non-linear and non-conformational linear epitopes may be performed by nuclear magnetic resonance spectroscopy (NMR) and X-ray crystallographic analysis. The determination of epitopes using X-ray techniques requires the protein-antibody complex to be crystallized, whereas NMR allows 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.

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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 by 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 focussing) or alternatively, by two-dimensional electrophoresis, the peptides can be transferred to immobilizing nylon or nitrocellulose membranes and incubated with sera raised 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

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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 among 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.

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Several computer-driven algorithms have now been devised to search for T-cell epitopes in proteins (Margalit *et al.*, 1987, Vajda and C. DeLisi, 1990; Altuvia *et al.*, 1994; Parker *et al.*, 1994, DeGroot *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 regions of each isolate's more variable proteins that should be included in a

20 multivalent vaccine

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 effective means of identifying immunogenic epitopes in diagnostic assays.

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The FpMer algorithm (Meister *et al.*, 1995; Gabriel *et al.*, 1995, DeGroot *et al.*, 1995)

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locates clustered MHC binding motifs in amino acid sequences of proteins, based upon 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, resulting in the generation of a motif-density histogram. Finally, the algorithm identifies protein regions in this histogram with a motif match density above an algorithm-defined cutoff density value, and produces a list of subsequences representing these clustered, 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 contains a "valid" motif, and furthermore, that the reiteration of identical motifs may contribute to peptide binding.

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Additional MHC binding motif-based algorithms have been described by Parker *et al.* (1994) and Altuvia *et al.* (1994). 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.* (1994) algorithm, known crystallographic structures may also be taken into consideration.

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, polymerase chain reaction (PCR) may be performed to amplify the epitope-encoding

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region 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.

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 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.

Conservative amino acid substitutions are well-known in the art. For example, one or more amino acid residues of a native SodC polypeptide of the present 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 related 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, cysteine, 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

The present invention is not limited by the source of the subject immunogen and clearly extends to isolated and recombinant polypeptides which are derived from a natural or a non-natural occurring source

- 15 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 suitable promoter wherein a genetic manipulation has been performed in order to achieve said expression. Accordingly, the term "recombinant polypeptide" clearly
- 20 encompasses polypeptides produced by the expression of genetic sequences contained in viral vectors, cosmids or plasmids 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
- 25 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 or ExoIII enzymes, site-directed mutagenesis, ligation, and amplification reactions. As will be known to those skilled in the art, additional techniques such as nucleic acid hybridisations and nucleotide sequence analysis may also be utilised in the
- 30 preparation of recombinant polypeptides, in confirming the identity of a nucleic acid molecule encoding a desired recombinant polypeptide and a genetic construct

comprising the 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 expression system or host cell. 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 alkylation reactions, amongst others, which may vary between cell lines used to produce the recombinant 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 in a 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 *Escherichia*, in particular a cell of *E. 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 or greater, 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. Such isolation 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.

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The purity of an isolated polypeptide of the present invention may be determined by any means known to those skilled in the art, including the degree of homogeneity of a protein preparation as assessed by SDS/polyacrylamide gel electrophoresis, 2-dimensional electrophoresis, or amino acid composition analysis or sequence analysis

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Preferably, the polypeptide of the present invention will be substantially homogeneous or substantially free of nonspecific proteins, as assessed by SDS/polyacrylamide gel electrophoresis, 2-dimensional electrophoresis, or amino acid composition analysis or sequence analysis.

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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.

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In a preferred embodiment, the isolated or recombinant polypeptide of the invention possesses the enzymatic or biological activity of a SOD polypeptide such as, for example, the *L. intracellularis* SodC polypeptide, or is at least derived from a SodC polypeptide or, alternatively, is immunologically cross-reactive with the *L. intracellularis* SodC polypeptide of the present invention.

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In a particularly preferred embodiment, the isolated or recombinant polypeptide of the invention is derived from *Lawsonia spp.* or other pathogenic agent associated with the onset and/or development of PPE and more preferably, the subject polypeptide is derived from *Lawsonia intracellularis*.

A B-cell or T-cell epitope of a SodC polypeptide or a derivative, homologue or analogue thereof may comprise any combination of the following:

- (i) the primary amino acid sequence of a SodC polypeptide, known in the art as a continuous non-conformational epitope,
- (ii) the secondary structure which a SodC polypeptide adopts, known in the art as a continuous conformational epitope,
- (iii) the tertiary structure which a SodC polypeptide adopts in contact with another region of the same polypeptide molecule, known in the art as a discontinuous conformational epitope, or
- (iv) the quaternary structure which a SodC polypeptide adopts in contact with a region of another polypeptide molecule, known in the art as a discontinuous conformational epitope.

20 Accordingly, immunogenic polypeptides or derivatives, homologues or analogues comprising the same, or substantially the same primary amino acid sequence are hereinafter defined as "immunogens which comprise a B-cell or T-cell epitope" or similar term.

25 Immunogenic polypeptides or derivatives, homologues, or analogues thereof comprising different primary amino acid sequences may comprise immunologically identical immunogens, because they possess conformational B-cell or T-cell epitopes that are recognised by the immune system of a host species to be identical. Such immunogenic polypeptides or derivatives, homologues or analogues thereof are hereinafter defined as "immunogens which mimic or cross-react with a B-cell or T-cell epitope" or similar term.

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Accordingly, the present invention extends to an immunogen which comprises, mimics, or cross-reacts with a B-cell or T-cell epitope of an isolated or recombinant polypeptide according to any one of the foregoing embodiments or a derivative, homologue or analogue thereof. In a particularly preferred embodiment, the present invention provides an immunogen which comprises, mimics, or cross-reacts with a B-cell or T-cell epitope of an isolated or recombinant polypeptide which in its native form is obtainable from a species of *Lawsonia* such as, but not limited to *L. intracellularis*, and which polypeptide preferably possesses SodC activity.

- 10 Preferably, such immunogenic polypeptides will not comprise a primary amino acid sequence which is highly-conserved between *L. intracellularis* and another non-pathogenic microorganism which is normally resident in the gut or other organ of an animal, in particular a porcine or avian animal. The significance of this exclusion to those embodiments of the invention wherein specificity is essential to performance (eg
- 15 vaccine and diagnostic applications) will be apparent to those skilled in the art.

