



No. 13-003443- -00000-0000

Fecha: 2013-01-09 16:31:46
Tra. 11 PATENTEIYII
Act. 411 PRESENTACION

Dep. 2020 DIR.NUEVASCR
Eve: 378 FASENACIONALI
Folios: 4



Industria y Comercio

SUPERINTENDENCIA

DELEGATURA DE PROPIEDAD INDUSTRIAL DIVISIÓN DE NUEVAS CREACIONES

SOLICITUD DE PATENTE DE INVENCION

TITULO: VACUNA Y MÉTODOS PARA REDUCIR UNA INFECCIÓN POR
CAMPYLOBACTER

SOLICITANTE: The Board Of Trustees Of The University Of Arkansas

DIRECCIÓN: 2404 North University Avenue, Little Rock, Ar 72207 Estados
Unidos De América

APODERADO: CARLOS R. OLARTE

BOGOTÁ 09 de enero de 2013

P2013/000010



PETITORIO - FORMULARIO ÚNICO DE SOLICITUD
PATENTE PCT
ENTRADA EN FASE NACIONAL

P2013/000010

SOLICITUD DE:

1

☒

Patente de Invención.

☐

Patente de Modelo de Utilidad.

☒

Capítulo I.

☐

Capítulo II.

2

SOLICITANTE
(71)

Nombre: The Board of Trustees of the University of Arkansas

Dirección: 2404 North University Avenue, Little Rock, AR 72207 Estados Unidos de América

Nacionalidad o Domicilio: Estadounidense

Lugar de Constitución: Estados Unidos de América.

Teléfono: _____

Fax: _____

E-mail: _____

IDENTIFICACIÓN

C.C. ☐ NIT ☐

C.E. ☐ Otro ☐

Cual _____

Número _____

REPRESENTANTE
O APODERADO

Nombre: CARLOS R. OLARTE

Dirección: Carrera 5 No. 34-03 Bogotá

Teléfono: 601-7700 Fax: 601-7799

E-mail: office@olartemoure.com

IDENTIFICACIÓN

C.C. ☒ NIT ☐

C.E. ☐ Otro ☐

Cual _____

Número 79.782.747Bta
TP 74.295

INVENTOR (ES)
(72)

Nombre: VER PAGINA ADJUNTA

Dirección: VER PAGINA ADJUNTA

Nacionalidad o Domicilio: VER PAGINA ADJUNTA

5

PCT (86)

Solicitud Internacional No. PCT/US2011/039832

Fecha Junio 9, 2011

Publicación Internacional No. WO 2011/156619A3

Fecha Diciembre 15, 2011

6

Título (54) VACUNA Y MÉTODOS PARA REDUCIR UNA INFECCIÓN POR CAMPYLOBACTER

Clasificación Internacional (51) A61K 39/02, C12N 1/20, C12N 15/00

Prioridad

SI

☒

NO

☐

(33) País de Origen

US

(32) Fecha

June 9, 2010

(31) Número de Solicitud

61/353,039

9

Comprobante de pago No. 13-

Fecha _____

10

ANEXOS

☒ x

Comprobante de pago de la tasa de presentación de la solicitud.

☒ x

Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica.

☒ x

Poderes, si fuere el caso.

☐

Documento de cesión del inventor al solicitante o a su causante.

☐

Declaraciones. Bajo la regla 4.17. (ii) del PCT.

☒ x

Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos).

☒ x

Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos).

☐

Copia del examen preliminar internacional efectuado por la Autoridad Internacional de Examen Preliminar (IPEA).

☐

Traducción del examen preliminar internacional efectuado por la IPEA.

☐

De ser el caso, copia del contrato de acceso.

☐

De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.

☐

De ser el caso, certificado de depósito del material biológico.

☐

Arte final 12 x 12.

☐

De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas parcial o totalmente con la invención de esta solicitud.

☒ x

De ser el caso, modificaciones efectuadas a la solicitud internacional.

☒ x

Otros. Búsqueda Internacional.

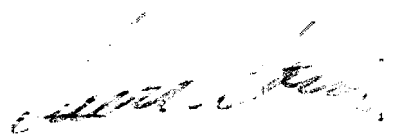
11

FIGURA CARACTERÍSTICA:

12

Solicito la concesión de la patente,

NOMBRE: CARLOS R. OLARTE

FIRMA: 

C.C. 79.782.747Bta T.P. 74.295

LISTA DE INVENTORES**PCT/US2011/039832****1. Billy HARGIS**

3229 Oakland Zion Road,
Fayetteville, AR 72703,
Estados Unidos de América
Nacionalidad: Estadounidense

2. Neil, R. PUMFORD

3196 Ozark Acres Drive,
Bentonville, AR 72712
Estados Unidos de América
Nacionalidad: Estadounidense

3. Young Min KWON,

288 E. Colorado Avenue,
Springdale, AR 72764
Estados Unidos de América
Nacionalidad: Estadounidense

4. Sherryll LAYTON,

6111 Bainbridge Drive,
Rogers AR 72758
Estados Unidos de América
Nacionalidad: Estadounidense

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
NIT : 800.176.089-2
- / -

Industria y Comercio
SUPERINTENDENCIA

RECIBO DE CAJA

No. 13 - 000763

Bogotá D.C., Enero 04 de 2013 - 14:28:53

RECIBIDO DE : OLARTE MOURE & ASOCIADOS NI 830.122.870

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR. PAGO
CONSIGNACION	BANCO DE BOGOTA	062754387	532931825	03/01/2013	6.910.000.00

*** Conceptos Pagados ***

CANT	RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
1	50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE INVENCIÓN	500.000.00	500.000.00
				\$500.000.00

SON: **QUINIENTOS MIL PESOS MONEDA CORRIENTE***

Responsable: _____

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

No. 13-003443- -00000-0000
Fecha: 2013-01-09 16:31:46 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 4

Industria y Comercio SUPERINTENDENCIA		PLANILLA DE RADICACIÓN - DIGITALIZACIÓN DIARIA RELACIÓN DE ANEXOS	
Dependencia:		Fecha: ____/____/____ AAAA MM DD	Recorrido
Tipo de Radicación: Entrada: ☐ Salida: ☐ Traslado: ☐ • Convenio: ☐			Planilla:
Radicador			

ID	No. Radicación	Consecutivo	Item
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Item	Unidad de Conservación
1	Bolsa
2	Caja
3	Libro
4	Sobre
5	Tomo
6	Otro
Item	SopORTE Documental
7	CD
8	Disquete
9	DVD
10	Folleto
11	Foto
12	Periódico
13	Plano
14	Publicación Periódica
15	USB
10	Otro

Observaciones:

AS 2006 05



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Folios: 4

PATENTE DE INVENCION ☐

MODELO DE UTILIDAD ☐

- ☐ Indicación que se solicita una patente.
- ☐ Datos de identificación del solicitante o de la persona que presenta la solicitud
- ☐ Descripción de la invención
- ☐ Dibujos de ser estos pertinentes
- ☐ Comprobante de pago de las tasas establecidas (De ser el caso formato de descuento)
- Completa ☐ Incompleta ☐

PATENTE DE INVENCION PCT ☒

MODELO DE UTILIDAD PCT ☐

Art.33 Decisión 486/00, Circular Única

- ☒ Indicación que se solicita una PCT
- ☐ Copia de la solicitud en español, tal como fue presentada inicialmente (capítulo descriptivo, reivindicatorio, resumen)
- ☐ Dibujos de ser estos pertinentes
- ☒ Comprobante de pago de las tasas establecidas (de ser el caso formato de descuento)
- Completa ☐ Incompleta ☐

DISEÑO INDUSTRIAL ☐

(Art. 119 Decisión 486/00)

- ☐ Indicación que se solicita Diseño Industrial
- ☐ Datos de identificación del solicitante o de la persona que presenta la solicitud
- ☐ Representación gráfica y fotográfica del Diseño Industrial o muestra del material que incorpora el diseño
- ☐ Comprobante de pago de las tasas establecidas
- Completa ☐ Incompleta ☐

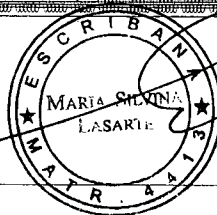
ESQUEMA DE TRAZADO ☐

Art. 92 Decisión 486/00)

- ☐ Indicación que se solicita un esquema de trazado
- ☐ Datos de identificación del solicitante o de la persona que presenta la solicitud
- ☐ Representación gráfica de un esquema de trazado
- ☐ Comprobante de pago de las tasas establecidas
- Completa ☐ Incompleta ☐



ACTUACION NOTARIAL



Pco 47336

N 015268968

FOLIO 346.- PRIMERA COPIA.- ESCRITURA NÚMERO: NOVENTA Y CINCO.- SUSTITUCIÓN PARCIAL DE PODER: MESSERER, Karen a favor de OLARTE, Carlos Reinaldo y otro del Poder otorgado por The Board of Trustees of the University of Arkansas.------

En la Ciudad Autónoma de Buenos Aires, Capital de la República Argentina, el día

VEINTE de **JULIO** del año **DOS MIL DOCE**, ante mí, María Silvina Lasarte,

Escribana Autorizante, titular del Registro Notarial Número 1.788, **COMPARECE:**

Karen MESSERER, argentina, nacida el 19 de noviembre de 1976, hija de Walter

Messerer y Elsa Onelia Romanelli, casada, titular del Documento Nacional de

Identidad Número 25.670.178, con domicilio en la Avenida Del Libertador 5.954,

Piso 7º, de esta Ciudad de Buenos Aires.- La compareciente es mayor de edad.-

INTERVIENE: por su propio derecho.- Y la compareciente **EXPRESA:**

PRIMERO: Que la Entidad **"The Board of Trustees of the University of**

Arkansas", con domicilio en 2404 N. University Avenue, Little Rock, AR 772207-

3608, Estados Unidos de América, le confirió, con fecha 3 de Octubre de 2.007, en

dicho país, un Poder, el cual mediante escritura número 93, del día de la fecha,

otorgada ante mí, al folio 340, protocolo corriente, de este Registro Notarial Número

1788, a mi cargo, fue incorporado y transcrito al mismo, cuya primera copia tengo

para este acto a la vista y en lo pertinente transcribiré en la presente.- **SEGUNDO:**

Manifiesta bajo juramento que el referido poder invocado se encuentra plenamente

vigente en virtud de no haberle sido revocado, suspendido ni limitado en ninguna de

sus partes.- **TERCERO:** En consecuencia, **DICE:** Que en uso de la facultad conferida

SUSTITUYE PARCIALMENTE el poder mencionado a favor de **Carlos Reinaldo**

Olarte y/o Juan Guillermo Moure Pérez, al solo y único objeto de que en nombre y

representación de la Entidad **"The Board of Trustees of the University of**

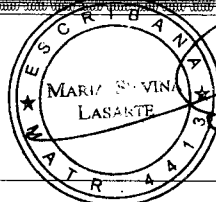


N 015268968

Arkansas”, y actuando en forma individual, indistinta y/o alternadamente, se
presenten **exclusivamente** ante las autoridades de **COLOMBIA**, que correspondan y
realicen los actos enumerados en el citado poder y con las facultades que surgen del
mismo, el que transcripto en lo pertinente dice: “FOLIO 340.- PRIMERA COPIA.-
ESCRITURA NÚMERO: NOVENTA Y TRES.- En la Ciudad Autónoma de Buenos
Aires, ..., el día VEINTE de JULIO del año DOS MIL DOCE, ante mí María Silvina
Lasarte, Escribana Autorizante, titular del Registro Notarial número 1788,
COMPARECE: Karen MESSERER, argentina, nacida el 19 de noviembre de 1976,
hija de Walter Messerer y Elsa Onelia Romanelli, casada, titular del Documento
Nacional de Identidad Número 25.670.178, con domicilio en la Avenida Del
Libertador 5.954, Piso 7º, de esta Ciudad de Buenos Aires.- La compareciente es
mayor de edad.- INTERVIENE: por su propio derecho.- Y la compareciente
EXPRESA: Que a los efectos que hubiere lugar, solicita de mí, la Autorizante, LA
INCORPORACIÓN y TRANSCRIPCIÓN en este protocolo del PODER que le fuera
conferido en Fayetteville, Arkansas, Estados Unidos de América, el día 3 de Octubre
de 2.007, por la Entidad “The Board of Trustees of the University of Arkansas”, con
domicilio en 2404 N. University Avenue, Little Rock, AR 772207-3608, Estados
Unidos de América, a favor de Gerardo Messerer y/o Dagmar Messerer y/o Karen
Messerer y/o Moeller & Co. S.A., indistintamente, sobre cuya plena vigencia
responde la requirente bajo juramento.- Yo, la Autorizante, hago constar: a) Que el
instrumento de poder cuya incorporación y transcripción a este protocolo solicita la
compareciente, se encuentra extendido en idioma castellano, certificada la firma por
notaria en idioma inglés, debidamente legalizado en idioma inglés (Sello de Apostilla
de la Convención de La Haya del 5 de octubre de 1961) y traducido en lo pertinente al
idioma nacional por la Traductora Pública Laura Thieberger.- b) Que la



ACTUACION NOTARIAL



N 015268969

1 compareciente en virtud de lo manifestado tiene interés legítimo en su requerimiento.-

2 En consecuencia, me hace entrega en este acto del original del instrumento de poder

3 relacionado con su respectiva traducción al idioma nacional, el que agrego a la

4 presente y transcripto dice: "... - PODER Los abajo firmante/s The Board of Trustees

5 of the University of Arkansas domiciliados en 2404 N. University Avenue, Little

6 Rock, AR 772207-3608, E.E.U.U. declaran por el presente otorgar a Gerardo

7 MESSERER y/o Dagmar MESSERER y/o Karen MESSERER y/o MOELLER & CO.

8 S.A. indistintamente poder general amplio y suficiente para recabar de las autoridades

9 ..., sud y centroamericanas ... que correspondan, la obtención de patentes de

10 invención, el registro y la renovación de marcas y modelos y/o derechos de propiedad

11 intelectual y/o nombres de dominio, a cuyo efecto los faculta/n para llevar a cabo ante

12 dichas autoridades todas las gestiones necesarias al objeto indicado, presentar

13 solicitudes, formular descripciones, oposiciones, protestos, declaraciones, apelaciones

14 y reclamos, oblar impuestos, justificar explotaciones, solicitar testimonios, aceptar

15 transferencias, recibir documentos y valores, percibir y hacer cuanto fuere necesario

16 ante las autoridades administrativas de cualquier orden. Por otra parte, les confieren

17 mandato para que en su nombre y representación puedan iniciar y proseguir cualquier

18 juicio que actualmente los que suscriben tengan o en el futuro tuvieran ..., en

19 Sudamérica, Centroamérica ..., con motivo de su nombre, marcas de fábrica o de

20 comercio, patentes de invención y modelos y/o de sus derechos de propiedad

21 intelectual que actualmente posean o adquieran en lo sucesivo, promover juicios de

22 cualquier jurisdicción, criminales, correccionales, civiles o comerciales, contra los

23 falsificadores, usurpadores o imitadores y contra los tenedores de artículos que

24 infrinjan su nombre, marcas o patentes de invención, seguir los juicios por oposición u

25 otros en que fueran parte como actores o demandados, defender el nombre, las



N 015268969

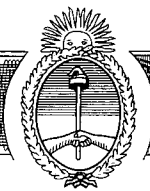
COPIA
FIEL

26 marcas, patentes y modelos y/o de derechos de propiedad intelectual y/o nombres de
27 dominio, haciendo las protestas del caso o diciendo de nulidad. Al efecto, podrán
28 presentarse con escritos, querellas, pedidos, y lo que fuere necesario ante cualquier
29 autoridad judicial, presentar testigos, documentos y demás justificativos, rendir
30 pruebas de cualquier especie, pedir nombramientos de peritos, pedir y absolver
31 posiciones, asistir a juicios verbales, declinar y prorrogar de jurisdicción, tachar,
32 recusar, apelar, transar, someter a arbitraje o mediación, hacer renunciaciones, exigir y dar
33 cauciones, pedir embargos preventivos, definitivos o inhibiciones y su levantamiento,
34 pedir la venta de los bienes de los deudores, solicitar y obtener el cumplimiento de
35 sentencias, prestar juramentos, decir de nulidad, desistir, percibir, otorgar recibos y en
36 general practicar todos los actos y diligencias que sean precisos al desempeño de este
37 mandato. Podrán asimismo substituir el presente poder en todo o en parte. Dado y
38 firmado en Fayetteville, Arkansas - Sigue fecha en idioma inglés - Sigue una firma
39 ilegible - Sigue texto en idioma inglés”.- ES COPIA FIEL, doy fe.- En hojas adjuntas
40 consta: a) La certificación notarial de la firma obrante en el poder en idioma inglés,
41 efectuada por la Escribana Pública del Condado de Washington, Estado de Arkansas,
42 Estados Unidos de América, Susie J. Engle; y b) La respectiva legalización de la firma
43 y sello de la nombrada Notaria (Sello de Apostilla de la Convención de La Haya del 5
44 de octubre de 1961), en idioma inglés, efectuada por el Secretario de Estado del
45 Estado de Arkansas, Estados Unidos de América, Charlie Daniels.- A continuación, en
46 hoja adjunta sigue la referida traducción al idioma nacional la que transcripta
47 textualmente, dice: “TRADUCCIÓN PÚBLICA – Sobre el margen izquierdo de un
48 Poder redactado en idioma español consta en idioma inglés: ...- Consta la firma del
49 solicitante con fecha 04 de octubre de 2007.- Adjunto: ... CERTIFICACIÓN
50 NOTARIAL. A los 3 días de octubre de 2007 ante mí, la Escribana Pública por el

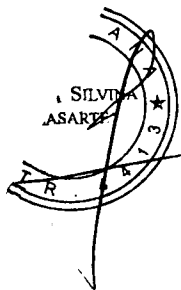


ACTUACION NOTARIAL

LEY 4824

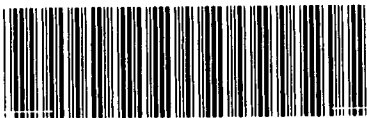


N 015268970



1 Estado de Arkansas, compareció personalmente la Dra. Lisa C. Childs, de mi
2 conocimiento, quien ha firmado el documento adjunto por estar facultada legalmente
3 para suscribir el mismo en nombre de "The Board of Trustees of the University of
4 Arkansas", de conformidad con los estatutos sociales.- Nombre: Susie J. Engle -
5 Consta la firma y el sello de la citada Escribano Público en el Condado de
6 Washington, Arkansas, cuya habilitación caduca el 29.06.2016.- Adjunto:
7 APOSTILLA.- (Convención de La Haya del 5 de octubre de 1961) - Estado de
8 Arkansas - Secretaría de Estado.- Solicitado para usar en Argentina.- 1. País: Estados
9 Unidos de Norte América.- El presente documento - 2. Está firmado por Susie J.
10 Engle - 3. En su calidad de Escribano Público, Condado de Washington, Estado de
11 Arkansas - 4. Estando provisto con el sello de la Escribano Público, Condado de
12 Washington, Estado de Arkansas - Confirmado - 5. En Little Rock, Arkansas - 6. El
13 11.10.2007 - 7. por el Secretario del Estado de Arkansas - 8. bajo el número
14 8098790002 - 9. Sello - Consta el sello del Estado de Arkansas.- 10. Firma - Consta
15 firma (aclarada) de Charlie Daniels, Secretario de Estado - Es traducción fiel del
16 documento adjunto de referencia, que en idioma inglés tengo a la vista y he vertido al
17 idioma español, en Buenos Aires, el 16.07.2012.- Hay una firma ilegible y un sello
18 que dice: "Laura Thieberger - Matrícula de Traductores Alemán- Francés - Tomo II
19 Folio 254 (Cap.) Tomo III 98- (La Plata) - Inglés - Tomo III: Folio 14 (Cap.) - Tomo
20 III Folio 329 (La Plata) - Colegio de Traductores Públicos N° 173".- ES COPIA FIEL,
21 doy fe.- A continuación hay un sello que dice: "Colegio de Traductores Públicos de la
22 Ciudad de Buenos Aires Corresponde a la Legalización N° 41840/12. Sigue una firma
23 ilegible. Sergio Alejandro Iervasi".- ...- A continuación sigue la legalización de la
24 firma de la nombrada traductora, ...- En este estado la compareciente DICE: Que en la
25 forma expresada deja INCORPORADO y TRANSCRIPTO en este Protocolo el Poder