- 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
- 20 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., NH₂-acetylation or COOH-terminal amidation, to provide additional means for
- 25 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 and either uncoupled or coupled to a carrier or solid support, are consequently within
- 30 the scope of the present invention

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Furthermore, the polypeptide can be immobilised to a polymeric carrier or support material

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 cytokine, such as an interleukin, fused to the subject polypeptide of the present invention, and genes encoding same.

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

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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 75% identical overall to SEQ ID NO.1, or is at least about 75% identical to at least 8 contiguous amino acids of SEQ ID NO:1. In a preferred embodiment, the immunogenic polypeptide of the present invention consists essentially of the amino acid sequence of SEQ ID NO:1, or the amino acid sequence

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encoded by the SodC-encoding nucleotide sequence present in pALK14 (ATCC 102155) or about the first forty two amino acids thereof

For the purposes of nomenclature, the amino acid sequence set forth in SEQ ID NO:1 represents the partial amino acid sequence of the SodC polypeptide comprising the C-terminal region, encoded by the 5'-end of the *Lawsonia intracellularis* sodC gene. The nucleotide sequence of the 5'-end of the sodC gene 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% identical 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 alignments, differences will arise in the positioning of non-identical residues, depending upon the algorithm used to perform the alignment. In the present context, reference to a 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.

As an alternative embodiment, the present invention provides an isolated or recombinant immunogenic polypeptide which comprises, mimics or cross-reacts with

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a B-cell or T-cell epitope of the SodC polypeptide derived from *Lawsonia* spp. wherein said isolated or recombinant immunogenic polypeptide comprises an amino acid sequence which comprises at least 5 contiguous amino acid residues of SEQ ID NO:1 or a homologue, analogue or derivative thereof.

Preferably, the isolated or recombinant immunogenic polypeptide of the invention comprises at least about 10 contiguous amino acids derived from SEQ ID NO:1, more preferably at least about 20 contiguous amino acid residues derived from SEQ ID NO:1, even more preferably at least about 30 contiguous amino acid residues derived from SEQ ID NO:1 and still even more preferably at least about 40 contiguous amino acid residues derived from SEQ ID NO:1.

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 1-10 amino acid residues and deletions will range from about 1-20 residues. Preferably amino acid substitutions will comprise conservative amino acid substitutions, such as those described *supra*.

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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 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.

Deletional variants are characterised by the removal of one or more amino acids from the sequence

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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 synthesis and the like, or by recombinant DNA manipulations. The manipulation of DNA 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 other site-directed mutagenesis protocol.

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"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 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 polypeptide of the present invention, and which therefore do not comprise the same primary amino acid sequence of said polypeptide, yet nevertheless mimic or cross-react with B-cell or T-cell epitope of *Lawsonia spp.* and preferably, mimic or cross-react with B-cell or T-cell epitope of *Lawsonia intracellularis*.

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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 (i.e., conformational) epitopes of the polypeptide of the present invention, because conformational epitopes are generally formed from non-contiguous regions in a polypeptide and 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-cyclopentylpentanoic acid (β AC⁵c) which has been shown to increase the immunogenic activity of a native polypeptide more than 20 times (Mierke *et al*, 1990; Portuguese *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 non-covalently bound to the amino acid sequence such as, for example, a reporter molecule which is bound thereto to facilitate its detection.

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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.

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 SodC polypeptides of other animal pathogens. Accordingly, peptides, oligopeptides 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. SodC 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.

In particular, the present inventors have shown that amino acids from about 1 to about 42 of the *Lawsonia intracellularis* SodC polypeptide, as set forth in SEQ ID NO:1, is not highly conserved compared to the corresponding region of the *Escherichia coli* SodC polypeptide, being only about 15% identical thereto. Accordingly, this region of the *L. intracellularis* SodC 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 *Lawsonia* spp. wherein said polypeptide comprises a sequence of amino acids which has at least about 50% sequence identity to about amino acid residues 1 to about amino acid residue 42 of the *L. intracellularis* SodC polypeptide as set forth in SEQ ID NO 1. Preferably, the percentage sequence identity to amino acids 1 to about 42 of SEQ ID NO 1 is at least about 60%, more preferably at least about 70%, even more

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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 42 of SEQ ID NO:1.

5 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 or otherwise related microorganism, said vaccine composition comprising:

- 10 (i) an immunogenic component which comprises an isolated or recombinant polypeptide having at least about 70% overall amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1 and/or at least about 50% amino acid sequence identity to amino acid residues 1 to about 42 of SEQ ID NO:1 or comprising at least 5 contiguous amino acids derived from SEQ ID NO:1 or an immunogenic homologue, analogue or derivative thereof which is immunologically cross-reactive with *Lawsonia intracellularis*; and
- 15 (ii) one or more carriers, diluents and/or adjuvants suitable for veterinary or pharmaceutical use

As used herein, the term "immunogenic component" refers to a peptide, polypeptide or a protein encoded by DNA from, or derived from, *L. intracellularis* or a related microorganism thereto 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 attenuated, killed or non-pathogenic isolates or forms of *L. intracellularis* or related microorganisms thereto which comprise or express said peptide, polypeptide or protein.

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 a 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

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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 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.

- 10 Preferably, the polypeptide component of the subject vaccine composition comprises 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
- 15 SEQ ID NO:1 or a homologue, analogue or derivative of the amino acid sequence set 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

- 20 recombinant form according to any of the embodiments described *supra* or exemplified 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.

- 25 Preferably, the immunogenic component has undergone at least one purification step 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 related microorganism, or from another culture in which the immunogenic component is recombinantly expressed. The purity of such a component which has the requisite
- 30 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

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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, polypeptides or proteins covering different or similar epitopes. In addition or, alternatively, a single polypeptide can be provided with multiple epitopes. The latter type of vaccine is referred to as a polyvalent vaccine. A multiple epitope includes two or more epitopes located within a peptide or polypeptide molecule.

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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.

15 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

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

Furthermore, the vaccine vector expressing the immunogenic component may be non-pathogenic or attenuated. Within the scope of this embodiment are cells that have been transfected with non-pathogenic or attenuated viruses encoding the
30 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 therein. 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.

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 SodC peptide, polypeptide or protein of the present invention from *L. intracellularis* in a manner effective to induce a protective immune response in the animal. For example, 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

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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 present invention which 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 set forth 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 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,

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- (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.

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, and/or 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 clarification of the parameters affecting hybridisation between nucleic acid molecules, 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 vector system.