120723 131875



23/07/2012

13 41 11

120723 360104



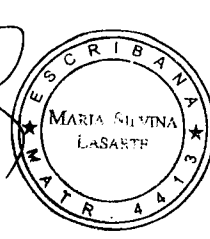
23/07/2012

13 41 11

15 01 02 68970



que agrego a la presente, solicitando de mí, la Escribana Autorizante, expida primera
copia de la presente ...- Constancias Notariales: Identidad de la compareciente
(Artículo 1.002 del Código Civil): Se justifica la misma por conocimiento de la
Autorizante.- ...- LEO esta escritura a la compareciente, quien así la otorga y en
prueba de conformidad, la firma ante mí, doy fe.- Karen MESSERER. Ante mí:
MARÍA SILVINA LASARTE. Escribana. Está mi sello. ...- ”.- **ES COPIA FIEL**, de
las partes pertinentes de la escritura de referencia, doy fe.- **CUARTO**: Finalmente la
compareciente expresa: a) Que el presente otorgamiento no significa renunciar al
ejercicio de las facultades sustituidas; b) Que los nombrados apoderados sustitutos no
podrán sustituir el presente poder en ninguna de sus partes; y c) Que el presente
instrumento no se encuentra alcanzado por el Impuesto de Sellos, en virtud de no
revestir el carácter de oneroso.- Constancias Notariales: Identidad de la
Compareciente (Artículo 1.002 del Código Civil): Se justifica la misma por
conocimiento de la Autorizante.- Informo a la compareciente respecto de los alcances
de las cláusulas contenidas en la presente escritura y del derecho de leer por sí este
instrumento.- LEO esta escritura a la compareciente, quien así la otorga y en prueba
de conformidad la firma ante mí, doy fe.- Karen MESSERER. Ante mí: MARÍA
SILVINA LASARTE. Escribana. Está mi sello. **CONCUERDA** con su escritura
matriz que pasó ante mí, al folio 346 del Registro Notarial número 1788, a mi cargo.
PARA LA PODERDANTE expido la presente **PRIMERA COPIA** en tres (3) sellos
de actuación notarial numerados correlativamente desde el número N 015268968 al
presente inclusive, que sello y firmo en la fecha y lugar de su otorgamiento, doy fe.-

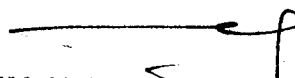


L 010914218

EL COLEGIO DE ESCRIBANOS de la Ciudad de Buenos Aires, Capital Federal de la República Argentina, en virtud de las facultades que le confiere la ley orgánica vigente, LEGALIZA la firma y sello del escribano MARIA SILVINA LASARTE obrantes en el documento anexo, presentado en el día de la fecha bajo el N° 120723360104/6 La presente legalización no juzga sobre el contenido y forma del documento.



Buenos Aires, Lunes 23 de Julio de 2012


ESC. SANTIAGO RAFAEL BLOUSSON
COLEGIO DE ESCRIBANOS
LEGALIZADOR

REPÚBLICA ARGENTINA

MINISTERIO *de*
RELACIONES EXTERIORES
COMERCIO INTERNACIONAL Y CULTO

(Convention de La Haye du 5 octobre 1961)

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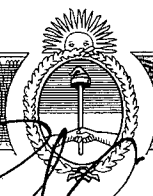
10 Firma

Amphiprion

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ACTUACION NOTARIAL



N 015335461

FOLIO 349.- PRIMERA COPIA.- ESCRITURA NÚMERO: NOVENTA Y SEIS.- SUSTITUCIÓN PARCIAL DE PODER: MESSERER, Karen a favor de OLARTE, Carlos Reinaldo y otro del Poder otorgado por The Texas A&M University System.------

En la Ciudad Autónoma de Buenos Aires, Capital de la República Argentina, el día

VEINTE de **JULIO** del año **DOS MIL DOCE**, ante mí, María Silvina Lasarte,

Escribana Autorizante, titular del Registro Notarial Número 1.788, **COMPARECE:**

Karen MESSERER, argentina, nacida el 19 de noviembre de 1976, hija de Walter

Messerer y Elsa Onelia Romanelli, casada, titular del Documento Nacional de

Identidad Número 25.670.178, con domicilio en la Avenida Del Libertador 5.954,

Piso 7º, de esta Ciudad de Buenos Aires.- La compareciente es mayor de edad.-

INTERVIENE: por su propio derecho.- Y la compareciente **EXPRESA:**

PRIMERO: Que la Sociedad **"The Texas A&M University System"**, con domicilio

en 3369 TAMU, College Station, TX 77843-3369, Estados Unidos de América, le

confirió, con fecha 28 de Abril de 2.011, en dicho país, un Poder, el cual mediante

escritura número 94, del día de la fecha, otorgada ante mí, al folio 343, protocolo

corriente, de este Registro Notarial Número 1788, a mi cargo, fue incorporado y

transcripto al mismo, cuya primera copia tengo para este acto a la vista y en lo

pertinente transcribiré en la presente.- **SEGUNDO:** Manifiesta bajo juramento que el

referido poder invocado se encuentra plenamente vigente en virtud de no haberle sido

revocado, suspendido ni limitado en ninguna de sus partes.- **TERCERO:** En

consecuencia, **DICE:** Que en uso de la facultad conferida **SUSTITUYE**

PARCIALMENTE el poder mencionado a favor de **Carlos Reinaldo Olarte y/o**

Juan Guillermo Moure Pérez, al solo y único objeto de que en nombre y

representación de la Sociedad **"The Texas A&M University System"**, y actuando en



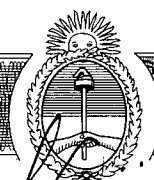
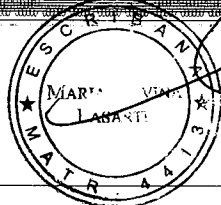
N 015335461

forma individual, indistinta y/o alternadamente, se presenten **exclusivamente** ante las
autoridades de **COLOMBIA**, que correspondan y realicen los actos enumerados en el
citado poder y con las facultades que surgen del mismo, el que transcripto en lo
pertinente dice: "FOLIO 343.- PRIMERA COPIA.- ESCRITURA NÚMERO:
NOVENTA Y CUATRO.- En la Ciudad Autónoma de Buenos Aires, ..., el día
VEINTE de JULIO del año DOS MIL DOCE, ante mí ..., COMPARECE: Karen
MESSERER, argentina, nacida el 19 de noviembre de 1976, hija de Walter Messerer
y Elsa Onelia Romanelli, casada, titular del Documento Nacional de Identidad
Número 25.670.178, con domicilio en la Avenida Del Libertador 5.954, Piso 7º, de
esta Ciudad de Buenos Aires.- La compareciente es mayor de edad.- INTERVIENE:
por su propio derecho.- Y la compareciente EXPRESA: Que a los efectos que hubiere
lugar, solicita de mí, la Autorizante, LA INCORPORACIÓN y TRANSCRIPCIÓN en
este protocolo del PODER que le fuera conferido en College Station, Texas, Estados
Unidos de América, el día 28 de Abril de 2.011, por la Sociedad "The Texas A&M
University System", con domicilio en 3369 TAMU, College Station, TX 77843-3369,
Estados Unidos de América, a favor de Gerardo Messerer y/o Dagmar Messerer y/o
Karen Messerer y/o Moeller & Co. S.A., indistintamente, sobre cuya plena vigencia
responde la requirente bajo juramento.- Yo, la Autorizante, hago constar: a) Que el
instrumento de poder cuya incorporación y transcripción a este protocolo solicita la
compareciente, se encuentra extendido en idioma castellano, certificada la firma por
notaria en idioma inglés, debidamente legalizado en idioma inglés (Sello de Apostilla
de la Convención de La Haya del 5 de octubre de 1961) y traducido en lo pertinente al
idioma nacional por la Traductora Pública Laura Thieberger.- b) Que la
compareciente en virtud de lo manifestado tiene interés legítimo en su requerimiento.-
En consecuencia, me hace entrega en este acto del original del instrumento de poder



ACTUACIÓN NOTARIAL

LEY 4680



N 015335462

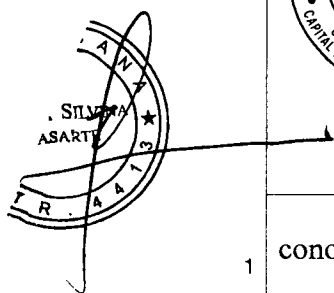
1 relacionado con su respectiva traducción al idioma nacional, el que agrego a la
2 presente y transcripto dice: "PODER Los abajo firmante/s The Texas A&M
3 University System domiciliados en 3369 TAMU, College Station, TX 77843-3369,
4 Estados Unidos de América declaran por el presente otorgar a Gerardo MESSERER
5 y/o Dagmar MESSERER y/o Karen MESSERER y/o MOELLER & CO. S.A.
6 indistintamente poder general amplio y suficiente para recabar de las autoridades ...,
7 sud y centroamericanas ... que correspondan, la obtención de patentes de invención,
8 el registro y la renovación de marcas y modelos y/o derechos de propiedad intelectual
9 y/o nombres de dominio, a cuyo efecto los faculta/n para llevar a cabo ante dichas
10 autoridades todas las gestiones necesarias al objeto indicado, presentar solicitudes,
11 formular descripciones, oposiciones, protestos, declaraciones, apelaciones y reclamos,
12 oblar impuestos, justificar explotaciones, solicitar testimonios, aceptar transferencias,
13 recibir documentos y valores, percibir y hacer cuanto fuere necesario ante las
14 autoridades administrativas de cualquier orden. Por otra parte, les confieren mandato
15 para que en su nombre y representación puedan iniciar y proseguir cualquier juicio
16 que actualmente los que suscriben tengan o en el futuro tuvieran ..., en Sudamérica,
17 Centroamérica ..., con motivo de su nombre, marcas de fábrica o de comercio,
18 patentes de invención y modelos y/o de sus derechos de propiedad intelectual que
19 actualmente posean o adquieran en lo sucesivo, promover juicios de cualquier
20 jurisdicción, criminales, correccionales, civiles o comerciales, contra los
21 falsificadores, usurpadores o imitadores y contra los tenedores de artículos que
22 infrinjan su nombre, marcas o patentes de invención, seguir los juicios por oposición u
23 otros en que fueran parte como actores o demandados, defender el nombre, las
24 marcas, patentes y modelos y/o de derechos de propiedad intelectual y/o nombres de
25 dominio, haciendo las protestas del caso o diciendo de nulidad. Al efecto, podrán



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presentarse con escritos, querellas, pedidos, y lo que fuere necesario ante cualquier
autoridad judicial, presentar testigos, documentos y demás justificativos, rendir
pruebas de cualquier especie, pedir nombramientos de peritos, pedir y absolver
posiciones, asistir a juicios verbales, declinar y prorrogar de jurisdicción, tachar,
recusar, apelar, transar, someter a arbitraje o mediación, hacer renunciaciones, exigir y dar
cauciones, pedir embargos preventivos, definitivos o inhibiciones y su levantamiento,
pedir la venta de los bienes de los deudores, solicitar y obtener el cumplimiento de
sentencias, prestar juramentos, decir de nulidad, desistir, percibir, otorgar recibos y en
general practicar todos los actos y diligencias que sean precisos al desempeño de este
mandato. Podrán asimismo sustituir el presente poder en todo o en parte. ... Dado y
firmado en College Station, Texas - Sigue texto en idioma inglés - Sigue una firma
ilegible - Sigue texto en idioma inglés".- ES COPIA FIEL, doy fe.- En hojas adjunta
consta: a) La certificación notarial de la firma obrante en el poder en idioma inglés,
efectuado por la Escribana Pública del Condado de Brazos, Estado de Texas, Estados
Unidos de América, Brenda A. Jones; y b) La respectiva legalización de la firma y
sello de la nombrada Notaria (Sello de Apostilla de la Convención de La Haya del 5
de octubre de 1961), en idioma inglés, efectuado por el Secretario de Estado del
Estado de Texas, Estados Unidos de América, Hope Andrade.- A continuación, en
hoja adjunta sigue la referida traducción al idioma nacional la que transcrita
textualmente, dice: "TRADUCCIÓN PÚBLICA – Sobre el margen izquierdo de un
Poder redactado en idioma español consta en idioma inglés: ...- Firmado en College
Station, Texas el 28 de Abril de 2011 – Adjunto: CERTIFICADO NOTARIAL – Yo,
la Escribana abajo firmante, Brenda Jones del Condado Brazos, Texas, certifico por el
presente la autenticidad de la firma estampada en mi presencia por el Dr Peter
Schuerman, domiciliado en 3369 Tamu, College Station, Texas 77843-3369, de mi



ACTUACIÓN NOTARIAL

LEY 402



N 015335463

1 conocimiento.- Certifico asimismo por el presente que Peter Schuerman está facultado
2 a actuar en nombre de Texas A&M University System.- Lugar y fecha: College
3 Station, Texas el 29.04.11.- Consta la firma y el sello de Brenda A. Jones, Escribano
4 Público, Estado de Texas, cuya habilitación caduca el 12.12.2011.- Adjunto:
5 APOSTILLA.- (Convención de La Haya del 5 de octubre de 1961) – Estado de Texas
6 - Secretaría de Estado.- Solicitado para usar en Argentina.- ...- 1. País: Estados Unidos
7 de Norte América.- El presente documento - 2.- Está firmado por Brenda A. Jones - 3.
8 En su calidad de Escribano Público, Estado de Texas - 4. Estando provisto con el sello
9 de Brenda A. Jones, Escribano Público, Estado de Texas, cuya habilitación caduca el
10 12.12.2011 – Confirmado - 5. En Austin, Texas - 6. El 10.05.2011 - 7. por el
11 Secretario del Estado de Texas - 8. bajo el número N-797555 - 9.- Sello – Consta el
12 sello del Secretario del Estado de Texas.- 10. Firma - Consta firma (aclarada): de
13 Hope Andrade, Secr. de Estado - Es traducción fiel del documento adjunto de
14 referencia que en idioma inglés tengo a la vista y he vertido al idioma español, en
15 Buenos Aires, el 16.07.2012.- Hay una firma ilegible y un sello que dice: “Laura
16 Thieberger - Matrícula de Traductores Alemán- Francés - Tomo II Folio 254 (Cap.)
17 Tomo III 98- (La Plata) – Inglés - Tomo III: Folio 14 (Cap.) - Tomo III Folio 329 (La
18 Plata) - Colegio de Traductores Públicos N° 173”.- ES COPIA FIEL, doy fe.- A
19 continuación hay un sello que dice: “Colegio de Traductores Públicos de la Ciudad de
20 Buenos Aires Corresponde a la Legalización N° 41839/12. Sigue una firma ilegible.
21 Sergio Alejandro Iervasi”.- ...- A continuación sigue la legalización de la firma de la
22 nombrada traductora, ...- En este estado la compareciente DICE: Que en la forma
23 expresada deja INCORPORADO y TRANSCRIPTO en este Protocolo el Poder que
24 agrego a la presente, solicitando de mí, la Escribana Autorizante, expida primera copia
25 de la presente ...- Constancias Notariales: Identidad de la compareciente (Artículo



23/07/2012

13-41-11

13 41 11

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1.002 del Código Civil): Se justifica la misma por conocimiento de la Autorizante.-
...- LEO esta escritura a la compareciente, quien así la otorga y en prueba de
conformidad, la firma ante mí, doy fe.- Karen MESSERER. Ante mí: MARÍA
SILVINA LASARTE. Escribana. Está mi sello. ...- ES COPIA FIEL, de las partes
pertinentes de la escritura de referencia, doy fe.- CUARTO: Finalmente la
compareciente expresa: a) Que el presente otorgamiento no significa renunciar al
ejercicio de las facultades sustituidas; b) Que los nombrados apoderados sustitutos no
podrán sustituir el presente poder en ninguna de sus partes; y c) Que el presente
instrumento no se encuentra alcanzado por el Impuesto de Sellos, en virtud de no
revestir el carácter de oneroso.- Constancias Notariales: Identidad de la
Compareciente (Artículo 1.002 del Código Civil): Se justifica la misma por
conocimiento de la Autorizante.- Informo a la compareciente respecto de los alcances
de las cláusulas contenidas en la presente escritura y del derecho de leer por sí este
instrumento.- LEO esta escritura a la compareciente, quien así la otorga y en prueba
de conformidad la firma ante mí, doy fe.- Karen MESSERER. Ante mí: MARÍA
SILVINA LASARTE. Escribana. Está mi sello. CONCUERDA con su escritura
matriz que pasó ante mí, al folio 349 del Registro Notarial número 1788, a mi cargo.
PARA LA PODERDANTE expido la presente PRIMERA COPIA en tres (3) sellos
de actuación notarial numerados correlativamente desde el número N 015335461 al
presente inclusive, que sello y firmo en la fecha y lugar de su otorgamiento, doy fe.-



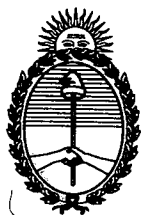
L 010914217

EL COLEGIO DE ESCRIBANOS de la Ciudad de Buenos Aires, Capital Federal de la República Argentina, en virtud de las facultades que le confiere la ley orgánica vigente, **LEGALIZA** la firma y sello del escribano **MARIA SILVINA LASARTE** obrantes en el documento anexo, presentado en el día de la fecha bajo el N° **120723360105/7**. La presente legalización no juzga sobre el contenido y forma del documento.