Reference herein to a "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a classical genomic gene, including the TATA box which is required for accurate transcription initiation, with or without a

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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 molecule, 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 expression and/or temporal expression of the said nucleic acid molecule.

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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 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 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 known in the art, some variation in this distance can also occur.

The prerequisite for producing intact polypeptides in bacteria such as *E. coli* is the use of a strong promoter with an effective ribosome binding site. Typical promoters suitable for expression in bacterial cells such as *E. coli* include, but are not limited to, the *lacZ* promoter, temperature-sensitive λ_{cl} or λ_{R} promoters, T7 promoter or the IPTG-inducible *lac* promoter. A number of other vector systems for expressing the nucleic

acid molecule of the invention in *E. coli* are well-known in the art and are described, for example, in Ausubel *et al* (1987) or Sambrook *et al* (1989). Numerous plasmids with suitable promoter sequences for expression in bacteria and efficient ribosome binding sites have been described, such as for example, pKC30 (λ , Shimatake and Rosenberg, 1981), pKK173-3 (*lac*: Amann and Brosius, 1985), pET-3 (T7: Studier and Moffat, 1986), the pFLEX series of expression vectors (Pfizer Inc., CT, USA) or the pQE series of expression vectors (Qiagen, CA), amongst others. Typical promoters suitable for expression in viruses of eukaryotic cells and eukaryotic cells include the SV40 late promoter, SV40 early promoter and cytomegalovirus (CMV) promoter, CMV IE (cytomegalovirus immediate early) promoter amongst others.

Means for introducing the isolated nucleic acid molecule or a genetic construct comprising same into a cell for expression of the immunogenic component of the vaccine composition are well-known to those skilled in the art. The technique used for a given organism depends on the known successful techniques. Means for introducing recombinant DNA into animal cells include microinjection, transfection mediated by DEAE-dextran, transfection mediated by liposomes such as by using lipofectamine (Gibco, MD, USA) and/or cellfectin (Gibco, MD, USA). PEG-mediated DNA uptake, electroporation and microparticle bombardment such as by using DNA-coated tungsten or gold particles (Agracetus Inc., WI, USA) amongst others.

The immunogenic component of a vaccine composition as contemplated herein exhibits excellent therapeutic activity, for example, in the treatment and/or prophylaxis of PPE when administered in an amount which depends on the particular case. For example, for recombinant peptide molecules, from about 0.5 μ g to about 20 mg may be administered, preferably from about 1 μ g to about 10 mg, more preferably from 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 5ml. 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

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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 polyoxyethylene 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, intravenous (where water soluble), intramuscular, subcutaneous, intranasal, intradermal or suppository routes or by implantation (e.g., using slow release 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.

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The vaccine composition may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof, or in oils. 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 syringability 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, ethanol, polyol (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 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

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are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients selected 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 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 *Lawsonia* strains which are antigenically-identical with respect to antigenic determinants produced by one or more loci. Quantitatively, serovars may be

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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 about 42 of SEQ ID NO.1.

15 The present invention extends further to vaccine compositions capable of conferring protection 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 about 42 of SEQ ID NO.1 or a homologue, 20 or analogue or derivative thereof which is immunologically cross-reactive thereto. 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 derivatives.

25 In a particularly preferred embodiment, the vaccine composition of the invention is intended for or suitable for the prophylaxis and/or treatment of infection in a porcine or avian animal and more preferably, for prophylaxis and/or treatment of a porcine animal for infection by *L. intracellularis*

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

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or avian animal and more preferably, for prophylaxis and/or treatment of a porcine animal for infection by *L. intracellularis*.

Accordingly, the present invention clearly extends to the use of the immunogenic polypeptide of the invention according to any one of the preceding embodiments or as exemplified herein in the preparation of a medicament for the treatment and/or prophylaxis of PPE in animals, particularly porcine or avian animals.

The invention further extends to a method of treatment and/or prophylaxis of PPE in an animal such as an avian or porcine animal, said method comprising administering the vaccine composition or the immunogenic polypeptide of the invention as described or exemplified herein to said animal for a time and under conditions sufficient for an immune response to occur thereto. Preferably, in the case of administration of a vaccine composition, the immune response to the immunogen is a protective immune response.

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 of the present invention, the only prerequisite is that the animal on which protection is conferred is capable of being infected with *Lawsonia intracellularis* and/or a related microorganism thereto 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 birds, game birds and caged birds.

5 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, hemolysin, FlgE, and OmpH polypeptides and homologues, analogues or derivatives thereof, in particular immunogenic variants or derivatives thereof, and nucleic acid molecules encoding same.

10 The isolated or recombinant SodC polypeptide of the invention or an immunologically-equivalent homologue, analogue or derivative thereof is also useful for the preparation of immunologically interactive molecules which are useful in the diagnosis of infection of an animal by *Lawsonia spp.*, in particular by *L. intracellularis* or a related organism thereto.

15 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 or 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.

25 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:

- 30 (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 which comprises an amino having at least about 50% overall

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sequence identity to amino acid residues 1 to 42 of SEQ ID NO:1;

(iii) a peptide comprising at least 5 contiguous amino acid residues derived from amino acids 1 to about 42 of SEQ ID NO 1, or

(iv) a homologue, analogue or derivative of (i) or (ii) or (iii) 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 forty two amino acids thereof.

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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, a mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with an immunogenic

15 form of the polypeptide of the present invention which elicits an antibody response in the mammal. Techniques for conferring immunogenicity on a polypeptide include 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 titres in plasma or serum. 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.

25

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

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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 immunogenically 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 also specifically reactive with a peptide that mimics or cross-reacts with a B-cell or T-cell epitope of the *Lawsonia intracellularis* SodC 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* SodC

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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 the 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* SodC 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 ELISA, radioimmunoassay, 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, analogue or derivative thereof, in a biological sample to diagnose the presence of *Lawsonia intracellularis* therein.

Accordingly, a further aspect of the invention provides a method of diagnosing infection of an animal by *Lawsonia intracellularis* or a related microorganism thereto, said method comprising the steps of contacting a biological sample derived from said animal with an immunologically interactive molecule which is capable of binding to a peptide, oligopeptide or polypeptide comprising the amino acid sequence set forth in SEQ ID NO 1 or a homologue, analogue or derivative thereof, for a time and under conditions sufficient for an antigen-antibody complex to form, and detecting said complex formation. According to this embodiment of the present invention, the immunologically interactive molecule is preferably an antibody molecule prepared against the *Lawsonia intracellularis* SodC polypeptide set forth in SEQ ID NO:1 or an analogue or derivative thereof

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The biological sample is one which might contain a polypeptide having an amino acid sequence set forth in SEQ ID NO:1 or a homologue, analogue or derivative thereof, in particular a biological sample derived from a porcine or avian host of the pathogen *Lawsonia intracellularis* or a related microorganism thereto, and can include any appropriate tissue or fluid sample from the animal. Preferred biological samples are derived from the ileum, caecum, small intestine, large intestine, whole serum or lymph nodes of the porcine or avian host animal being tested. Alternatively or in addition the biological test sample may comprise faeces or a rectal swab derived from the animal.

- 10 To distinguish *L. intracellularis* from other microorganisms resident in the gut or other organ of an animal, the antibodies should not be prepared against highly-conserved epitopes of SodC such as those regions of at least 5 amino acids in length which are conserved between *L. intracellularis* and *E. coli* as set forth in Figure 1.
- 15 Conventional immunoassays can be used to perform this embodiment of the invention. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. These, of course, include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as the traditional competitive binding assays. These assays also include direct
20 binding of a labelled antibody to a target. It will be readily apparent to the skilled 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.
- 25 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
30 antibody complex to form, and detecting said complex formation. This embodiment differs from the embodiment described *supra* in that it relies upon the

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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 present embodiment. This embodiment of the invention can also utilise derivatives of blood and serum which comprise immunologically interactive molecules such as, 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 having at least about 70% identical 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% identity to amino acid residues from about position 1 to about position 42 of SEQ ID NO:1;
- (iii) a peptide comprising at least 5 contiguous amino acids derived from SEQ ID NO:1, or
- (iv) 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 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* SodC 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* SodC polypeptide having an amino acid sequence set forth in SEQ ID NO. 1 or encoding about the first forty-two amino acid residues thereof.

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It is within the scope of the invention to encompass polymeric forms of the immunogenic 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, 10 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 thereof

15 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 thereto is at least about 20 80%. Still more preferably, the percentage sequence identity is at least about 90%. Yet still more preferably, the percentage sequence identity is at least about 95%.

In a preferred embodiment, the nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO:2, or the SodC-encoding nucleotide sequence 25 present in pALK14 (ATCC 207155), or a degenerate variant thereof, and complements thereof

In determining whether or not two nucleotide sequences fall within these percentage 30 side comparison or multiple alignment of sequences. In such comparisons or alignments, differences may arise in the positioning of non-identical residues,

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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 BESTFIT programme or other appropriate programme of the Computer Genetics Group, Inc., University Research Park, Madison, Wisconsin, United States of America (Devereaux *et al.*, 1984).

10 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 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

15 complementary nucleotide sequence thereto.

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

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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 wash 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, and/or 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 washing may vary depending upon the nature of the

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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 clarification of the parameters affecting hybridisation between nucleic acid molecules, reference is found in pages 2.10.8 to 2.10.16. of Ausubel *et al.* (1987), which is herein incorporated by reference.

The present invention clearly encompasses genetic constructs comprising the subject nucleic acid molecule in an expressible format suitable for the preparation of a recombinant immunogenic polypeptide of the present invention, such as for use in recombinant univalent or polyvalent recombinant vaccines.

In such 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 3'-end of a primary mRNA transcript. Terminator sequences may be isolated from the genetic 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 with it. In a non-limiting embodiment, the vector is plasmid pALK14 (ATCC Accession No. 207155).

The genetic constructs of the present invention are particularly useful for producing

the proteinaceous immunogenic component of the vaccine, composition described herein or for use in a DNA vaccine

A range of genetic diagnostic assays to detect infection of an animal by *Lawsonia intracellularis* 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.

10 Accordingly, a still 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
15 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
20 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 being used to amplify a nucleotide sequence from *Lawsonia intracellularis* or a
25 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
30 a complementary nucleotide sequence thereto

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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.

- 10 For the 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
15 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
20 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,
25 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
30 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

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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 introduced into 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.

- 10 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
15 about the first 42 amino acids thereof.

- Probes or primers can comprise inosine, adenine, guanine, thymidine, cytidine or uracil residues or functional analogues or derivatives thereof that are capable of being incorporated into a polynucleotide molecule, provided that the resulting probe or
20 primer is capable of hybridising under at least low stringency conditions to SEQ ID NO 2 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.

- The biological sample according to this aspect of the invention includes any organ,
25 tissue, cell or exudate which contains or is likely to contain *Lawsonia intracellulans* 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
30 described herein

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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 SodC polypeptide of the present invention or a SodC-like polypeptide.

- 5 The present invention clearly contemplates diagnostic assays which are capable of both genus-specific and species-specific detection. Accordingly, in one embodiment, the 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
- 10 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 amino acid residues from about position 1 to about position 42 of the *Lawsonia*
- 15 *intracellularis* SodC polypeptide set forth in SEQ ID NO.1, 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-
- 20 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.

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- 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.
- 30 The invention further encompasses the use of different assay formats of said nucleic acid-based detection means, including restriction fragment length polymorphism

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(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 (iPCR), *in situ* polymerase chain reaction and reverse transcription polymerase chain reaction (RT-PCR), amongst others.

Where 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., 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 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.

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 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 said membrane 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 nucleic acid comprising the *Lawsonia spp.* nucleotide sequences complementary to the probe will hybridise thereto and become immobilised in that region of the membrane to 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 or purified DNA. Additional variations of this detection means which utilise the nucleotide sequences described herein are clearly encompassed by the present

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invention

Wherein the detection means is a RFLP, nucleic acid derived from the biological 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 support, wherein said pattern is preferably specific for a particular *Lawsonia* species to enable the user to distinguish between different species of the bacterium.

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 same, 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 SodC-encoding genetic sequences in said sample, or a part or fragment thereof, are enzymically-amplified.

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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 varied to promote hybridisation

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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

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Those skilled in the art will also be aware that, in one format, PCR provides for the

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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
5 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
10 DNA is amplified, different length amplification products are produced from different *Lawsonia* spp. The amplification products can be 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 described. According to this embodiment, a specific pattern of amplified
15 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
20 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
25 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
30 piperidine. The cleaved nucleic acid may be analysed using denaturing polyacrylamide gel electrophoresis, followed by standard nucleic acid hybridisation as

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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.