Buenos Aires, Lunes 23 de Julio de 2012

ESC. SANTIAGO RAFAEL BLOUSSON
COLEGIO DE ESCRIBANOS
LEGALIZADOR



REPÚBLICA ARGENTINA

MINISTERIO *de*
RELACIONES EXTERIORES
COMERCIO INTERNACIONAL Y CULTO

APOSTILE

(Convention de La Haye du 5 octobre 1961)

1. País: REPÚBLICA ARGENTINA
El presente documento público DE LEGALIZACIÓN
2. Ha sido firmado por SANTIAGO RAFAEL BLOUSSON
3. Quien actúa en calidad de LEGALIZADOR
4. Lleva el sello/timbre del COLEGIO DE ESCRIBANOS DE LA CIUDAD DE BUENOS AIRES.
5. En LA CIUDAD DE BUENOS AIRES 6. El día 23/07/2012
7. Por EL COLEGIO DE ESCRIBANOS DE LA CIUDAD DE BUENOS AIRES
MINISTERIO DE RELACIONES EXTERIORES, COMERCIO INTERNACIONAL Y CULTO (Convenio 02/09/2003).
8. Bajo el Número: 120723131879
9. Sello/Timbre:



10-1000-2

Autograph

COLEGIO DE
CONSE

[illegible]

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



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15 December 2011 (15.12.2011)

PCT

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9 June 2011 (09.06.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/353,039 9 June 2010 (09.06.2010) US

(71) Applicant (for all designated States except US): **THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ARKANSAS** [US/US]; 2404 North University Avenue, Little Rock, AR 72207 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HARGIS, Billy** [US/US]; 3229 Oakland Zion Road, Fayetteville, AR 72703 (US). **PUMFORD, Neil, R.** [US/US]; 3196 Ozark Acres Drive, Bentonville, AR 72712 (US). **KWON, Young, Min** [US/US]; 288 E. Colorado Avenue, Springdale, AR 72764 (US). **LAYTON, Sherryll** [US/US]; 6111 Bainbridge Drive, Rogers, AR 72758 (US).

(74) Agents: **VANHEYNINGEN, Tambryn K.** et al.; Andrus, Sceales, Starke & Sawall, LLP, 100 East Wisconsin Avenue, Suite 1100, Milwaukee, WI 53202 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report (Rule 48.2(g))

— with sequence listing part of description (Rule 5.2(a))

(54) Title: VACCINE AND METHODS TO REDUCE *CAMPYLOBACTER* INFECTION

(57) Abstract: Vaccine vectors and methods for enhancing resistance to *Campylobacter* infection or for enhancing the immune response to *Campylobacter* are provided herein. The vaccine vectors include a first polynucleotide which encodes an antigenic polypeptide selected from SEQ ID NO: 7-9 or a fragment thereof. The vector may also include an immunostimulatory polypeptide. The methods include administering the vaccine vectors to a subject.



WO 2011/156619 A2

VACCINE AND METHODS TO REDUCE *CAMPYLOBACTER* INFECTION

CROSS-REFERENCE TO RELATED APPLICATIONS

This patent application claims the benefit of priority of United States Provisional
5 Patent Application No. 61/353,039, filed June 9, 2010, which is incorporated herein by
reference in its entirety.

STATEMENT REGRADING FEDERALLY SPONSORED RESEARCH

This invention was made with government support under grant number 208-35-
10 201-04-683, awarded by the USDA/NRI. The government has certain rights in the
invention.

SEQUENCE LISTING

A Sequence Listing accompanies this application and is incorporated herein by
15 reference in its entirety. The Sequence Listing was filed with the application as a text file
on June 9, 2011.

BACKGROUND

The repertoire of safe and cost-effective vaccines for generation of mucosal
20 immunity against a variety of agents is limited. The leading bacterial cause of human
gastrointestinal disease worldwide is *Campylobacter*. Bacterial gastroenteritis continues
to pose a significant threat to the general public in the United States and abroad for the
foreseeable future. Infections with *Campylobacter jejuni* occur more frequently than the
more publicized infections from *Salmonella* species or *Escherichia coli* O157:H7. The
25 actual burden of illness of *Campylobacter* gastroenteritis nationwide is 500-850
infections/100,000 persons per year.

Not only is *Campylobacter* the leading cause of bacterial gastroenteritis, but *C.*
jejuni has been associated with the neuropathological disease Guillain-Barré Syndrome
(GBS). This life-threatening disease may be an immune response to ganglioside-like
30 structures on certain *C. jejuni* strains leading to an autoimmune response against nerve

cells. Although GBS is the most important chronic sequelae, *Campylobacter* infection is also associated with a reactive arthritis, which may progress to Reiter's syndrome.

Vaccination against *Campylobacter* has had limited success using killed whole-cell or protein based vaccines. In addition, there are concerns regarding the development of Guillain-Barré syndrome or other sequelae from killed whole-cell vaccination. A successful vaccine would need to be cost-effective, safe, orally effective, and be produced in large quantities in a very short time-period. At the present time there is no such vaccine.

SUMMARY

Vectors and methods for enhancing resistance to *Campylobacter* infection or enhancing the immune response to *Campylobacter* are provided herein.

In one aspect, vectors including a first polynucleotide sequence encoding an antigenic polypeptide not natively associated with the vector are provided. The antigenic polypeptide may be SEQ ID NO: 7 (cjaD; cj0113; GVSITVEGNCDEWGTDEYNQA), SEQ ID NO: 8(cjaA; cj0982; KDIVLDAEIGGVAKGKDGKEK) or SEQ ID NO: 9(ACE 393; cj0420; KVALGVAVPKDSNITSVEDLKDKTLLLNKGTTADA) or a fragment thereof. The vector may also include an immunostimulatory polypeptide not natively associated with the vector. The vaccine vector is capable of eliciting an immune response from a vaccinated subject that includes an IgA antibody response against *Campylobacter*. The response may be protective against challenge with *Campylobacter*.

In another aspect, vectors including a first polynucleotide sequence encoding an antigenic polypeptide not natively associated with the vector and a second polynucleotide sequence encoding an immunostimulatory polypeptide are provided. The antigenic polypeptides may be a fragment of SEQ ID NO: 1 (cjaD), SEQ ID NO: 2 (cjaA) or SEQ ID NO: 3 (ACE393). The vaccine vector is capable of eliciting an immune response from a vaccinated subject that includes an IgA antibody response against *Campylobacter*. The response may be protective against challenge with *Campylobacter*.

In still another aspect, pharmaceutical compositions comprising the vectors provided herein in a pharmaceutically acceptable carrier are disclosed.

In yet another aspect, methods of enhancing an immune response directed to *Campylobacter* in a subject are provided. The methods include administering an effective amount of the vectors provided herein to a subject. In one embodiment, the enhanced immune response includes an IgA antibody response and the response may be protective.

In still a further aspect, methods of enhancing resistance to *Campylobacter* infection are provided herein. The methods include administering an effective amount of the vectors disclosed herein to the subject such that the subject is resistant to infection after subsequent exposure to *Campylobacter*. In one embodiment the enhanced immune response includes an IgA antibody response and the response may be protective.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a graph showing the standard curve for the quantitative PCR showing the number of cultivable *Campylobacter jejuni* on the x-axis as determined by conventional culturing and determination of colony forming units (CFU). The cycle number at which the fluorescence crossed the threshold in the quantitative PCR is shown on the y axis.

Figure 2 is a graph showing the log cfu/g ileum content in chicks vaccinated with saline, or Salmonella vectors expressing *Campylobacter* peptides cj0420 (SEQ ID NO: 9), cj0113 (SEQ ID NO: 7) or cj0982 (SEQ ID NO: 8) (10^8 cfu/chick), 11 days after challenge with *C. jejuni* (5×10^7 cfu/ml). The quantitative PCR was performed on total DNA extracted by conventional methods from mucosal linings of the ileum. The results are presented as mean $\pm$ SEM (n=10). Groups with different lower case letters are significantly different ($p < 0.05$).

Figure 3 is a graph showing the log cfu/g ileum content in chicks vaccinated with saline, the Salmonella vector without an antigenic polypeptide insert or the Salmonella vector with the cj0113 insert (SEQ ID NO: 7) (10^8 cfu/chick), 11 days after challenge with *C. jejuni* (1×10^7 cfu/ml). The quantitative PCR was performed on total DNA extracted by conventional methods from mucosal linings of the ileum. The results are presented as mean $\pm$ SEM (n=10) and the * indicates a significant difference ($P < 0.05$).

Figure 4 is a graph showing the relative levels of anti-*Campylobacter* IgG (S/P ratio) as measured by ELISA at days 21 and 32 after administration of the indicated vectors (10^8 cfu/chick). Groups with different lower case letters are significantly different ($P < 0.05$).

5 Figure 5 is a graph showing the relative levels of anti-*Campylobacter* sIgA (S/P ratio) in the mucosa of the ileum on day 32 post-vaccination with the indicated vectors (10^8 cfu/chick). Groups with different lower case letters are significantly different ($P < 0.05$).

10 Figure 6 is a graph showing the serum IgG levels (S/P ratio) at days 21 and 31 post vaccination and sIgA levels (S/P ratio) in the mucosa of the ileum at day 32 post vaccination with saline, the *Salmonella* vector without an insert or the vector with the antigenic polypeptide, cj0113 (SEQ ID NO: 7) (10^8 cfu/chick). A * indicates a significant difference from controls ($P < 0.05$).

15 Figure 7 is a graph showing the *C. jejuni* specific serum IgG antibody levels 10 days post vaccination by oral gavage with either *Bacillus subtilis* backbone strain (BSBB) or cj0113 (SEQ ID NO: 7) *Bacillus subtilis* vectored vaccine candidate at 10^8 cfu/chick. Data are presented as mean $\pm$ SEM with the * indicating a significant difference ($P < 0.05$) from both controls.

20 Figure 8 is a graph showing the *C. jejuni* specific secretory IgA antibody levels 10 days post vaccination by oral gavage with either *Bacillus subtilis* backbone strain (BSBB) or cj0113 (SEQ ID NO: 7) *Bacillus subtilis* vectored vaccine candidate at 10^8 cfu/chick. The mucosa was collected in the ileum region. Data are presented as mean $\pm$ SEM with the * indicating a significant difference ($P < 0.05$) from both controls.

25 Figure 9 is a graph showing the $\log_{10}$ CFU of *C. jejuni* per gram of ileum content as enumerated by quantitative PCR. Birds vaccinated with either *Bacillus subtilis* backbone strain (BSBB) or *Bacillus subtilis* vector expressing cj0113 (SEQ ID NO: 7), were challenged with *C. jejuni* at 1×10^8 cfu/chick then enumerated 10 days after by PCR. qPCR was performed on total DNA extracted by conventional methods from mucosal linings of the ileum. The results are presented as mean $\log_{10}$ cfu/gram of ileum content $\pm$ SEM (n=10) and the * indicates a significant difference ($P < 0.05$) from control.

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Figure 10 is a graph showing the log₁₀ CFU of *C. jejuni* per gram of turkey ileum content as enumerated by quantitative PCR. Turkeys vaccinated with either backbone strain or cj0113 (SEQ ID NO: 7) *Salmonella* vectored vaccine, were challenged with *C. coli* at 1x10⁸ cfu/chick then enumerated 12 days after by PCR. qPCR was performed on
5 total DNA extracted by conventional methods from mucosal linings of the ileum. The results are presented as mean log₁₀ cfu/gram of ileum content $\pm$ SEM (n=10) and the * indicates a significant difference (P < 0.05) from control.

DETAILED DESCRIPTION

10 Vaccine vectors that elicit mucosal, humoral, and cell-mediated immune responses against multiple serovars of *Campylobacter* offer a promising approach to limit *Campylobacter* gastroenteritis. This project utilizes a novel approach in the development of vaccines by inserting polynucleotide sequences encoding non-native linear epitopes (antigenic polypeptides). The antigenic polypeptides may be used in combination with
15 an immunostimulatory polypeptide such as CD154 (CD40L) or HMGB1 (high mobility group box 1) in the vaccine vector. The antigenic polypeptide and the immunostimulatory polypeptide are suitably not polypeptide found natively associated with the vector. The epitope or antigenic polypeptide and the immunostimulatory polypeptide may be expressed on the surface of recombinant vectors. The vectors may
20 be bacterial, viral or even liposome vectors. The vectors may be live, live and attenuated, or killed prior to administration. Substantial preliminary data, such as that shown in the Examples, demonstrates that *Salmonella* or *Bacillus* constructs expressing a foreign epitope are able to rapidly induce high titer epitope-specific antibodies *in vivo*. Furthermore, co-expression of surface CD154 or HMGB1 effectively enhanced the
25 antibody response against the foreign epitope.

Recombinant DNA technologies enable relatively easy manipulation of many bacterial and viral species. Some bacteria and viruses are mildly or non-pathogenic, but are capable of generating a robust immune response. These bacteria and viruses make attractive vaccine vectors for eliciting an immune response to a heterologous, non-native,
30 or foreign antigen. Bacterial or viral vaccine vectors may mimic the natural infection and produce robust and long lasting immunity. Vaccine vectors are often relatively

inexpensive to produce and administer. In addition, such vectors can often carry more than one antigen and may provide protection against multiple infectious agents.

Polynucleotides encoding polypeptide antigens from any number of pathogenic organisms may be inserted into the vaccine vector and expressed to generate antigenic polypeptides. An antigenic polypeptide is a polypeptide that is capable of being specifically recognized by the adaptive immune system. An antigenic polypeptide includes any polypeptide that is immunogenic. The antigenic polypeptides include, but are not limited to, antigens that are pathogen-related, allergen-related, tumor-related or disease-related. Pathogens include viral, parasitic, fungal and bacterial pathogens as well as protein pathogens such as the prions.

The antigenic polypeptides may be full-length proteins or portions thereof. It is well established that immune system recognition of many proteins is based on a relatively small number of amino acids, often referred to as the epitope. Epitopes may be only 8-10 amino acids. Thus, the antigenic polypeptides described herein may be full-length proteins, 8 amino acid long epitopes or any portion between these extremes. In fact the antigenic polypeptide may include more than one epitope from a single pathogen or protein. Suitably the antigenic polypeptide is a polypeptide that is not natively associated with the vector. Not natively associated includes antigenic polypeptides that may also occur natively in the vector, but that are being expressed recombinantly as an epitope, are being expressed in combination with a different polypeptide as a fusion protein to allow for differential display and differential enhancement of the immune response as compared to the natively expressed polypeptide.

Multiple copies of the same epitope or multiple epitopes from different proteins may be included in the vaccine vector. It is envisioned that several epitopes or antigens from the same or different pathogens or diseases may be administered in combination in a single vaccine vector to generate an enhanced immune response against multiple antigens. Recombinant vaccine vectors may encode antigens from multiple pathogenic microorganisms, viruses or tumor associated antigens. Administration of vaccine vectors capable of expressing multiple antigens has the advantage of inducing immunity against two or more diseases at the same time.

The polynucleotides may be inserted into the chromosome of the vaccine vector or encoded on plasmids or other extrachromosomal DNA. Polynucleotides encoding epitopes may be expressed independently (i.e., operably linked to a promoter functional in the vector) or may be inserted into a vaccine vector polynucleotide (i.e., a native polynucleotide or a non-native polynucleotide) that is expressed in the vector. Suitably, the vaccine vector polynucleotide encodes a polypeptide expressed on the surface of the vaccine vector such as a transmembrane protein. The polynucleotide encoding the antigenic polypeptide may be inserted into the vaccine vector polynucleotide sequence in frame to allow expression of the antigenic polypeptide on the surface of the vector. For example, the polynucleotide encoding the antigenic polypeptide may be inserted in frame into a bacterial polynucleotide in a region encoding an external loop region of a transmembrane protein such that the vector polynucleotide sequence remains in frame. See the Examples below in which the antigenic polypeptides are inserted into an external loop of the *lamB* gene of the *Salmonella enteritidis* vector.

Alternatively, the polynucleotide encoding the antigenic polypeptide may be inserted into a secreted polypeptide. Those of skill in the art will appreciate that the polynucleotide encoding the antigenic polypeptide could be inserted in a wide variety of vaccine vector polynucleotides to provide expression and presentation of the antigenic polypeptide to the immune cells of a subject treated with the vaccine vector. In the Examples, several *Campylobacter* polynucleotides were inserted into the *lamB* coding sequence of *Salmonella enteritidis*. The resulting recombinant bacteria express the inserted antigenic polypeptides on the surface of the bacteria. The polynucleotides may be inserted in *CotB* of *Bacillus subtilis* such that the recombinant bacteria expressed the inserted antigenic polypeptides in spores or into *slp* for surface expression in vegetative bacteria.

The vectors may include a polynucleotide encoding full length *Campylobacter* proteins including *cjaD* (SEQ ID NO: 1), *cjaA* (SEQ ID NO: 2) and ACE393 (SEQ ID NO: 3) or an antigenic polypeptide of these proteins. In the Examples, antigenic polypeptides derived from the full-length proteins were used as follows: SEQ ID NO: 7 (a *cjaD* polypeptide called cj0113); SEQ ID NO: 8 (a *cjaA* polypeptide called cj0982); and SEQ ID NO: 9 (an ACE 393 polypeptide called cj0420). The polynucleotides used

in the Examples are provided as SEQ ID NOs: 4-6, respectively. The polynucleotides used in the Examples had the antigenic polypeptides of SEQ ID NOs 7-9 separated by serine linkers and linked to CD154 amino acids 140-149 (three amino acids before, after and in between the antigenic polypeptide and the immunostimulatory polypeptide).

5 Suitably, the portion of the antigenic polypeptide inserted into the vaccine vector is immunogenic or antigenic. An immunogenic fragment is a peptide or polypeptide capable of eliciting a cellular or humoral immune response. Suitably, an antigenic polypeptide may be the full-length protein, or suitably may be 20 or more amino acids, 15 or more amino acids, 10 or more amino acids or 8 or more amino acids of the full-
10 length sequence. Suitably the immune response generated against the target pathogen is a protective immune response. A protective immune response is a response capable of blocking or reducing morbidity or mortality caused by subsequent infection with the target pathogen, namely *Campylobacter*.

One of skill in the art will appreciate that any of these polynucleotide sequences
15 may be used in combination with any other antigenic polypeptide including polypeptides from other heterologous pathogens or organisms and may also be used in conjunction with polynucleotides encoding immunostimulatory polypeptides such as a polypeptide of CD154 or HMGB1 such as is described in International Application Nos. PCT/US07/078785 and PCT/US2011/022062 both of which are incorporated herein by
20 reference in their entireties.

Polynucleotides encoding immunostimulatory polypeptides that are homologous to proteins of the subject and capable of stimulating the immune system to respond to the foreign epitope may also be inserted into a vector. As described in more detail below, the vector may include a CD154 polypeptide that is capable of binding CD40 in the subject
25 and stimulating the subject to respond to the vector and its associated foreign antigenic polypeptide. In addition, a vector may include a HMGB1 polypeptide or a functional fragment thereof. As described above with regard to antigenic polypeptides, polynucleotides encoding these polypeptides may be inserted into the chromosome of the vector or maintained extrachromosomally. One of skill in the art will appreciate that
30 these polynucleotides can be inserted in a variety of vector polynucleotides for expression in different parts of the vector or for secretion of the polypeptides.

The polynucleotide encoding an immunostimulatory polypeptide capable of enhancing the immune response to a non-native antigenic polypeptide may also encode the antigenic polypeptide. The polynucleotide encoding an immunostimulatory polypeptide may be linked to the polynucleotide encoding the antigenic polypeptide, such that in the vaccine vector the immunostimulatory polypeptide and the foreign antigenic polypeptide are present on the same polynucleotide. For example, the antigenic polypeptide and the immunostimulatory polypeptide may be portions of a fusion protein. In the Examples, a polynucleotide encoding a polypeptide of CD154 that is capable of binding to CD40 also encodes an antigenic polypeptide from *cjaD*, *cjaA* or ACE 393 of *Campylobacter*. See SEQ ID NOS: 10-12 in the attached sequence listing for some examples of potential polypeptide sequences and SEQ ID NOs: 4-6 for polynucleotide sequences which encode for optional serine linkers between the antigenic polypeptide, the immunostimulatory polypeptide and the host polypeptide.