- 5 According to this embodiment, the use of a single end-labelled probe allows unequivocal 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

- 10 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

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,

- 15 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.

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

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

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

The amplification reaction detection means described *supra* can be further coupled to a classical hybridisation reaction detection means to further enhance sensitivity and

- 30 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

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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.

- 5 A further 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

15 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*, 1997). An example of a suitable antibody is monoclonal antibody G4 available from
20 the University of Edinburgh, UK.

EXAMPLE 2 ISOLATION OF *Lawsonia intracellularis* BACTERIA FROM THE INFECTED PIG ILEUM

- 25 *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
30 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

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20 approximately one ml

PURIFICATION OF *Lawsonia intracellularis* GENOMIC DNA

Briefly, the *L. intracellularis* cells were pelleted by centrifugation at 14,000 x g at 4°C for 15 min. 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

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EXAMPLE 5

ANALYSIS OF *L. intracellularis* EXPRESSING PHAGE CLONES

Phagemid DNA from positive λZAP Express phage clones was isolated by *in vivo* excision, by the conditions recommended by the manufacturer (Stratagene).

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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).

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The nucleotide sequence of the *sodC* gene of *L. intracellularis* was obtained from the clone p98LI50 identified in the preceding Example. DNA sequencing was performed by the Dye-terminator method of automated sequencing (ABI Biosystems, CA, USA) The nucleotide sequence of the complete coding region of the *sodC* gene is set

15 out in SEQ ID NO: 2.

EXAMPLE 6

IDENTIFICATION OF *L. Intracellularis* COMPONENTS

Sequence similarity of the DNA molecules encoding putative vaccine candidates identified from Example 4 and 5, was identified using the BLAST algorithm (Gish and States, 1993). Amino acid residues 43 to 57 and 62 to 64 of the *Lawsonia intracellularis* SodC sequence set forth in SEQ ID NO.1 have very high sequence similarity to the *Escherichia coli* SodC polypeptides (Figure 1). Unique regions, in particular residues 1 to 42 of the *Lawsonia intracellularis* SodC polypeptide are
25 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

EXPRESSION OF THE FULL-LENGTH SodC PROTEIN

The primary sequence of the native SodC protein appears to match the sequence requirements for both a standard secretory signal peptide cleavage site and a prokaryotic lipoprotein cleavage site. Both cleavage sites are located at Ala26-Cys27 of the amino acid sequence set forth in SEQ ID NO: 1

The expression of certain proteins can often be improved by fusing a short leader peptide or a "protective peptide" to the N-terminus of the target protein. This protective peptide protects recombinant proteins from proteolysis (Sung *et al.*, 1986; 1987 and United States Patent No. 5,460,954).

To express the SodC protein, the nucleotide DNA sequence encoding the putative signal peptide of the SodC protein was removed from the full-length SodC gene (SEQ ID NO 2), to yield a signal sequence-deficient SodC polypeptide-encoding sequence, designated SS(-)SodC. Second, a nucleotide sequence encoding the protective peptide leader amino acid sequence MGTITTTTSL (PP: SEQ ID NO: 3) was fused to the 5'-end of SS(-)SodC, to yield PP-SS(-)SodC. Cloning details are presented in Example 8 below.

EXAMPLE 8

AMPLIFICATION OF SodC

Template DNA for SodC was plasmid p98LI50. Plasmid p98LI50 was excised from Lambda ZAP Express (Stratagene Cloning Systems, La Jolla, California) and is a pBK-CMV derivative, which was identified by screening a *L. intracellularis* genomic lambda libraries with α -*L. intracellularis* pig antisera, as described in Example 4.

The PCR amplifications consisted of 0.1 ng of plasmid template, 1 μ M each of the forward primer (RA167: 5' GGCCATGGGTACCACCACCACCACCTCTCTGTC TTTACTTCAGAAGTCCATATG 3'; SEQ ID NO: 4); and the reverse primer (RA175: 5' GGCTCTAGAGGTATATAAATATAGAGGTATG 3'; SEQ ID NO: 5); 7.5 units

KlenTaqI polymerase (Ab Peptides, Inc., St. Louis, Missouri) 0.075 units *Pfu* polymerase (Stratagene Cloning Systems, La Jolla, California) 1 x PC2 (KlenTaqI) buffer and 0.2 mM dNTPs in a 50 μ l volume. PCR was carried out in 4 stages: (i) 95°C for 5 min (5'); (ii) 94°C for 1 min, 58°C for 30 seconds, 72°C for 1.5 min, x 33 cycles; (iii) 72°C for 10 min, (iv) hold at 4°C

The PCR fragment containing the *SodC* gene of *L. intracellularis* was subcloned into pCR2.1-TOPO (Invitrogen Corp., Carlsbad, CA) and designated pALK14. The pCR2.1-TOPO intermediate plasmid was digested with *Nco*I and *Eco*RI and the 0.6 kb fragment excised therefrom was gel-purified and sub-cloned into *Nco*I-*Eco*RI-digested pET28b, to produce the PP-SS(-)*SodC* expression plasmid, pRL032. In this plasmid, the ATG start codon of PP-SS(-)*SodC* is directly downstream from the ribosome-binding site. The expression of the PP-SS(-)*SodC* protein from this plasmid is under control of the T7 promoter, which is inducible by IPTG. The plasmid was introduced into *E. coli* BL21 (DE3) cells for expression of the modified *SodC* protein.

MICROORGANISM DEPOSITS

- 20 The plasmid pALK14 was deposited with the American Type Culture Collection (ATCC) at 10801 University Boulevard, Manassas, VA 20110, USA on 11th March, 1999 and was assigned ATCC Accession No.207155

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WE CLAIM

1. An isolated or recombinant immunogenic polypeptide which comprises, mimics or cross-reacts with a B-cell or T-cell epitope of a *Lawsonia spp* SodC 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 *Lawsonia spp* is *L. intracellularis*.
5. The isolated or recombinant immunogenic polypeptide of claim 3 wherein the *Lawsonia spp* is *L. 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 42 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 a *Lawsonia spp* SodC polypeptide.
7. The isolated or recombinant immunogenic polypeptide of claim 6 capable of

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

The isolated or recombinant immunogenic polypeptide of claim 7 capable of conferring 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 *L. intracellularis*.

12. The isolated or recombinant immunogenic polypeptide of claim 10 wherein the *Lawsonia spp.* is *L. 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 SodC-encoding nucleotide sequence of pALK14 (ATCC 207155) 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 essentially of the amino acid sequence of SEQ ID NO. 1 or the amino acid sequence encoded by the SodC-encoding nucleotide sequence of pALK14 (ATCC 207155).

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

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or avian 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 42 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 42 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 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 42 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

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

23. 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 SodC-encoding nucleotide sequence of pALK14 (ATCC 207155).

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 42 of SEQ ID NO: 1.

26. The vaccine composition of claim 25, wherein the immunogenic component consists essentially of about amino acid 1 to about amino acid 42 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 42 of SEQ ID NO: 1 or an immunogenic homologue, analogue or derivative thereof which is immunologically cross-reactive with *Lawsonia intracellularis*;

(ii) a second immunogenic component comprising an antigenic *L. intracellularis* 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 immunogenic 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) incubating, 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 SodC polypeptide or a derivative of a SodC 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 SodC 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 SodC polypeptide or derivative thereof comprises about amino acid 1 to about amino acid 42 of SEQ ID NO: 1

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34. 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, lymph nodes, ileum, caecum, small intestine, large intestine, faeces or a rectal swab derived from a porcine animal

36. 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 blood or serum derived from said animal with the immunogenic polypeptide of claim 30 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,

(ii) 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 42 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.*

38. The isolated nucleic acid molecule of claim 37, wherein the peptide, oligopeptide

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comprises the amino acid sequence set forth in SEQ ID NO: 1 or about amino acid residue 1 to about amino acid residue 42 thereof, or the amino acid sequence encoded by the SodC-encoding nucleotide sequence of pALK14 (ATCC 207155), 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 42 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 42 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, lymph nodes, ileum, caecum, small intestine, large intestine, faeces or a rectal swab derived from a porcine 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 pALK14 (ATCC Accession No. 207155).
- 48 The combination vaccine according to claim 27 wherein the second immunogenic component comprises an antigenic *L. intracellularis* peptide, polypeptide or protein selected from the group consisting of OmpH, FlgE, hemolysin and autolysin

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ABSTRACT

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 SodC peptide, polypeptide or protein that is particularly useful as an antigen in vaccine preparation for conferring humoral immunity against *Lawsonia intracellularis* and related 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 diagnostic agents and procedures for detecting *Lawsonia intracellularis* or similar or otherwise related microorganisms:

1991-1992

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- 1 -

SEQUENCE LISTING

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Patent No. 5,000,000

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196. 194

-2-

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PROPEKMMROLAWSON:4 PRV 10/5/78

- 3 -

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TRADUCCIÓN OFICIAL No. 00/154 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.

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FECHA DE SOLICITUD: Mayo 13 1999

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Fecha: Mayo 13, 1999

Página 1

(Al margen códigos de barras JC595 U.S. PTO, 05/13/99, jc541 U.S. PTO,
60/133989, 05/13/99)

(Sello al margen en 2 páginas: 60133989.051399)

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WASHINGTON D.C. 20231

ATENCIÓN: SOLICITUD PROVISIONAL DE PATENTE

Señor:

**Esta es una petición para presentar una Solicitud Provisional de Patente bajo 37 CFR
§ 1.53 (c).**

Para: GEN NOVEDOSO LAWSONIA Y SUS USOS V

Nombre del Primer Inventor: Michael Panaccio

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Adjuntos se encuentran:

- (X) Solicitud en 73 páginas con 4 páginas de listados secuenciales.
- (X) 1 hoja de dibujos
- (X) Se adjunta un cheque por el monto de \$150 para cubrir los derechos de presentación.
- (X) Una postal de devolución pre-pagada
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Fue esta invención hecha por una agencia del Gobierno de los Estados Unidos o bajo contrato con una agencia del Gobierno de los Estados Unidos?

TRADUCCION OFICIAL No. 00 / 154

JUAN PABLO PIEDRAHITA AGUILERA

TRADUCTOR E INTERPRETE OFICIAL

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SOLICITUD PROVISIONAL DE PATENTE
CARÁTULA

Caso No. DAVIE74.001PRF

Fecha: Mayo 13, 1999

Página 2

(X) No.

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

(X) Favor enviar la correspondencia a:

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Presentado respetuosamente,

(Firmado)

Daniel E. Altman

Registro No. 34,115

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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.

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REIVINDICACIONES

1. Un polipéptido inmunogénico aislado o recombinante que comprende, imita o reacciona con un epítipo de célula B o célula T de un polipéptido de *Lawsonia spp.* SodC.
2. El polipéptido inmunogénico aislado o recombinante de la reivindicación 1 capaz de lograr la producción de anticuerpos contra *Lawsonia spp.*, cuando se administra a un animal porcino o un ave.
3. El polipéptido inmunogénico aislado o recombinante de la reivindicación 1 capaz de conferir una respuesta inmune protectora contra *Lawsonia spp.* cuando se administra a un ave o un porcino.
4. El polipéptido inmunogénico aislado o recombinante de la reivindicación 2 donde la *Lawsonia spp.* es *Lawsonia intracellularis*.
5. El polipéptido inmunogénico aislado o recombinante de la reivindicación 3 donde la *Lawsonia spp.* es *Lawsonia intracellularis*.
6. Un polipéptido inmunogénico aislado o recombinante seleccionado de lo siguiente:
 - (i) un péptido, oligopéptido o polipéptido que comprende una secuencia de aminoácido que tiene por lo menos un 70% de identidad de secuencia general con la secuencia de aminoácido que se observa en la SEC ID NO: 1;
 - (ii) un péptido, oligopéptido o polipéptido que comprende una secuencia de aminoácido que tiene por lo menos aproximadamente 50% de identidad general de secuencia con los residuos 1 a 42 de la SEC ID NO: 1; o
 - (iii) un homólogo, análogo o derivado de (i) o (ii) que imita un epítipo de célula B o de célula T de un polipéptido de *Lawsonia spp.* SodC