In the Examples, the polynucleotide encoding the *Campylobacter* antigenic polypeptides and the polynucleotide encoding the immunostimulatory polypeptide are both inserted in the outer loop of the transmembrane *lamB* gene. Those of skill in the art will appreciate that vector polynucleotides encoding other transmembrane proteins may also be used. In addition, the antigenic polynucleotides may be extrachromosomal or secreted by the vector. In the Examples, the polynucleotide encoding the *Campylobacter* cj0113 antigen (SEQ ID NO: 7) and the immunostimulatory peptide HMGB1 (SEQ ID NO:20) were expressed from a plasmid carried by a *Bacillus* vector and expressed on the cell surface.

Suitably, the CD154 polypeptide is fewer than 50 amino acids long, more suitably fewer than 40, fewer than 30 or fewer than 20 amino acids in length. The polypeptide may be between 10 and 15 amino acids, between 10 and 20 amino acids or between 10 and 25 amino acids in length. The CD154 sequence and CD40 binding region are not highly conserved among various species. The CD154 sequences of chicken and human are provided in SEQ ID NO: 13 and SEQ ID NO: 14, respectively.

The CD40 binding regions of CD154 have been determined for a number of species, including human, chicken, duck, mouse and cattle and are shown in SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO:18, and SEQ ID NO:19, respectively.

Although there is variability in the sequences in the CD40 binding region between species, cross-species binding of CD154 to CD40 has been reported. For example, the human CD154 polypeptide was able to enhance the immune response in chickens. Therefore, one may practice the invention using species specific CD154 polypeptides or a heterologous CD154 polypeptide.

The HMGB1 (High Mobility Group Box-1) protein was first identified as a DNA-binding protein critical for DNA structure and stability. It is a ubiquitously expressed nuclear protein that binds DNA with no sequence specificity. The protein is highly conserved and found in plants to mammals. The zebrafish, chicken and human HMGB1 amino acid sequences are provided in SEQ ID NO: 28, SEQ ID NO: 20 and SEQ ID NO: 27, respectively. The sequence throughout mammals is highly conserved with 98% amino acid identity and the amino acid changes are conservative. Thus an HMGB1 protein from one species can likely substitute for that from another species functionally. The full-length HMGB1 protein or a portion thereof may be used as the HMGB1 polypeptide in the vaccine vectors described herein. HMGB1 has two DNA binding regions termed A box as shown in SEQ ID NO: 21 and 22 and B box as shown in SEQ ID NO: 23 and 24. See Andersson and Tracey, *Annu. Rev. Immunol.* 2011, 29:139-162, which is incorporated herein by reference in its entirety.

HMGB1 is a mediator of inflammation and serves as a signal of nuclear damage, such as from necrotic cells. HMGB1 can also be actively secreted by cells of the monocyte/macrophage lineage in a process requiring acetylation of the protein, translocation across the nucleus and secretion. Extracellular HMGB1 acts as a potent mediator of inflammation by signaling via the Receptor for Advanced Glycated End-products (RAGE) and via members of the Toll-like Receptor family (TLR), in particular TLR4. The RAGE binding activity has been identified and requires the polypeptide of SEQ ID NO: 25. TLR4 binding requires the cysteine at position 106 of SEQ ID NO: 20, which is found in the B box region of HMGB1.

The inflammatory activities of HMGB1 do not require the full-length protein and functional fragments have been identified. The B box has been shown to be sufficient to mediate the pro-inflammatory effects of HMGB1 and thus SEQ ID NO: 23 and 24 are HMGB1 polypeptides or functional fragments thereof within the context of the present

invention. In addition, the RAGE binding site and the pro-inflammatory cytokine activity have been mapped to SEQ ID NO: 25 and SEQ ID NO: 26, respectively. Thus, these polypeptides are functional fragments of HMGB1 polypeptides in the context of the present invention.

5 Those of skill in the art are capable of identifying HMGB1 polypeptides and fragments thereof capable of stimulating pro-inflammatory cytokine activity, using methods such as those in International Publication No. WO02 092004, which is incorporated herein by reference in its entirety. Suitably, the HMGB1 polypeptide includes the RAGE binding domain at amino acids 150-183 of SEQ ID NO:20 (SEQ ID
10 NO: 25 or a homolog thereof) and the pro-inflammatory cytokine activity domain between amino acids 89-109 of SEQ ID NO: 20 (SEQ ID NO: 26 or a homolog thereof). In particular, HMGB1 polypeptides and functional fragments or homologs thereof include polypeptides identical to, or at least 99% identical, at least 98% identical, at least 95% identical, at least 90% identical, at least 85% identical, or at least 80% identical to
15 the HMGB1 polypeptides of SEQ ID NOs: 20-28.

One of skill in the art will appreciate that the HMGB1 polypeptide could be used to enhance the immune response to more than one antigenic polypeptide present in a vector. The polypeptide from HMGB1 stimulates an immune response at least in part by activating dendritic cells and macrophages and thus stimulating production of IL-1, IL-6,
20 IFN- γ and TNF- α . Suitably, HMGB1 may be expressed on the surface of the vector.

At least a portion of the antigenic polypeptide and at least a portion of the HMGB1 polypeptide or another immunostimulatory polypeptide may be present on the surface of the vaccine vector. Present on the surface of the vaccine vector includes polypeptides that are comprised within a transmembrane protein, interacting with,
25 covalently or chemically cross-linked to a transmembrane protein, a membrane lipid or membrane anchored carbohydrate. A polypeptide can be comprised within a transmembrane protein by having the amino acids comprising the polypeptide linked via a peptide bond to the N-terminus, C-terminus or anywhere within the transmembrane protein (i.e. inserted between two amino acids of the transmembrane protein or in place
30 of one or more amino acids of the transmembrane protein (i.e. deletion-insertion). Suitably, the polypeptides may be inserted into an external loop of a transmembrane

protein. Suitable transmembrane proteins are *cotB* and *lamB*, but those of skill in the art will appreciate many suitable transmembrane proteins are available.

Alternatively, the polypeptides may be covalently or chemically linked to proteins, lipids or carbohydrates in the membrane, or capsid if a viral vector is being used through methods available to persons of skill in the art. For example, di-sulfide bonds or biotin – avidin cross-linking could be used to present the antigenic and HMGB1 polypeptides on the surface of a vaccine vector. Suitably, the antigenic polypeptide and the HMGB1 polypeptide are part of a fusion protein. The two polypeptides may be directly linked via a peptide bond or may be separated by a linker or a section of a third protein into which they are inserted.

In the Examples, some of the vectors have the *Campylobacter* antigenic polypeptides (cj0113, cj0420 and cj0982) and the immunostimulatory polypeptide (CD154 amino acids 140-149 or HMGB1 or a functional fragment thereof) encoded on the same polynucleotide (*lamB*) such that the sequences are in frame with each other and with the *Salmonella* polynucleotide in which they were inserted. In some embodiments, linkers may be added between the polynucleotide sequences encoding the antigenic polypeptide and the immunostimulatory polypeptide such that in the expressed polypeptide several amino acids separate the two polypeptides. The linker may be 3 nucleotides encoding a single amino acid, or may be much longer, e.g. 30 nucleotides encoding 10 or more amino acids. In the Examples a 9 nucleotide linker was used and encoded for three serine residues. Those of skill in the art will readily envision many other types of linkers that could be used.

In addition, the polynucleotides may be present in a single copy or in multiple copies. For example, three copies of the antigenic polypeptide and three copies of the immunostimulatory polypeptide may be found in the same external loop of a transmembrane protein or expressed within several different vector proteins. In alternative embodiments, the immunostimulatory polypeptide and the antigenic polypeptide may be encoded by distinct polynucleotides.

Potential vaccine vectors for use in the methods include, but are not limited to, *Bacillus*, *Salmonella* (*Salmonella enteritidis*), *Shigella*, *Escherichia* (*E. coli*), *Yersinia*, *Bordetella*, *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Vibrio* (*Vibrio cholerae*), *Listeria*,

adenovirus, poxvirus, herpesvirus, alphavirus, and adeno-associated virus. Suitably, the vaccine vector is a GRAS organism. The vaccine vector may be inactivated or killed such that it is not capable of replicating. Methods of inactivating or killing bacterial or viral vaccine vectors are known to those of skill in the art and include, but are not limited to methods such as formalin inactivation, antibiotic-based inactivation, heat treatment and ethanol treatment. In some embodiments the vaccine vector may be a liposome based vector.

Compositions comprising the vector and a pharmaceutically acceptable carrier are also provided. A pharmaceutically acceptable carrier is any carrier suitable for *in vivo* administration. The pharmaceutically acceptable carrier may include water, buffered solutions, glucose solutions or bacterial culture fluids. Additional components of the compositions may suitably include excipients such as stabilizers, preservatives, diluents, emulsifiers and lubricants. Examples of pharmaceutically acceptable carriers or diluents include stabilizers such as carbohydrates (e.g., sorbitol, mannitol, starch, sucrose, glucose, dextran), proteins such as albumin or casein, protein-containing agents such as bovine serum or skimmed milk and buffers (e.g., phosphate buffer). Especially when such stabilizers are added to the compositions, the composition is suitable for freeze-drying or spray-drying.

Methods of enhancing immune responses to *Campylobacter* in a subject by administering the vectors described herein are also provided. The vector may contain a HMGB1 polypeptide or a CD154 polypeptide capable of stimulating the immune response to the vector and the antigenic polypeptides described above. The vector is administered to a subject in an amount effective to enhance the immune response of the subject to the non-native antigenic polypeptides. Suitably the immune response to challenge with *Campylobacter* is enhanced.

Enhancing an immune response includes, but is not limited to enhancing antibody responses. Suitably the IgA response is enhanced, more suitably the secretory IgA response is enhanced after administration of the vector as compared to a control. The control may be the same subject prior to administration of the vector, a comparable subject administered a vector alone or a vector expressing an irrelevant or a non-*Campylobacter* antigenic polypeptide. The antibody response, suitably the IgA response,

may be increased as much as two fold, three fold, four fold, five fold or more as compared to the response of a control subject. The enhanced immune response may also result in a reduction of the ability of *Campylobacter* to grow or replicate and colonize the subject after administration of the vectors described herein. Such a reduction may be tested by challenging a subject administered the vector with a *Campylobacter* infection and monitoring the ability of the bacteria to colonize and replicate, i.e. infect, the subject as compared to a control subject. The growth of *Campylobacter* in the subject may be reduced by 1 log, 2 logs, 3 logs, 4 logs, 5 logs or even more. The growth of *Campylobacter* in a subject administered the vector may be below the level of detection.

In addition, methods of enhancing resistance to *Campylobacter* infection are disclosed. Briefly, the methods comprise administering to a subject the vectors described above comprising *Campylobacter* antigenic polypeptides in an amount effective to elicit an immune response. Enhancing resistance to *Campylobacter* infection includes but is not limited to reducing the incidence of *Campylobacter* infections, limiting the spread of *Campylobacter* infections from one host to another, reducing *Campylobacter* replication in the subject, invasion or spread within a single host, reduced morbidity associated with *Campylobacter* infections, and reduced duration of a *Campylobacter* infection.

Administration of the vector may prevent the subject from contracting *Campylobacter* or from exhibiting any outward signs of disease, such as gastroenteritis or GBS. Increased resistance to *Campylobacter* may also include increased antibody production, suitably IgA production. The antibody response, suitably the IgA response, may be increased as much as two fold, three fold, four fold, five fold or more as compared to the response of a control subject. The enhanced immune response may also result in a reduction of the ability of *Campylobacter* to grow or replicate and colonize the subject after administration of the vectors described herein. Such a reduction may be tested by challenging a subject administered the vector with a *Campylobacter* infection and monitoring the ability of the bacteria to colonize and replicate, i.e. infect, the subject as compared to a control subject. The growth of *Campylobacter* in the subject may be reduced by 1 log, 2 logs, 3 logs, 4 logs, 5 logs or even more. The growth of *Campylobacter* in a subject administered the vector may be below the level of detection.

The antigenic polypeptides for use in all the methods described herein may be from cjaD, cjaA or ACE 393 as discussed above. The insertion of the antigenic polypeptides into the vector may be accomplished in a variety of ways known to those of skill in the art, including but not limited to the scarless site-directed mutation system described in International Patent Publication No. WO2008/036675, which is incorporated herein by reference in its entirety. The vector may be a bacterium engineered to express *Campylobacter* antigenic polypeptides in conjunction with polynucleotides capable of enhancing the immune response as discussed above. In particular, a polypeptide of CD154 or HMGB1 may be expressed by the vector to enhance the immune response of the subject to the antigenic polypeptides. The vectors used in these methods may be attenuated or killed prior to administration or use in the methods.

The useful dosage to be administered will vary depending on the age, weight and species of the subject, the mode and route of administration and the type of pathogen against which an immune response is sought. The composition may be administered in any dose of vector sufficient to evoke an immune response. For bacterial vectors, it is envisioned that doses ranging from 10^3 to 10^{10} bacteria, from 10^4 to 10^9 bacteria, or from 10^5 to 10^7 bacteria are suitable. The composition may be administered only once or may be administered two or more times to increase the immune response. For example, the composition may be administered two or more times separated by one week, two weeks, or by three or more weeks. The bacteria vectors are suitably viable prior to administration, but in some embodiments the bacteria vectors may be killed prior to administration. In some embodiments, the bacteria vectors may be able to replicate in the subject, while in other embodiments the bacteria vectors may be attenuated and/or may not be capable of replicating in the subject.

For administration to animals or humans, the compositions may be administered by a variety of means including, but not limited to, intranasally, mucosally, by spraying, intradermally, parenterally, subcutaneously, orally, by aerosol or intramuscularly. Eye-drop administration or addition to drinking water or food are additionally suitable means of administration. For chickens, the compositions may be administered *in ovo*.

With regard to the methods, a subject includes, but is not limited to, a vertebrate, suitably a mammal, suitably a human, or birds, suitably poultry such as chickens or

turkeys. Other animal models of infection may also be used. Enhancing an immune response includes, but is not limited to, inducing a therapeutic or prophylactic effect that is mediated by the immune system of the subject. For example, an immune response is enhanced if the subject is protected from subsequent infection with *Campylobacter*.

5 Specifically, enhancing an immune response may include enhanced production of antibodies, such as demonstrated in Figures 4-8, enhanced class switching of antibody heavy chains, maturation of antigen presenting cells, stimulation of helper T cells, stimulation of cytolytic T cells or induction of T and B cell memory. In the Examples, an increase in the amount of secretory IgA was seen after administration of the vector and
10 was correlated with protection from subsequent *Campylobacter* infection.

It is envisioned that several epitopes or antigens from the same or different pathogens may be administered in combination in a single vector to generate an enhanced immune response against multiple antigens and their associated pathogens. Recombinant vaccine vectors may encode antigens from multiple pathogenic microorganisms, viruses
15 or tumor associated antigens. Administration of vaccine vectors capable of expressing multiple antigens has the advantage of inducing immunity against two or more diseases at the same time.

Heterologous polynucleotides encoding antigens can be inserted in the vaccine vector genome at any non-essential site or alternatively may be carried on a plasmid
20 using methods well known in the art. One suitable site for insertion of polynucleotides is within external portions of transmembrane proteins or coupled to sequences which target the heterologous polynucleotide for secretory pathways. One example of a suitable transmembrane protein for insertion of polynucleotides is the *lamB* gene of Salmonella. Heterologous polynucleotides include, but are not limited to, polynucleotides encoding
25 antigens selected from pathogenic microorganisms or viruses other than the vaccine vector, i.e., non-native polynucleotides encoding non-native polypeptides.

The following examples are meant only to be illustrative and are not meant as limitations on the scope of the invention or of the appended claims. All references cited herein are hereby incorporated by reference in their entirety.

Examples

Attenuation of Salmonella Vaccine Candidate Strains

Salmonella enteritidis phage type 13A (*S. enteritidis*) was attenuated by introducing defined, irreversible deletion mutations in the *aroA* and/or *htrA* gene of the *S. enteritidis* genome as previously described (available as ATCC Deposit Nos: PTA-7871, PTA-7872 and PTA-7873). Briefly, the target gene sequence in the bacterial genome of *S. enteritidis* was replaced with the kanamycin-resistant (Km^R) gene sequence. This was performed using 3S-PCR and electroporation of the 3S-PCR products into electrocompetent *Salmonella* cells containing the pKD46 plasmid. The resulting cell mixture was plated on LB agar plates supplemented with Km to select for positive clones containing a Km^R gene. The Km^R gene was inserted into the genomic region containing the genes of interest (*aroA* or *htrA*) by flanking the Km^R gene with sequences homologous to the genes of interest. Once Km^R mutants were obtained, the deletion mutations were confirmed by PCR and DNA sequencing. All Km^R genes were removed before epitope insertion was started.

Construction of Recombinant Vaccine Candidates

Three potential candidate antigenic polypeptides were selected: Omp18/cjaD (cj0113), cjaA (cj0982) and ACE393 (cj0420). The polypeptides selected were as follows: cj0113 (GVSITVEGNCDEWGTDEYNQAWMTTSYAPTS; SEQ ID NO: 10), cj0982c (KDIVLDAEIGGVAKGKDGKEKWMTTTSYAPTS; SEQ ID NO: 11), and cj0420 (KVALGVAVPKDSNITSVEDLKDKTLLLNGTADAWMTTSYAPTS; SEQ ID NO: 12), all inserts additionally contain a sequence for amino acids 140-149 of CD154.

Recombinant *S. enteritidis* strains containing stable integrated copies of cj0113-CD154 (cj0113), cj0420-CD154 (cj0420) or cj0982c-CD154 (cj0982) were constructed using the method of Cox et al. Scarless and site-directed mutagenesis in *Salmonella enteritidis* chromosome. BMC Biotechnol 2007;7:59. Briefly, an I-SceI enzyme site along with a Km^R gene was introduced into Loop 9 of the *lamB* gene by design of a PCR product which had the I-SceI enzyme site and Km^R gene flanked by approximately 200-300 base pairs of DNA on each side, homologous to the up and downstream regions of Loop 9. Primers used are shown in Table I below. The PCR product was electroporated

into electrocompetent attenuated *Salmonella* cells containing the pKD46 plasmid and the resulting cell mixture plated on LB agar plates supplemented with Km to select for positive clones now containing a Km^R gene. After the Sce-I/Km mutation was made in Loop 9, this region was replaced by a codon-optimized foreign epitope DNA sequence

5 (Burns DM, Beacham IR. Rare codons in *E. coli* and *S. typhimurium* signal sequences. FEBS Lett 1985;189(2):318-24.). This second 3S-PCR reaction produced the foreign epitope insert flanked by Loop 9 up and downstream regions, and the resulting PCR product was electroporated into electrocompetent SE13A containing the Sce-I/Km mutation described above. Plasmid pBC-I-SceI was also electroporated into the cells

10 along with the insert as the plasmid produces the I-SceI enzyme which recognizes and cleaves a sequence creating a gap at the I-SceI enzyme site in the Loop 9 region of the LamB gene where the foreign epitope sequences inserted into the SE13A genome. The plasmid also carries with it a chloramphenicol (Cm) resistant gene (Cm^R) as the inserts that will replace the Km^R gene the mutations must have a new selection marker to

15 counter-select against the previous I-SceI/Km mutation. After electroporation, cells were plated on LB agar plates containing 25 µg/mL Cm for the selection of positive mutants.