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7. El polipéptido inmunogénico aislado o recombinante de la reivindicación 6 capaz de lograr la producción de anticuerpos contra *Lawsonia spp*, cuando se administra en un animal porcino o un ave.
8. El polipéptido inmunogénico aislado o recombinante de la reivindicación 7 capaz de conferir una respuesta inmune protectora contra *Lawsonia spp* cuando se administra a un ave o un porcino.
9. El polipéptido inmunogénico aislado o recombinante de la reivindicación 8 capaz de inducir inmunidad humoral contra *Lawsonia spp* cuando se administra a un ave.
10. El polipéptido inmunogénico aislado o recombinante de la reivindicación 9 capaz de inducir inmunidad humoral contra *Lawsonia spp* cuando se administra a un animal porcino.
11. El polipéptido inmunogénico aislado o recombinante de la reivindicación 8 donde la *Lawsonia spp* es *Lawsonia intracellularis*.
12. El polipéptido inmunogénico aislado o recombinante de la reivindicación 10 donde la *Lawsonia spp* es *Lawsonia intracellularis*.
13. El polipéptido inmunogénico aislado o recombinante de la reivindicación 6 que comprende la secuencia de aminoácido que se observa en la SEC ID NO: 1 o la secuencia de aminoácido codificada por la secuencia de nucleótido que codifica el SodC de pALK13 (ATCC 207196) y es capaz de obtener la producción de anticuerpos contra *Lawsonia intracellularis* cuando se administra a un ave o un animal porcino.
14. El polipéptido inmunogénico aislado o recombinante de la reivindicación 13 que consiste esencialmente en la secuencia de aminoácido de la SEC ID NO: 1 o la secuencia de aminoácido codificada por la secuencia de nucleótido que codifica el SodC de pALK14 (ATCC 207155).

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15. El polipéptido inmunogénico aislado o recombinante de la reivindicación 13 o 14 capaz de inducir una respuesta inmune protectora contra *Lawsonia intracellularis* en un ave o un porcino.
16. El polipéptido inmunogénico aislado o recombinante de la reivindicación 15 capaz de inducir una respuesta inmune protectora contra *Lawsonia intracellularis* en un porcino.
17. El polipéptido inmunogénico aislado o recombinante de la reivindicación 6 que comprende los residuos de aminoácido de aproximadamente 1 hasta aproximadamente 42 de la SEC ID NO: 1 y es capaz de lograr la producción de anticuerpos contra *Lawsonia intracellularis* cuando se administra a un ave o un porcino.
18. El polipéptido aislado inmunogénico o recombinante de la reivindicación 17 que consiste esencialmente en aproximadamente el aminoácido 1 hasta aproximadamente el aminoácido 42 de la SEC ID NO: 1.
19. El polipéptido inmunogénico aislado o recombinante de la reivindicación 17 capaz de inducir una respuesta inmune protectora contra *Lawsonia intracellularis* en un animal porcino o un ave.
20. El polipéptido inmunogénico aislado o recombinante de la reivindicación 19 capaz de inducir una respuesta inmune protectora contra *Lawsonia intracellularis* en un animal porcino.
21. Una composición para vacuna para la profilaxis o el tratamiento de infección de un animal con *Lawsonia spp.* dicha composición para vacuna comprende un componente inmunogénico que comprende un polipéptido aislado o recombinante que tiene por lo menos aproximadamente 70% de identidad general de secuencia con la secuencia de aminoácido que se observa en la SEC ID NO: 1, o por lo menos aproximadamente 50% de identidad general de secuencia con los residuos de aminoácido 1 a 42 de la

SEC ID NO: 1 o un homólogo, análogo o derivado inmunogénico de ella que es reactivo a nivel inmunológico con *Lawsonia intracellularis*; y uno o más portadores, diluyentes o adyuvantes apropiados para uso veterinario o farmacéutico.

22. La composición para vacuna de acuerdo con la reivindicación 21 donde la *Lawsonia spp* es *L. intracellularis*.
23. La composición para vacuna de acuerdo con la reivindicación 22 donde el componente inmunogénico comprende un polipéptido recombinante o aislado que comprende la secuencia de aminoácido que se observa en la SEC ID NO: 1 o la secuencia de aminoácido codificada por la secuencia de nucleótido que codifica el SodC de pALK14 (ATCC 207155).
24. La composición para vacuna de la reivindicación 23 donde el componente inmunogénico consiste esencialmente en la secuencia de aminoácido de la SEC ID NO: 1.
25. La composición de vacuna de acuerdo con la reivindicación 22 donde el componente inmunogénico comprende un polipéptido aislado o recombinante que comprende aproximadamente del residuo de aminoácido 1 hasta aproximadamente el residuo de aminoácido 42 de la SEC ID NO: 1.
26. La composición de vacuna de la reivindicación 25 donde el componente inmunogénico consiste esencialmente en aproximadamente del residuo de aminoácido 1 hasta aproximadamente el residuo de aminoácido 42 de la SEC ID NO: 1.
27. Una composición de combinación para vacuna para la profilaxis o el tratamiento de la infección de un animal con *Lawsonia spp.*, dicha composición para vacuna comprende:
 - (i) un primero componente inmunogénico que comprende un polipéptido aislado o recombinante que tiene por lo menos

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aproximadamente 70% de identidad general de secuencia con la secuencia de aminoácido que se observa en la SEC ID NO: 1, o por lo menos aproximadamente un 50% de identidad general de secuencia con los residuos de aminoácido del 1 al 42 de la SEC ID NO: 1, o un homólogo, análogo inmunogénico, o derivado de ellos que es reactivo inmunológicamente con *Lawsonia intracellularis*.

- (ii) un segundo componente inmunogénico que comprende un péptido, polipéptido o proteína de *L. intracellularis* antigénico; y
 - (iii) uno o más portadores, diluyentes o adyuvantes apropiados para uso veterinario o farmacéutico.
28. Un vector para vacuna que comprende, en una forma expresable, una molécula aislada de ácido nucleico que tiene una secuencia de nucleótido que codifica un polipéptido inmunogénico recombinante o aislado que comprende la secuencia de aminoácido que se observa en la SEC ID NO: 1, de modo tal que dicho polipéptido es expresable a un nivel suficiente para conferir inmunidad contra *Lawsonia spp.* cuando se administra a un porcino o un ave.
29. El vector para vacuna de la reivindicación 28, donde el polipéptido inmunogénico es expresado utilizando los pasos de:
- (i) colocar una molécula aislada de ácido nucleico que comprende la secuencia de nucleótido que se observa en la SEC ID NO: 2 o una variante degenerada, un homólogo, análogo o derivado de ella que tiene por lo menos aproximadamente 70% de identidad de secuencia, en conexión operable con una secuencia promotora;
 - (ii) introducir la molécula de ácido nucleico aislada y la secuencia promotora del paso (a) en el vector para vacuna; y

- (iii) incubar, criar y propagar el vector para vacuna durante y tiempo y bajo condiciones suficientes para que ocurra la expresión del polipéptido inmunogénico codificado por dicha molécula de ácido nucleico.
30. El vector para vacuna de la reivindicación 28 donde la *Lawsonia spp* es *L. intracellularis*.
31. Una molécula de anticuerpo monoclonal o policlonal que es capaz de unirse específicamente a un polipéptido de SodC o un derivado de un polipéptido de SodC que deriva de la *Lawsonia spp* y tiene por lo menos aproximadamente 70% de identidad general de secuencia con la secuencia de aminoácido que se observa en la SEC ID NO: 1.
32. La molécula de anticuerpo de la reivindicación 31 donde el polipéptido de SodC o derivado del mismo comprende la secuencia de aminoácido que se observa en la SEC ID NO: 1.
33. La molécula de anticuerpo de la reivindicación 31 donde el polipéptido de SodC o derivado del mismo comprende desde aproximadamente el aminoácido 1 hasta aproximadamente el aminoácido 42 de la SEC ID NO: 1.
34. Un método para diagnosticar la infección de un porcino o un ave con *Lawsonia intracellularis* o un microorganismo que es inmunológicamente reactivo a ella, dicho método comprende los pasos de contactar una muestra biológica derivada de dicho animal con la molécula de anticuerpo de la reivindicación 25 durante un tiempo y bajo condiciones suficientes para formar un complejo antígeno-anticuerpo, y luego detectar dicha formación del complejo.

35. El método de la reivindicación 34 donde la muestra biológica comprende suero, ganglios linfáticos, íleo, ciego, intestino delgado, intestino grueso, heces o raspado rectal derivados de un animal porcino.
36. Un método para identificar si un porcino o un ave ha sufrido o no de una infección en el pasado, o actualmente esta infectado, con *Lawsonia intracellularis* o un microorganismo que es inmunológicamente reactivo a ella, dicho método comprende contactar sangre o suero derivados de dicho animal con el polipéptido inmunogénico de la reivindicación 1 durante un tiempo y bajo condiciones suficientes para formar un complejo antígeno-anticuerpo, y luego detectar dicha formación del complejo.
37. Una molécula aislada de ácido nucleico que comprende una secuencia de nucleótidos que codifica, o es complementaria con una molécula de ácido nucleico que codifica un péptido, oligopéptido o polipéptido seleccionados del grupo que consiste en:
- (i) un péptido, oligopéptido o polipéptido que comprende una secuencia de aminoácido que tiene por lo menos un 70% de identidad de secuencia general con la secuencia de aminoácido que se observa en la SEC ID NO: 1;
 - (ii) un péptido, oligopéptido o polipéptido que comprende una secuencia de aminoácido que comprende un amino que tiene por lo menos aproximadamente 50% de identidad general de secuencia con el residuo de aminoácido 1 hasta aproximadamente el residuo de aminoácido 42 de la SEC ID NO: 1.
 - (iii) un homólogo, análogo o derivado de (i) o (ii) que imita un epítopo de célula B o de célula T de un polipéptido de *Lawsonia spp.*
38. La molécula aislada de ácido nucleico de la reivindicación 37 donde el péptido, oligopéptido o polipéptido comprende la secuencia de aminoácido

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que se observa en la SEC ID NO: 1, o aproximadamente del residuo de aminoácido 1 hasta aproximadamente el residuo de aminoácido 42 de ella o la secuencia de aminoácido codificada por la secuencia de nucleótido que codifica el SodC de pALK14 (ATCC 207155) o un epítopo de la célula B o la célula T de ellos

39. La molécula aislada de ácido nucleico de la reivindicación 38 que comprende la secuencia de nucleótido que se observa en la SEC ID NO: 2 o una secuencia de nucleótido complementaria con ella, o una variante degenerada de ella.
40. La molécula aislada de ácido nucleico de la reivindicación 39 que consiste esencialmente en la secuencia de nucleótido de la SEC ID NO: 2 o una variante degenerada de ella.
41. La molécula aislada de ácido nucleico de la reivindicación 38 que comprende la porción de la secuencia de nucleótido de SEC ID NO: 2, o una variante degenerada de ella, que codifica aproximadamente desde el residuo de aminoácido 1 hasta aproximadamente el residuo de aminoácido 42 de la SEC ID NO: 2.
42. La molécula aislada de ácido nucleico de la reivindicación 41 que consiste esencialmente en esa porción de la secuencia de nucleótido de SEC ID NO: 2, o una variante degenerada de ella, que codifica aproximadamente desde el residuo de aminoácido 1 hasta aproximadamente el residuo de aminoácido 42 de la SEC ID NO: 2.
43. Un método para detectar *Lawsonia intracellularis* o un microorganismo relacionado en una muestra biológica de un sujeto porcino o un ave, dicho método comprende los pasos de hibridar una o más sondas o cebadores derivados de la secuencia de nucleótido que se observa en la SEC ID NO:

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2 o una secuencia de nucleótido complementaria a dicha muestra y luego detectar dicha hibridación usando medios de detección.

44. El método de la reivindicación 43 donde la muestra biológica comprende suero, ganglios linfáticos, hígado, ciego, intestino delgado, intestino grueso, heces o raspado rectal derivados de un animal porcino.
45. El método de la reivindicación 34 donde el medio de detección comprende cualquier hibridación o reacción de amplificación basadas en el ácido nucleico.
46. Una sonda o cebador que tiene por lo menos aproximadamente 15 nucleótidos contiguos en longitud que derivan de la SEC ID NO: 2 o una secuencia de nucleótido complementaria con ella.
47. Un plásmido designado pILK14 (Acceso de ATCC No. 251155).
48. La vacuna de combinación de acuerdo con la reivindicación 27 donde el segundo componente inmunogénico comprende un péptido, polipéptido o proteína antigénica de *L. intracellum* seleccionados del grupo que consiste en OmpH, FigE, hemolisina y autolisina.

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