Table I: PCR Primers

Primer	Amplified Region	Primer Sequence (SEQ ID NO)
lam-up-f	Loop 9 up	5'TGTACAAGTGGACGCCAATC 3' (SEQ ID NO: 29)
lam-up-r	Loop 9 up	5'GTTATCGCCGCTTTGATATAGCC3' (SEQ ID NO: 30)
lam-dn-f	Loop 9 dn	5'ATTTCCCGTTATGCCGAGC3' (SEQ ID NO: 31)
lam-dn-r	Loop 9 dn	5'GTTAAACAGAGGGCGACGAG 3'(SEQ ID NO: 32)
Km-f	I-SceI/Km ^R gene	5'GCTATATCAAAGACGGCGATAAC TAACTATAACGGTCCTAAGGTAGCGA ATTTCGGGGATCCGTCGA 3'(SEQ ID NO: 33)
Km-r	I-SceI/Km ^R gene	5'GCTGCGGCATAACGGGAAA TGTAGGCTGGAGCTGCTTCG 3' (SEQ ID NO: 34)
Kan4f	Inside Km ^R gene	5'CAAAAGCGCTCTGAAGTTC 3' (SEQ ID NO: 35)
Kan4r	Inside Km ^R gene	5'GCGTGAGGGGATCTTGAAGT 3' (SEQ ID NO: 36)
lam 3f	Outer regions of loop 9	5'GCCATCTCGCTTGGTGATAA 3' (SEQ ID NO: 37)
lam 3r	Outer regions of loop 9	5'CGCTGGTATTTGCGGTACA 3' (SEQ ID NO: 38)
Cj0113f	Insert with loop 9 up	5'TTCATCGGTACCCCAATCATCACAGTTACCTTCAACGGTGATGCTAACACCGGAGGAGGAGT TATCGCCGCTTTGATATAGCC3' (SEQ ID NO: 39)
Cj0113r	Insert with loop 9 down	5'ATGAATGGGGTACCGATGAATATAACAGGCGTCCTCCTCTGGATGACCACCTCCTATGCG CCGACCTCCTCCTCCTCCATTTCGGTTATGCCGAGC3' (SEQ ID NO: 40)
Cj0420f	Insert with loop 9 up	5'ATCTTTACCTTCGCAACACCACCGATTTCCGCAATCCAGAACGATATCTTTGGAGGAGGAGT TATCGCCGCTTTGATATAGCC3' (SEQ ID NO: 41)
Cj0420r	Insert with loop 9 down	5'GTGTTGCGAAAGGTAAGATGGTAAAGAAAAATCTCCTCCTGGATGACCACCTCCTATGC GCCGACCTCCTCCTCCTCCATTTCGGTTATGCCGAGC3' (SEQ ID NO: 42)

Cj0982c-f	Insert with loop 9 up	5'GGTTTTATCTTTTCAGATCTTCAACGCTGGTGATGTTGCTATCTTTCGGAACCGCAACACCCA GCGCAACTTTGGAGGAGGAGTTATCGCCGTCCTTGATATAGCC3' (SEQ ID NO: 43)
Cj0982c-r	Insert with loop 9 down	5'AAGATCTGAAAGATAAAACCCTGCTGCTGAACAAAGGTACCACCGCGGATGCGTCCTCCTC CTGGATGACCACCTCCTATGCGCCGACCTCCTCCTCCTCCATTCCCGTTATGCCGCAGC3' (SEQ ID NO: 44)

Once positive mutation/inserts were suspected, PCR and DNA sequencing were performed to confirm that the insertion sequences are present and correct.

Challenge with Campylobacter jejuni

5 Three wild-type isolates of *C. jejuni* from broiler chickens were individually grown to log-phase growth, combined, serially diluted and spread plated for conventional culture enumeration as previously described (Cole et al. Effect of aeration and storage temperature on *Campylobacter* concentrations in poultry semen. Poult Sci 2004;83:1734-8.). These were diluted to approximately 10^7 to 10^8 cfu/ml for challenge by oral gavage
10 using spectrophotometric density and comparison to a previously-generated standard curve. Empirically determined cfu administered are reported for each of experiment involving challenge (see below).

Vaccination Study 1

In the first immunization study, 210 day-of-hatch broiler chicks were obtained
15 from a local commercial hatchery and randomly assigned to one of four treatment groups: saline only (Negative control), or one of three vaccine candidate groups: cj0113, cj0420 or cj0982; n=50/pen. Each treatment group was housed in an individual floor pen on fresh pine litter and provided water and feed *ad libitum*. On day-of-hatch, all chicks in each treatment group were inoculated, via oral gavage, with 0.25mL of a solution
20 containing approximately 10^8 cfu/mL of the appropriate treatment. On day 21 post-hatch, all birds in each treatment group were challenged with *C. jejuni*, via oral gavage, with 0.25mL of a solution containing 1×10^7 cfu/ml. On days 3, 11, 21 (prior to booster inoculation) and 32 post-hatch, 10-15 birds from each treatment group were humanely killed and their liver, spleen and cecal tonsils aseptically removed for the determination
25 of organ invasion, colonization and clearance of the *Salmonella* vaccine vector strains. Also, on days 21 and 32 post-hatch, ileum sections were removed and processed for use in qRT-PCR and on day 32 a separate ileum sample was removed and diluted 1:5 in saline and was used to test for secretory immunoglobulin A (sIgA). In addition, blood

samples were collected from 10 birds per treatment group and the serum was used for determining antibody response on days 21 and 32 post-vaccination.

Vaccination Study 2

In experiment 2, 110 day-of-hatch broiler chicks were obtained from a local commercial hatchery and randomly assigned to one of two treatment groups: saline only (vehicle control) or *Salmonella* vaccine candidate, cj0113, (n=55/pen). Each treatment group was housed in an individual floor pen on fresh pine litter and provided water and feed *ad libitum*. On day-of-hatch, all chicks in each treatment group were inoculated via oral gavage with 0.25mL of a solution containing approximately 10^8 cfu/mL of the appropriate treatment. On day 21 post-hatch, all birds in each treatment group were challenged with *C. jejuni*, via oral gavage, with 0.25 mL of a solution containing 1×10^7 cfu/mL. On days 3, 11, 21 (prior to booster inoculation) and 32 post-hatch, 10-15 birds from each treatment group were humanely killed and their liver, spleen and cecal tonsils aseptically removed for the determination of organ invasion, colonization and clearance of the *Salmonella* vaccine vector strains. Also, on days 21 and 32 post-hatch ileum sections were removed and processed for use in qRT-PCR. In addition, blood samples were collected from 10 birds per treatment group and the serum was used for determining antibody response on days 21 and 32 post-hatch.

Vaccination Study 3

A third experiment was similar to vaccination experiment 2 (described above) except with the addition of a third group of *S. enteritidis* 13A *aroA/htrA* without the *Campylobacter* epitope (SE13A) as a control for the oral vaccination of the vector itself. All sample collections were the same as vaccination study 2 except on day 32 post-hatch an additional section of ileum was used to harvest the mucosal layer for sIgA as in experiment 1.

Measurement of Campylobacter Antibody Response

Serum collected from birds in both immunization studies was used in an ELISA to determine relative antibody responses. Briefly, individual wells of a 96-well plate were coated with *C. jejuni*. Antigen adhesion was allowed to proceed overnight at 4°C, the plates were then washed and blocked with Superblock (Pierce) for 1 hour at room temperature. Plates were then incubated for 2 hours with a 1:50 dilution of the previously

collected sera. The plates were rinsed again followed by incubation with a Peroxidase-labeled anti-chicken IgG secondary antibody (Jackson Immunolaboratories) for an additional hour. After subsequent rinsing, the plates were developed using a peroxidase substrate kit (BD OptEIA, Fisher Scientific) and absorbances were read on a spectrophotometer at 450nm. Each plate contained a positive control and negative control where a pooled sample from vaccinated chicks and pre-immune chicken serum, respectively, replaced the serum from the treatment groups. The absorbance obtained for the positive control, negative control and experimental samples were used to calculate Sample to Positive control ratios (S/P ratios) using the following calculation: (sample mean – negative control mean) / (positive control mean – negative control mean) (Brown et al. Detection of antibodies to *Mycoplasma gallisepticum* in egg yolk versus serum samples. J Clin Microbiol 1991;29(12):2901-3 and Davies et al. Evaluation of the use of pooled serum, pooled muscle tissue fluid (meat juice) and pooled faeces for monitoring pig herds for *Salmonella*. J Appl Microbiol 2003;95(5):1016-25.). The ELISA method used for detection of sIgA was similar to the above described assay for serum immunoglobulin except we used goat anti-chicken IgA conjugated with horseradish peroxidase (GenTex) in place of the anti-chicken IgG antibody conjugate.

DNA Isolation and Quantitative PCR for C. jejuni

Total DNA extraction from ileal samples was achieved using the QIAmp DNA Stool Mini Kit (Qiagen). The manufacturer's included protocol was modified slightly in the following ways: ileal contents were removed to include the mucosal layer and diluted 1:5(w/v) with ice cold PBS + .05% Tween 20; one ml of the slurry was added to 1ml of the included ASL Buffer in a 2.0ml microcentrifuge tube, vortexed and heated to 70°C for 5 minutes. Subsequently, the manufacturer's recommendations were followed to the last step when the DNA was eluted into a final volume of 50ul.

Quantitative determination of *C. jejuni* was accomplished using a previously published method with slight modifications (Skanseng et al. Comparison of chicken gut colonisation by the pathogens *Campylobacter jejuni* and *Clostridium perfringens* by real-time quantitative PCR. Mol Cell Probes 2006;20(5):269-79). The assay was optimized for use on the MX3005P (Agilent Technology) and Brilliant II QPCR master mix

(Agilent Technologies) all other mixture components, primers, probe and cycling conditions remained as published.

A standard curve (Figure 1) was prepared using a pure culture of *C. jejuni* serially diluted 10-fold and added to a constant background of ileal content; total DNA isolation was done as previously described.

Statistical Analysis

Data were analyzed using Student's two-tailed *t-test* assuming unequal variances to compare the difference between groups and controls using JMPTM statistic software. A value of $P < 0.05$ was considered significant.

Results

An excellent correlation of quantification of *C. jejuni* using conventional microbiological enumeration techniques verses the qPCR was found (Fig. 1) with a greater than 99% correlation between the two methods. In experiment 1, we observed significant levels of colonization by the three candidate vectored vaccines within the cecal tonsils by day 3 post-vaccination; as well as significant invasion of the internal organs by the cj0113 expressing vector at the same time point (Table II). However, by day 11 post-vaccination, there was a decline in the amount of colonization of all three vectors and by day 21 post-vaccination, the vectors had been completely cleared from the cecal tonsils as well as the internal organs (Table II). We observed the same trend in our follow up vaccination study (experiment 2), using vector-expressed cj0113 as our vaccine candidate, as shown by the data presented in Table II.

Table II.

Percentage of colonization, invasion and clearance of liver, spleen or cecal tonsils by *Salmonella* following vaccination with one of three *Salmonella* vectored vaccine candidates or saline gavage.

	Liver/Spleen				Cecal Tonsils			
	Day 3	Day 11	Day 21	Day 32	Day 3	Day 11	Day 21	Day 32
Experiment 1								
Saline	0	0	0	0	0	0	0	0
cj0420	0	0	0	0	60	0	0	0
cj0113	50	0	0	0	100	40	0	0
cj0982	0	0	0	0	70	20	0	0
Experiment 2								
Saline	0	0	0	0	0	0	0	0
cj0113	0	20	0	0	50	40	0	0

In experiments 1 and 2 incidence of the attenuated recombinant *Salmonella* vaccine vector is represented as the percentage of positive liver, spleen, or cecal tonsils out of 10 birds. Chicks were orally gavaged with approximately 10^8 cfu of the appropriate treatment on day-of-hatch and. On days 3, 11, 21 and 32 post-hatch, 10 birds from each treatment group were euthanatized, and the livers, spleens, and ceca tonsils were collected for the determination (+/-) of the attenuated recombinant *Salmonella* vaccine vectors. The liver and spleen of each bird was pooled and assayed as one sample.

Chickens were challenged with *C. jejuni* on day 21 post vaccination. Ileal mucosal samples were obtained on days 21 and 32 post vaccination (days 0 and 11 post challenge) and used for DNA sample preparation to enumerate *C. jejuni* within the gut as described above. Vaccination with vector candidates cj0420 and cj0982 caused an approximate 1 log and 2 log reduction ($P < 0.05$), respectively, in the level of *C. jejuni* present in the ileal samples. Using the cj0113 vaccine candidate, there was a marked 4.8 log reduction ($P < 0.05$) of *C. jejuni* in the ileum compared to the control birds (Fig. 2).

In experiment 2, a repeat of the primary immunization study was done with only the vaccine candidate expressing cj0113. In this study, qPCR data revealed an approximate 5 log reduction of *C. jejuni* in cj0113 SE-vectored vaccine administered to birds when compared to the birds receiving saline only (Table III). Additionally, in experiment 3 vaccination with the cj0113 vector caused an approximate 4 log reduction,

to below detectable levels, of *C. jejuni* as compared with the saline or *Salmonella* parent strain which contained no epitope insert (Fig. 3).

Table III.

- 5 Enumeration of *Campylobacter jejuni* by quantitative PCR in chicks 11 days following *Campylobacter* challenge in Experiment 2 (n=10).

	Mean <i>C. jejuni</i> Log ₁₀ cfu/gm ileum	SD ^a	SE ^b
Saline	5.00	0.98	0.44
cj0113	0.00	0.00	0.00

- 10 In experiment 2 *Campylobacter jejuni* quantification was determined in chicks receiving saline or the *Salmonella* vectored vaccine candidate, cj0113 at 10⁸ cfu/chick by quantitative PCR 11 days after receiving a *C. jejuni* challenge dose of approximately 10⁷ cfu/ml. qPCR was performed on total DNA extracted by conventional methods from mucosal linings of the ileum. The results are presented as mean log₁₀ cfu/gram of ileum content with standard deviation^a and standard error^b (n=10).

15

- Serum samples collected in each experiment on Days 21 and 32 post- vaccination were used to determine *C. jejuni*- specific IgG antibodies. In the first experiment all three vaccine candidates (cj0420, cj0113, cj0982) caused significantly higher antibody levels at both time points when compared to the group which received only saline (Fig. 4). Also
 20 in the first experiment, the group vaccinated with cj0113 showed significantly higher antibody titers when compared to cj0420 and cj0982 (Fig. 4). An ELISA was also used to determine mucosal sIgA antibody levels specific for *Campylobacter*. These data indicate that the vaccine vector cj0113 caused a significant increase in the levels of sIgA when compared to the saline group and the two groups receiving either cj0420 or cj0982
 25 (Fig. 5). The results from the second and third study in which only cj0113 was used as a vaccine candidate showed results similar to experiment 1 with vaccinated birds having significantly higher levels of antigen-specific IgG and sIgA antibodies to *C. jejuni* when compared to the birds receiving only saline (Data for Experiment 3 are shown in Fig. 6).

Also, in the third experiment the antibody levels for the backbone strain (SE13) were similar to saline controls (Fig. 6).

Bacillus vectored vaccination study

5 *Production of Heterologous Proteins for Vegetative Cell Expression*

Plasmid pHT10 purchased from MoBioTec/Boca Scientific, Boca Raton, FL (Nguyen et al., 2007) was transformed at the multiple cloning site by addition of a *Bacillus subtilis* codon optimized insertion sequence for cj0113 and HMGB1 (SEQ ID NO: 4 and 20, respectively). DNA sequencing was done to confirm correct sequence
10 insertion. The newly modified plasmid was then transformed into *Bacillus*. Briefly, *Bacillus* cultures were grown overnight at 37°C in HS media (Spizizen's medium supplemented with 0.5% glucose, 50µg/ml DL-tryptophan, 50µg/ml uracil, 0.02% casein hydrolysate, 0.1% yeast extract, 8µg/ml arginine, 0.4µg/ml histidine, 1mM MgSO₄). Inoculate 20ml LS medium (Spizizen's medium supplemented with 0.5% glucose,
15 5µg/ml DL-tryptophan, 5µg/ml uracil, 0.01% casein hydrolysate, 0.1% yeast extract, 1mM MgSO₄, 2.5mM MgCl₂, 0.5mM CaCl₂) with 1 ml overnight culture and incubate with shaking for 3-4 hours at 30 °C. Withdraw 1 ml of LS culture and add 10µl of 0.1M EGTA and incubate at room temperature for 5 minutes. Add 1-2 µg plasmid DNA, shake for 2 hours at 37°C, and plate on LB plates with selective antibiotics. These transformed
20 *Bacillus* spp. now produce heterologous epitope sequences from *Campylobacter* (cj0113) and HMGB1 when induced with 1mM IPTG.

Vaccination study

In the vaccination challenge, 100 day-of-hatch broiler chicks were obtained from a local commercial hatchery and randomly assigned to one of four treatment groups:
25 saline only, *Bacillus* vector alone (BSBB) or 10⁶ or 10⁸ *Bacillus* vaccine candidate, cj0113, (n=25/pen). Each treatment group was housed in an individual floor pen on fresh pine litter and provided water and feed *ad libitum*. On day-of-hatch, all chicks in each treatment group were inoculated via oral gavage with 0.25mL of a solution containing approximately 10⁶ cfu/mL of the backbone vector strain or the Cj0113 *Bacillus* vector.
30 On day 10 birds received a booster vaccination of the same treatment they received on

day of hatch. On day 21 post-hatch, all birds in each treatment group were challenged with *C. jejuni*, via oral gavage, with 0.25 mL of a solution containing 1×10^7 cfu/mL prepared as described above. On days 3, 11, 21 (prior to booster inoculation) and 32 post-hatch, 10-15 birds from each treatment group were humanely killed and their liver, spleen and cecal tonsils aseptically removed for the determination of organ invasion, colonization and clearance of the vaccine vector strains. Also, on days 21 and 36 post-hatch ileum sections were removed and processed for use in qRT-PCR. In addition, blood samples were collected from 15 birds per treatment group and the serum was used for determining antibody response on days 21 and 36 post-hatch.

Results

Serum samples collected on Day 36 post- vaccination were used to determine *C. jejuni*- specific IgG antibodies. Vaccination with the bacillus vector expressing the cj0113 polypeptide caused significantly higher antibody levels when compared to the group which received only saline or empty vector (Fig. 7). An ELISA was also used to determine mucosal sIgA antibody levels specific for *Campylobacter* at day 36 post vaccination. These data indicate that the vaccine vector expressing cj0113 caused a significant increase in the levels of sIgA when compared to the empty vector (Fig. 8).

Chickens were challenged with *C. jejuni* on day 24 post vaccination. Ileal mucosal samples were obtained on days 24 and 36 post vaccination (days 0 and 11 post challenge) and used for DNA sample preparation to enumerate *C. jejuni* within the gut as described above. Vaccination with the Bacillus cj0113 vector caused an approximate 3 log reduction ($P < 0.05$) in the level of *C. jejuni* present in the ileal samples (Fig. 9).

Vaccination study in Turkey poults

Since the *Salmonella*-vectored Cj0113 vaccine has been effective in reducing *Campylobacter* recovery after challenge in chickens we hypothesized that the vaccine may also work in poults. Therefore, to further evaluate the use of this epitope delivery system, an experiment was designed to test the effectiveness of the vaccine against *C. coli* challenge in turkey poults.

The Δ SE-cj0113 vaccine was constructed as described above. *Salmonella enteritidis* phage type 13A (SE13A) was used as the backbone strain for this vaccine candidate. This isolate was double attenuated by irreversible gene deletions in the *aroA* and *htrA* genes as previously described. Recombinant strains containing these deletions were then modified further to incorporate the cj0113 insert and an immunostimulatory molecule, CD-154 (Δ SE-cj0113). These sequences were integrated as previously described.

In this experiment, 70 poult were obtained from a local hatchery. They were randomly assigned to one of two treatment groups and tagged. Thirty poult were orally gavaged with 10^8 cfu/poult Δ SE-cj0113 and the remaining poult were sham treated with saline. On day 21, poult were challenged with 1.5×10^8 cfu/poult of *C. coli* by oral gavage. Liver, spleen and cecal tonsils were aseptically removed on day 3 (N=10), day 21 (N=10) and day 35 (N=10) for detection of vector recovery by enrichment in tetrathionate broth and plating on Brilliant Green agar. On days 21 and 35, ingesta (N=10) and tissue (N=5) samples were collected for further analysis. Ingesta from the ileum of cultured poult were analyzed using qPCR for enumeration of *C. coli*. Tissue samples from the same region were collected and total RNA was extracted. Interferon-gamma and TNF-alpha like vaccine responses were evaluated.

Results

As previously shown in chickens, the poult developed a significant immune response following vaccination with *Salmonella*-vectored Cj0113 vaccine. After challenge, there was a about a five-log reduction in *Campylobacter coli* in the ileum as compared to vector only controls (Fig. 10). Vaccination with the *Salmonella*-vectored Cj0113 vaccine does appear to work in turkeys similar to the chickens.

CLAIMS

We claim:

1. A vector comprising a first polynucleotide sequence encoding an antigenic polypeptide of SEQ ID NO: 7 (cjaD; cj0113), SEQ ID NO: 8 (cjaA; cj0982) or SEQ ID NO: 9 (ACE 393; cj0420) or a fragment thereof, the first polynucleotide sequence not natively associated with the vector.
5
2. The vector of claim 1, further comprising a second polynucleotide sequence encoding an immunostimulatory polypeptide not natively associated with the vector.
10
3. A vector comprising a first polynucleotide sequence encoding an antigenic polypeptide not natively associated with the vector and a second polynucleotide sequence encoding an immunostimulatory polypeptide, wherein the antigenic polypeptide is a fragment of SEQ ID NO: 1(cjaD; cj0113), SEQ ID NO: 2(cjaAcj0982) or SEQ ID NO: 3(ACE393; cj0420).
15
4. The vector of any one of claims 1-3, wherein the vector is a bacterium.
5. The vector of claim 4, wherein the bacterium is from a genus selected from
20 Salmonella, Escherichia, Bacillus or Lactobacillus.
6. The vector of claim 4, wherein the first polynucleotide sequence and the second polynucleotide sequence are integrated into the genome of the bacterium.
- 25 7. The vector of any one of claims 2-6, wherein the vector comprises more than one copy of the first polynucleotide sequence, more than one copy of the second polynucleotide sequence or more than one copy of the first and second polynucleotide sequence.
8. The vector of any one of claims 2-7, wherein the first polynucleotide sequence is
30 linked in frame to the second polynucleotide sequence.

9. The vector of any one of claims 2-8, wherein the antigenic polypeptide and the immunostimulatory polypeptide are expressed on the surface of the vector.

10. The vector of claim 9, wherein the first polynucleotide sequence and the second
5 polynucleotide sequence are inserted within a third polynucleotide sequence encoding an external portion of a transmembrane protein.

11. The vector of any one of claims 2-10, wherein the immunostimulatory polypeptide is a CD154 polypeptide or an HMGB1 polypeptide.
10

12. The vector of claim 11, wherein the CD154 polypeptide is capable of binding CD40, the CD154 polypeptide having fewer than 50 amino acids and comprising amino acids 140-149 of SEQ ID NO:13 or a homolog thereof.

13. The vector of claim 12, wherein the CD154 polypeptide comprises a polypeptide
15 selected from SEQ ID NOS: 15-19.

14. The vector of claim 11, wherein the HMGB1 polypeptide comprises a polypeptide selected from at least one of SEQ ID NOS: 20-28 or a fragment of at least one of SEQ ID NOS: 20-28.

15. The vector of any one of claims 1-14, wherein the first polynucleotide sequence is
20 SEQ ID NO: 7 or a fragment thereof.

16. The vector of any one of claims 1-15, wherein the first polynucleotide sequence is SEQ ID NO: 8, SEQ ID NO: 9, a fragment of SEQ ID NO: 8, a fragment of SEQ ID NO: 9 or a combination thereof.

17. The vector of any one of claims 1-16, wherein administration of the vector to a
25 subject is capable of eliciting an antibody response.

18. The vector of claim 17, wherein the antibody response is an IgA response.

19. The vector of any one of claims 1-18, wherein the vector is capable of protecting a vaccinated subject from subsequent infection with *Campylobacter* spp..
20. A pharmaceutical composition comprising the vector of any one of claims 1-19 and a pharmaceutically acceptable carrier.
- 5 21. A method of enhancing an immune response in a subject comprising administering the vector of any of claims 1-19 to the subject in an amount effective to enhance the immune response of the subject.
22. The method of claim 21, wherein the immune response includes an enhanced antibody response.
- 10 23. The method of claim 22, wherein the soluble IgA antibody response is enhanced.
24. The method of claim 23, wherein the IgA response after administration of the vector is increased at least three fold relative to the IgA response of a subject administered a control vector.
25. The method of any one of claims 21-23, further comprising challenging the
15 subject with *Campylobacter* spp., wherein the growth of the *Campylobacter* in the subject after challenge is reduced by at 2 logs in subjects administered the vector as compared to subjects administered a control vector.
26. A method of enhancing resistance to *Campylobacter* infection in a subject comprising administering the vector of any of claims 1-19 to the subject in an amount
20 effective to enhance resistance to *Campylobacter* infection after subsequent exposure to *Campylobacter* spp..
27. The method of any one of claims 21-26, wherein the vector is attenuated.
28. The method of claim 27, wherein the vector is killed prior to administration.
29. The method of any one of claims 26-28, wherein resistance is enhanced by
25 increased antibody production.

30. The method of claim 29, wherein the increased antibody response comprises an increased IgA response.

31. The method of any one of claims 26-30, wherein increased resistance is characterized in that subsequent infection with *Campylobacter* spp. results in decreased
5 *Campylobacter* growth as compared to a control.

32. The method of claim 31, wherein *Campylobacter* growth is reduced by at least 2 logs as compared to *Campylobacter* growth after administration of a control vector.

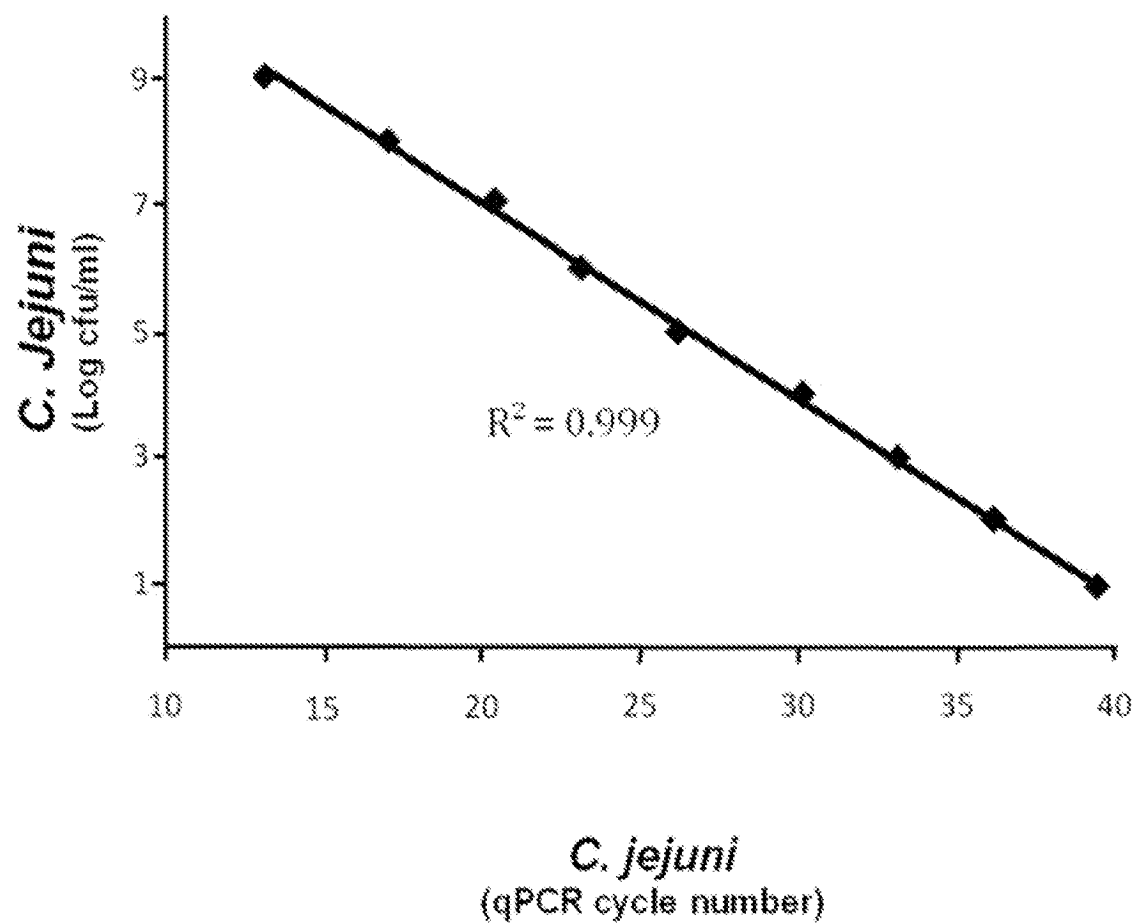


Figure 1.

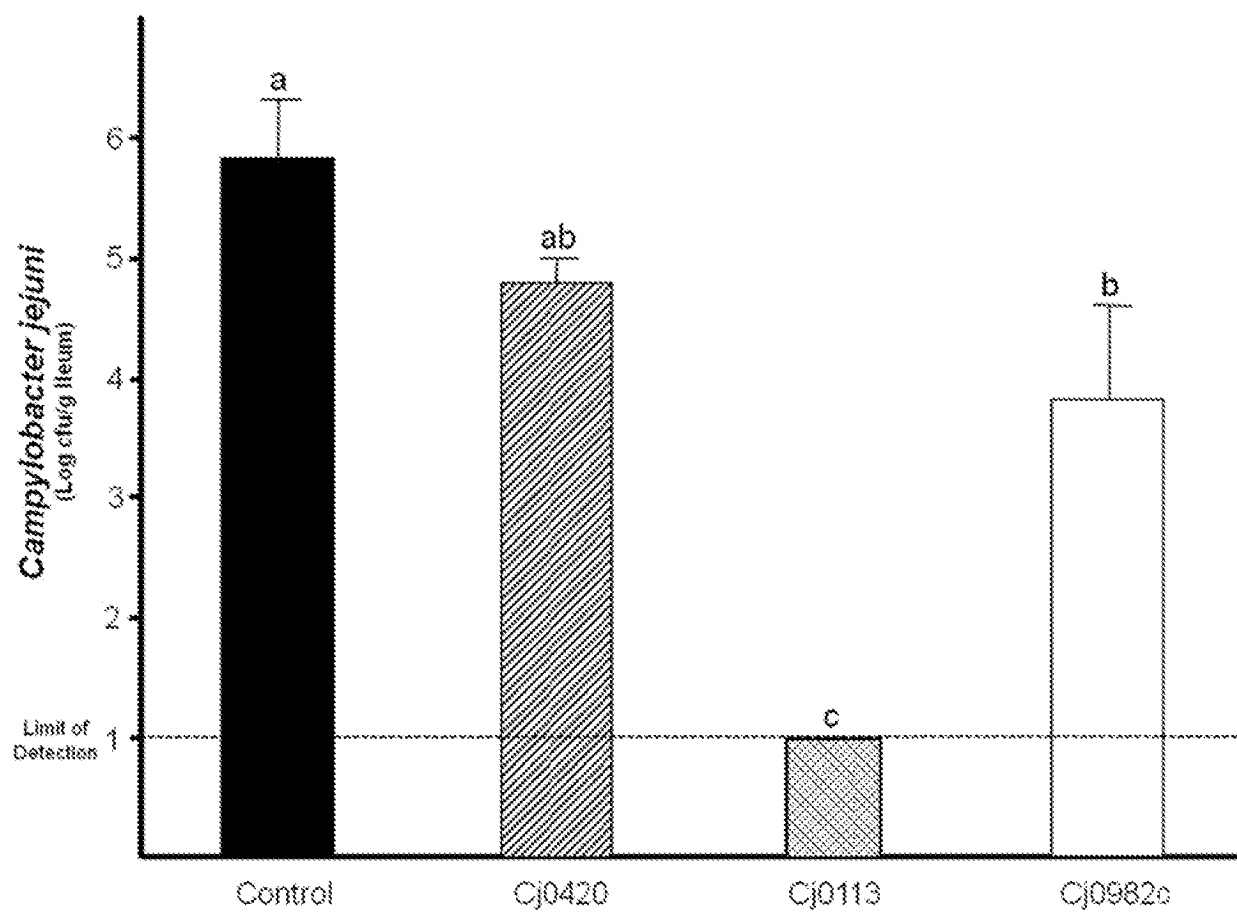


Figure 2.

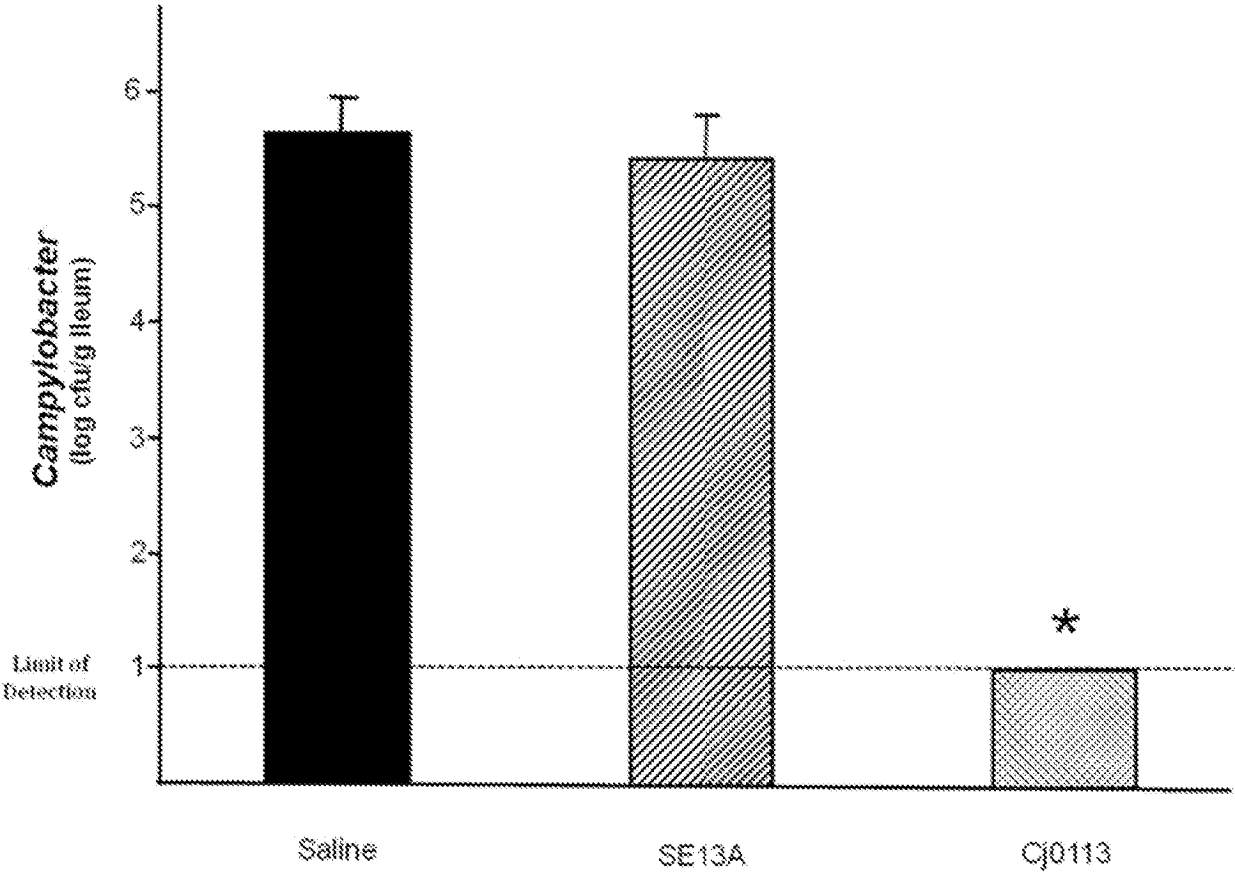


Figure 3.

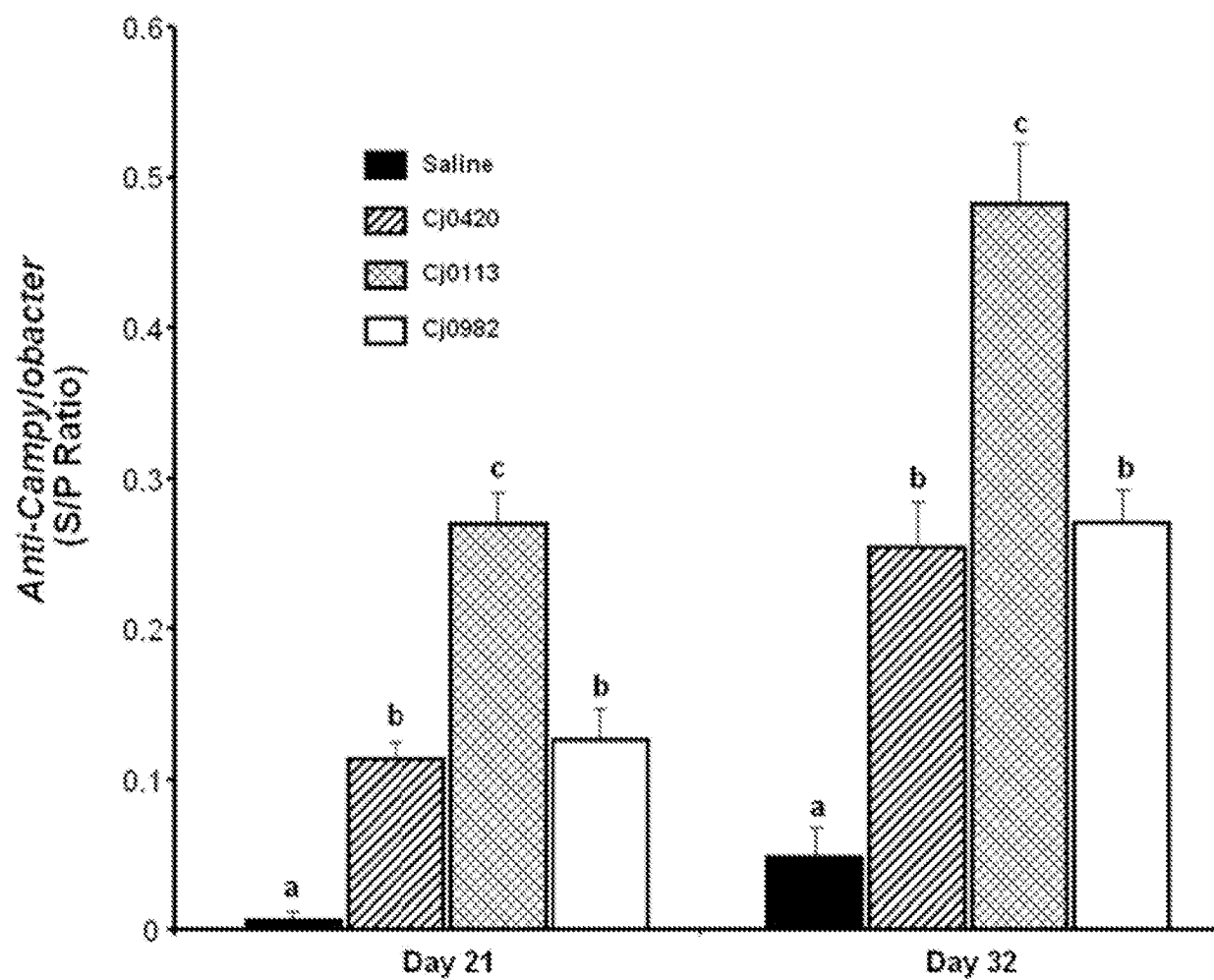


Figure 4.

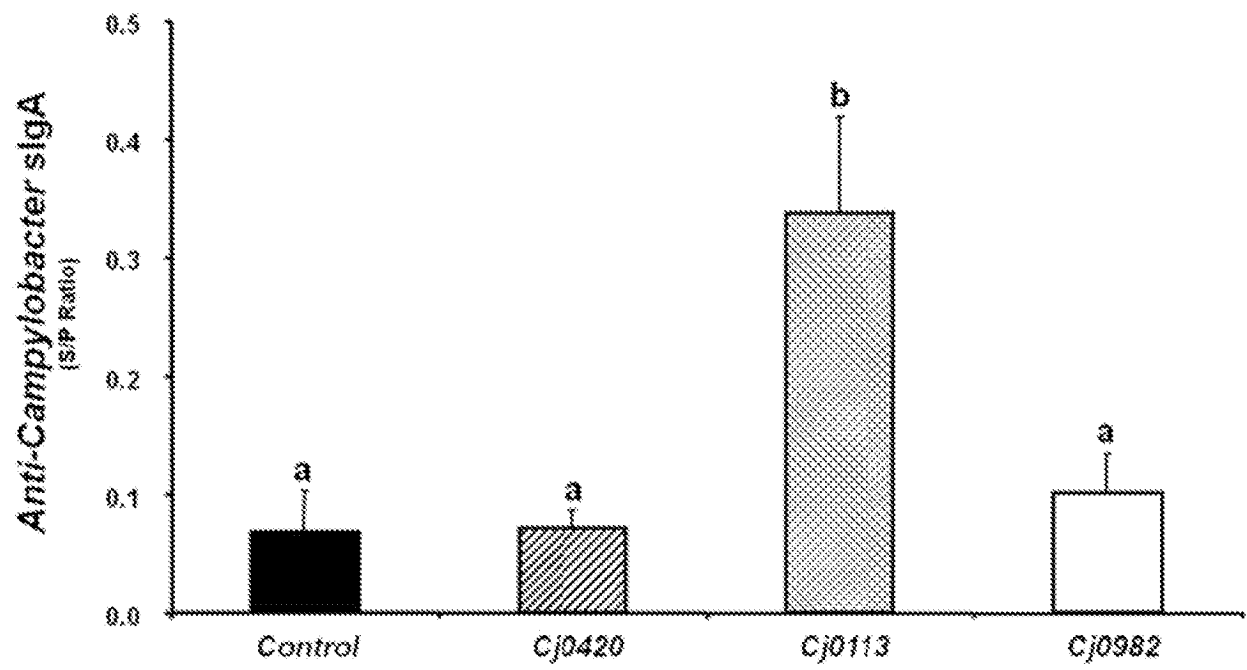


Figure 5.

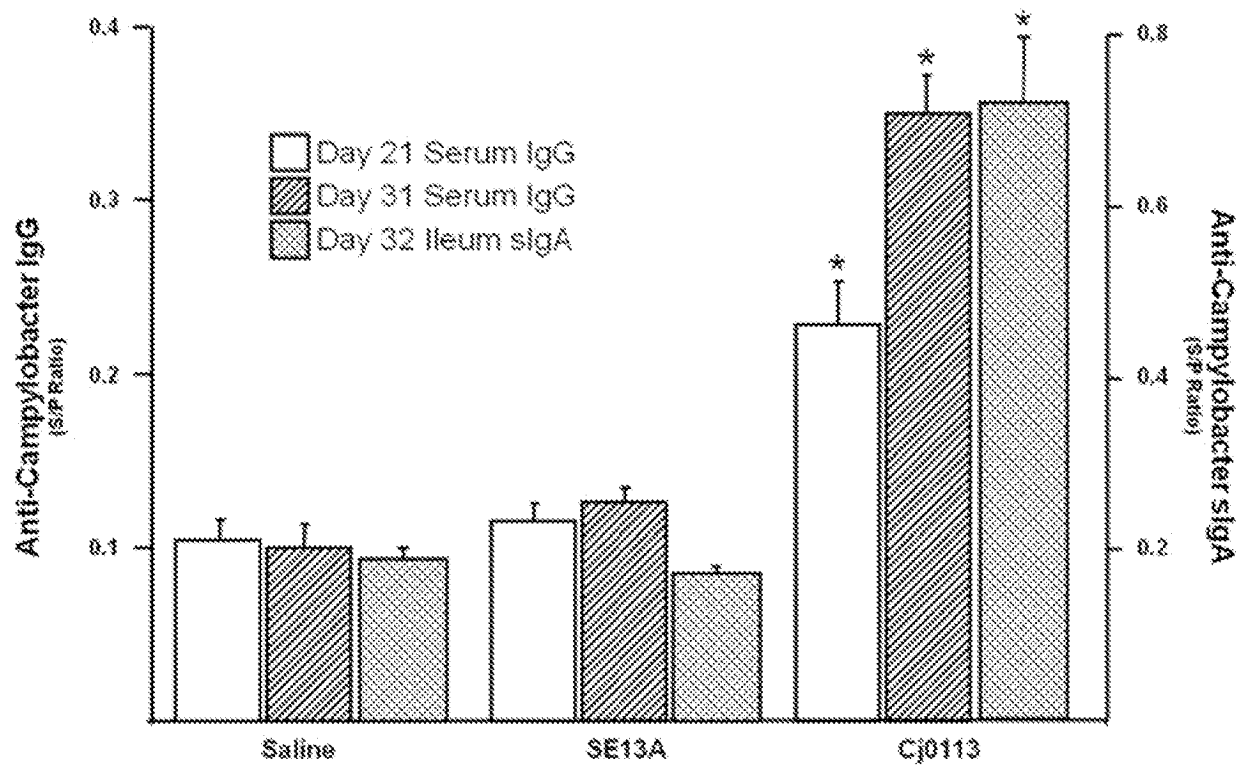


Figure 6.

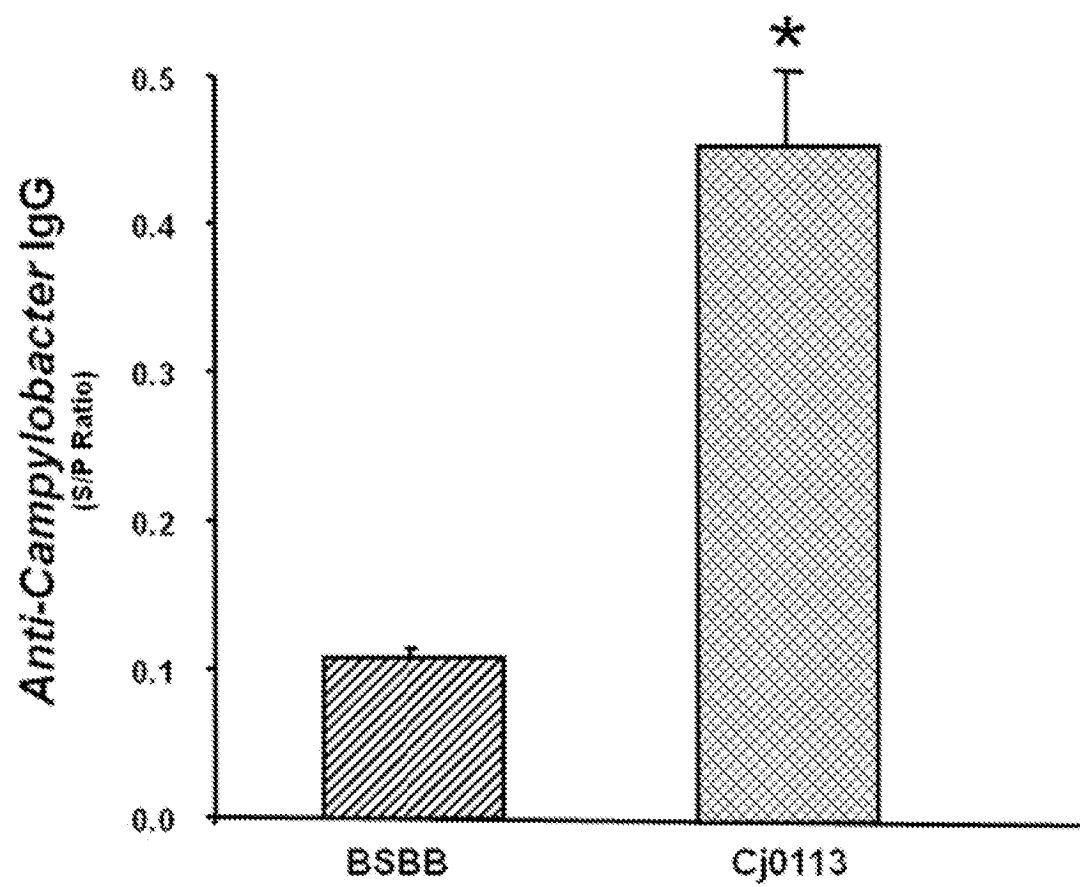


Figure 7.

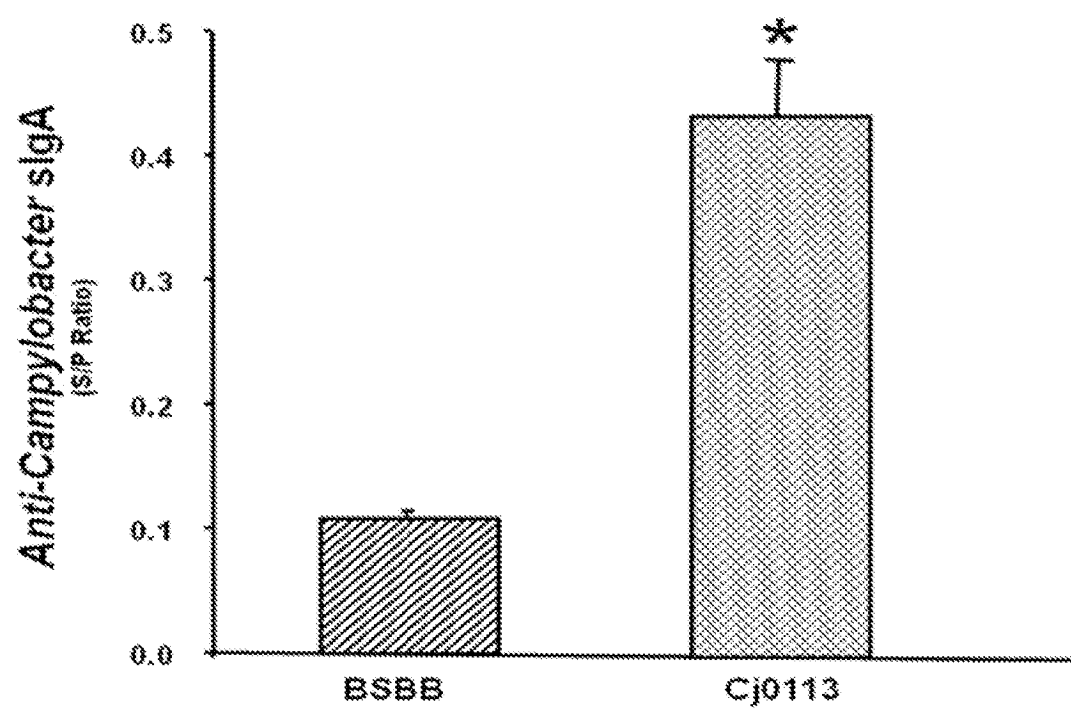


Figure 8.

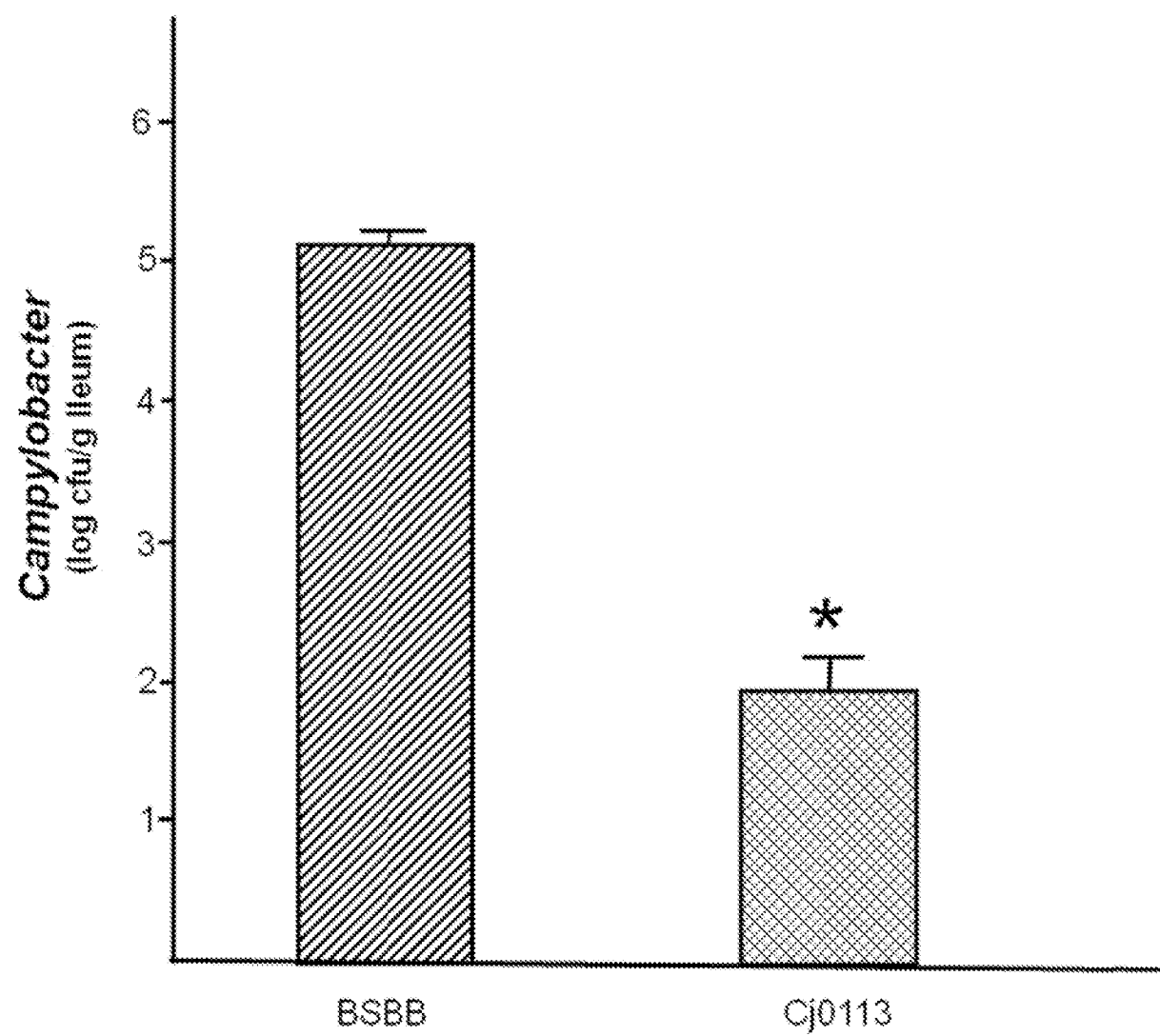


Figure 9.

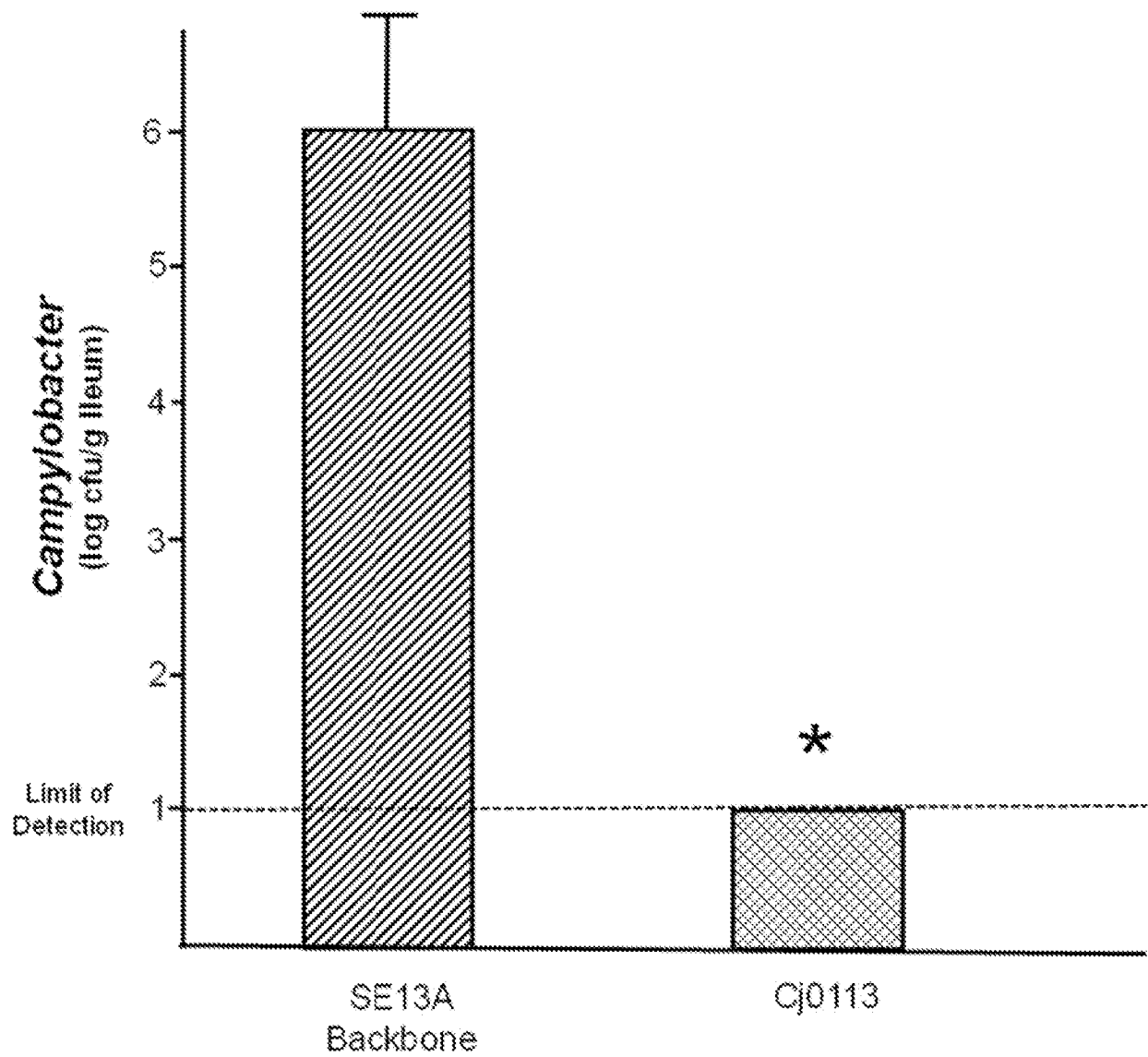


Figure 10.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/39832

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8):- A61K 39/02; C12N 1/20; C12N 15/00 (2011.01)

USPC - 424/200.1, 424/234.1, 424/190.1; 435/252.3; 435/320.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A61K 39/02; C12N 01/20; C12N 15/00 (2011.01)

USPC ? 424/200.1, 424/234.1, 424/190.1; 435/252.3; 435/320.1; 514/44R; 435/69.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

IPC(8) - A61K 39/02; C12N 01/20; C12N 15/00 (2011.01) - see keyword below

USPC ? 424/200.1, 424/234.1, 424/190.1; 435/252.3; 435/320.1; 514/44R; 435/69.3 - see keyword below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWEST(USPT,PGPB,EPAB,JPAB); Medline, Google: Campylobacter jejuni, C. jejuni, cjaD, cjaA; ACE 393, cj0982c, cj0113, cj0420, vector, express, encoding, polynucleotide, polypeptide, vaccine, attenuated, bacteria, Salmonella, Escherichia, Bacillus, Lactobacillus, immunostimulatory, CD154, CD40L, HMGB1, genome, chromosome, integrated, insert, intro

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2007/0128183 A1 (MEINKE et al.) 07 June 2007 (07.06.2007), Abstract, para [0001], [0046], [0047], [0054], [0057], [0098], [0099], [0110], [0122], [0147], [0148], [0149], [0200], [0209], [0218],[0301], and SEQ ID NO: 1209	1 ----- 2-6
Y	US 2010/0112002 A1 (LIEN et al.) 06 May 2010 (06.05.2010), para [0012], [0031], [0051], [0060], [0061], [0064], and [0078]	2-6
A	Skerra, Use of the tetracycline promoter for the tightly regulated production of a murine antibody fragment in Escherichia coli. Gene. 1994, Vol. 151, p.131?135. Abstract [online]. [Retrieved on 2011.11.01]. Retrieved from the Internet: <URL: http://www.ncbi.nlm.nih.gov/pubmed/7828861 > Abstract	1-6
A	WYSZYNSKA et al. Oral immunization of chickens with avirulent Salmonella vaccine strain carrying C. jejuni 72Dz/92 cjaA gene elicits specific humoral immune response associated with protection against challenge with wild-type Campylobacter. Vaccine. 2004, Vol. 22, p. 1379?1389. Entire documentation, especially Abstract; pg 1382, col 1, para 2; pg 1383, col 1, lower para, col 2, lower para, and Fig 1; and pg 1385, col 1	1-6
A	US 2007/0249553 A1 (NEWELL et al.) 25 October 2007 (25.10.2007), para [0001], and SEQ ID NO: 14, which is 100% identical to the claimed SEQ ID NO: 3, and comprises a region between amino acid residues 130-150, that is 100% identical to the claimed SEQ ID NO: 8	1-6
A	UniProt_A3ZI25, CjaA protein, Last modified April 3, 2007 [online]. [Retrieved on 2011.11.01]. Retrieved from the Internet: <URL: http://www.uniprot.org/uniprot/A3ZI25 > the sequence is 100% identical to the claimed SEQ ID NO: 2, and comprises a region between amino acid residues 127-161, that is 100% identical to the claimed SEQ ID NO: 9.	1-6

☒ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

01 November 2011 (01.11.2011)

Date of mailing of the international search report

23 NOV 2011

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/39832

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	LAYTON et al. Evaluation of Salmonella-Vectored Campylobacter Peptide Epitopes for Reduction of Campylobacter jejuni in Broiler Chickens. Clin Vaccine Immunol. 2011, Vol. 18(3), p. 449-54. Epub 2010 Dec 22. Entire documentation, especially Abstract; pg 450, col 1, para 2, and col 2, para 1 and para 3	1-6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/39832

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

a. (means)

☐

on paper

☒

in electronic form

b. (time)

☐

in the international application as filed

☒

together with the international application in electronic form

☐

subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/39832

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 7-32
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

2011-06-09_5658-00102_sequence_Listing
SEQUENCE LISTING

<110> THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ARKANSAS
<120> VACCINE AND METHODS TO REDUCE CAMPYLOBACTER INFECTION
<130> 5658-00102
<150> US 61/353,039
<151> 2010-06-09
<160> 44
<170> PatentIn version 3.5
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<213> Campylobacter jejuni cjaD
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20 25 30
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35 40 45
Lys Ile Ser Gln Leu Asn Asp Thr Leu Asn Lys Val Tyr Phe Asp Phe
50 55 60
Asp Lys Phe Asn Ile Arg Pro Asp Met Gln Asn Val Val Ser Thr Asn
65 70 75 80
Ala Asn Ile Phe Asn Thr Glu Val Ser Gly Val Ser Ile Thr Val Glu
85 90 95
Gly Asn Cys Asp Glu Trp Gly Thr Asp Glu Tyr Asn Gln Ala Leu Gly
100 105 110
Leu Lys Arg Ala Lys Ala Val Lys Glu Ala Leu Ile Ala Lys Gly Val
115 120 125
Asn Ala Asp Arg Ile Ala Val Lys Ser Tyr Gly Glu Thr Asn Pro Val
130 135 140
Cys Thr Glu Lys Thr Lys Ala Cys Asp Ala Gln Asn Arg Arg Ala Glu
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Phe Lys Leu Ser Arg

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<210> 2
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<400> 2

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 20 25 30

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 35 40 45

Lys Pro Pro Phe Gly Tyr Val Asp Glu Lys Gly Asn Asn Gln Gly Tyr
 50 55 60

Asp Ile Ala Leu Ala Lys Arg Ile Ala Lys Glu Leu Phe Gly Asp Glu
 65 70 75 80

Asn Lys Val Gln Phe Val Leu Val Glu Ala Ala Asn Arg Val Glu Phe
 85 90 95

Leu Lys Ser Asn Lys Val Asp Ile Ile Leu Ala Asn Phe Thr Gln Thr
 100 105 110

Pro Gln Arg Ala Glu Gln Val Asp Phe Cys Ser Pro Tyr Met Lys Val
 115 120 125

Ala Leu Gly Val Ala Val Pro Lys Asp Ser Asn Ile Thr Ser Val Glu
 130 135 140

Asp Leu Lys Asp Lys Thr Leu Leu Leu Asn Lys Gly Thr Thr Ala Asp
 145 150 155 160

Ala Tyr Phe Thr Gln Asn Tyr Pro Asn Ile Lys Thr Leu Lys Tyr Asp
 165 170 175

Gln Asn Thr Glu Thr Phe Ala Ala Leu Met Asp Lys Arg Gly Asp Ala
 180 185 190

Leu Ser His Asp Asn Thr Leu Leu Phe Ala Trp Val Lys Asp His Pro
 195 200 205

Asp Phe Lys Met Gly Ile Lys Glu Leu Gly Asn Lys Asp Val Ile Ala
 Page 2

210

Pro Ala Val Lys Lys Gly Asp Lys Glu Leu Lys Glu Phe Ile Asp Asn
225 230 235 240

Leu Ile Ile Lys Leu Gly Gln Glu Gln Phe Phe His Lys Ala Tyr Asp
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Glu Thr Leu Lys Ala His Phe Gly Asp Asp Val Lys Ala Asp Asp Val
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<210> 3
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<212> PRT
<213> Campylobacter jejuni ACE 393

<400> 3

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20 25 30

Val Gly Phe Lys Ile Lys His Leu Gln Ile Ser Asn Val Lys Gly Asn
35 40 45

Phe Lys Asp Tyr Ser Ala Val Ile Asp Phe Asp Pro Ala Ser Ala Glu
50 55 60

Phe Lys Lys Leu Asp Val Thr Ile Lys Ile Ala Ser Val Asn Thr Glu
65 70 75 80

Asn Gln Thr Arg Asp Asn His Leu Gln Gln Asp Asp Phe Phe Lys Ala
85 90 95

Lys Lys Tyr Pro Asp Met Thr Phe Thr Met Lys Lys Tyr Glu Lys Ile
100 105 110

Asp Asn Glu Lys Gly Lys Met Thr Gly Thr Leu Thr Ile Ala Gly Val
115 120 125

Ser Lys Asp Ile Val Leu Asp Ala Glu Ile Gly Gly Val Ala Lys Gly
130 135 140

Lys Asp Gly Lys Glu Lys Ile Gly Phe Ser Leu Asn Gly Lys Ile Lys
Page 3

145

150

155

160

Arg Ser Asp Phe Lys Phe Ala Thr Ser Thr Ser Thr Ile Thr Leu Ser
165 170 175

Asp Asp Ile Asn Leu Asn Ile Glu Val Glu Ala Asn Glu Lys
180 185 190

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tcctggatga ccacctccta tgcgccgacc tcctcctcct cc 162

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<213> Campylobacter jejuni ACE 393

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Glu Tyr Asn Gln Ala
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<210> 8
<211> 21

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<212> PRT

<213> Campylobacter jejuni Cj0982

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1 5 10 15

Asp Gly Lys Glu Lys
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<210> 9

<211> 35

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<213> Campylobacter jejuni Cj0420

<400> 9

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Val Glu Asp Leu Lys Asp Lys Thr Leu Leu Leu Asn Lys Gly Thr Thr
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Ala Asp Ala
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<211> 31

<212> PRT

<213> Artificial Sequence

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<223> Synthetic peptide: Cj0113-CD154

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Glu Tyr Asn Gln Ala Trp Met Thr Thr Ser Tyr Ala Pro Thr Ser
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<210> 11

<211> 31

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide: Cj0982-CD154

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Asp Gly Lys Glu Lys Trp Met Thr Thr Ser Tyr Ala Pro Thr Ser
20 25 30

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<212> PRT
<213> Artificial Sequence

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1 5 10 15

Val Glu Asp Leu Lys Asp Lys Thr Leu Leu Leu Asn Lys Gly Thr Thr
20 25 30

Ala Asp Ala Trp Met Thr Thr Ser Tyr Ala Pro Thr Ser
35 40 45

<210> 13
<211> 272
<212> PRT
<213> Gallus gallus

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<221> misc_feature
<223> CD154 chicken

<400> 13

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1 5 10 15

Ser Pro Ser Thr Met Lys Met Phe Met Cys Phe Leu Ser Val Phe Met
20 25 30

Val Val Gln Thr Ile Gly Thr Val Leu Phe Cys Leu Tyr Leu His Met
35 40 45

Lys Met Asp Lys Met Glu Glu Val Leu Ser Leu Asn Glu Asp Tyr Ile
50 55 60

Phe Leu Arg Lys Val Gln Lys Cys Gln Thr Gly Glu Asp Gln Lys Ser
65 70 75 80

Thr Leu Leu Asp Cys Glu Lys Val Leu Lys Gly Phe Gln Asp Leu Gln
85 90 95

2011-06-09_5658-00102_Sequence_Listing

Cys Lys Asp Arg Thr Ala Ser Glu Glu Leu Pro Lys Phe Glu Met His
100 105 110

Arg Gly His Glu His Pro His Leu Lys Ser Arg Asn Glu Thr Ser Val
115 120 125

Ala Glu Glu Lys Arg Gln Pro Ile Ala Thr His Leu Ala Gly Val Lys
130 135 140

Ser Asn Thr Thr Val Arg Val Leu Lys Trp Met Thr Thr Ser Tyr Ala
145 150 155 160

Pro Thr Ser Ser Leu Ile Ser Tyr His Glu Gly Lys Leu Lys Val Glu
165 170 175

Lys Ala Gly Leu Tyr Tyr Ile Tyr Ser Gln Val Ser Phe Cys Thr Lys
180 185 190

Ala Ala Ala Ser Ala Pro Phe Thr Leu Tyr Ile Tyr Leu Tyr Leu Pro
195 200 205

Met Glu Glu Asp Arg Leu Leu Met Lys Gly Leu Asp Thr His Ser Thr
210 215 220

Ser Thr Ala Leu Cys Glu Leu Gln Ser Ile Arg Glu Gly Gly Val Phe
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Glu Leu Arg Gln Gly Asp Met Val Phe Val Asn Val Thr Asp Ser Thr
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<221> misc_feature
<223> CD154 human

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20 25 30

2011-06-09_5658-00102_Sequence_Listing

Ile Thr Gln Met Ile Gly Ser Ala Leu Phe Ala Val Tyr Leu His Arg
35 40 45

Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp Phe Val
50 55 60

Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser Leu Ser
65 70 75 80

Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe Val Lys
85 90 95

Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser Phe Glu
100 105 110

Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser
115 120 125

Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly
130 135 140

Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys Gln
145 150 155 160

Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val Thr
165 170 175

Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile Ala Ser
180 185 190

Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu Arg Ala
195 200 205

Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser Ile His
210 215 220

Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe Val Asn
225 230 235 240

Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr Ser Phe
245 250 255

Gly Leu Leu Lys Leu
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<210> 15

<211> 11

2011-06-09_5658-00102_Sequence_Listing

<212> PRT
<213> Homo sapiens

<220>
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<223> CD154 partial human

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<210> 16
<211> 11
<212> PRT
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1 5 10

<210> 17
<211> 10
<212> PRT
<213> Anas sp.

<220>
<221> misc_feature
<223> Duck CD154 peptide

<400> 17

Trp Asn Lys Thr Ser Tyr Ala Pro Met Asn
1 5 10

<210> 18
<211> 10
<212> PRT
<213> Mus sp.

<220>
<221> misc_feature
<223> Mouse CD154 peptide

<400> 18

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1 5 10

<210> 19

2011-06-09_5658-00102_Sequence_Listing

<211> 10
<212> PRT
<213> Bos taurus

<220>
<221> misc_feature
<223> Cow CD154 peptide

<400> 19

Trp Ala Pro Lys Gly Tyr Tyr Thr Leu Ser
1 5 10

<210> 20
<211> 190
<212> PRT
<213> Gallus gallus

<220>
<221> misc_feature
<223> Chicken HMGB1 amino acid

<400> 20

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1 5 10 15

Ala Phe Phe Val Gln Thr Cys Arg Glu Glu His Lys Lys Lys His Pro
20 25 30

Asp Ala Ser Val Asn Phe Ser Glu Phe Ser Lys Lys Cys Ser Glu Arg
35 40 45

Trp Lys Thr Met Ser Ser Lys Glu Lys Gly Lys Phe Glu Asp Met Ala
50 55 60

Lys Ala Asp Lys Leu Arg Tyr Glu Lys Glu Met Lys Asn Tyr Val Pro
65 70 75 80

Pro Lys Gly Glu Thr Lys Lys Lys Phe Lys Asp Pro Asn Ala Pro Lys
85 90 95

Arg Pro Pro Ser Ala Phe Phe Leu Phe Cys Ser Glu Phe Arg Pro Lys
100 105 110

Ile Lys Gly Glu His Pro Gly Leu Ser Ile Gly Asp Val Ala Lys Lys
115 120 125

Leu Gly Glu Met Trp Asn Asn Thr Ala Ala Asp Asp Lys Gln Pro Tyr
130 135 140

2011-06-09_5658-00102_Sequence_Listing

Glu Lys Lys Ala Ala Lys Leu Lys Glu Lys Tyr Glu Lys Asp Ile Ala
145 150 155 160

Ala Tyr Arg Ala Lys Gly Lys Val Asp Ala Gly Lys Lys Val Val Ala
165 170 175

Lys Ala Glu Lys Ser Lys Lys Lys Lys Glu Glu Glu Glu Asp
180 185 190

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<211> 85
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<220>
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20 25 30

Asp Ala Ser Val Asn Phe Ser Glu Phe Ser Lys Lys Cys Ser Glu Arg
35 40 45

Trp Lys Thr Met Ser Ser Lys Glu Lys Gly Lys Phe Glu Asp Met Ala
50 55 60

Lys Ala Asp Lys Leu Arg Tyr Glu Lys Glu Met Lys Asn Tyr Val Pro
65 70 75 80

Pro Lys Gly Glu Thr
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<210> 22
<211> 54
<212> PRT
<213> Artificial Sequence

<220>
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<400> 22

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1 5 10 15

Arg Trp Lys Thr Met Ser Ser Lys Glu Lys Gly Lys Phe Glu Asp Met
20 25 30

2011-06-09_5658-00102_Sequence_Listing

Ala Lys Ala Asp Lys Leu Arg Tyr Glu Lys Glu Met Lys Asn Tyr Val
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Pro Pro Lys Gly Glu Thr
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<400> 23

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Cys Ser Glu Phe Arg Pro Lys Ile Lys Gly Glu His Pro Gly Leu Ser
 20 25 30

Ile Gly Asp Val Ala Lys Lys Leu Gly Glu Met Trp Asn Asn Thr Ala
 35 40 45

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 50 55 60

Lys Tyr Glu Lys Asp Ile Ala Ala Tyr
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 <211> 69
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 <213> Artificial Sequence

<220>
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 20 25 30

Val Ala Lys Lys Leu Gly Glu Met Trp Asn Asn Thr Ala Ala Asp Asp
 35 40 45

Lys Gln Pro Tyr Glu Lys Lys Ala Ala Lys Leu Lys Glu Lys Tyr Glu
 50 55 60

2011-06-09_5658-00102_Sequence_Listing

Lys Asp Ile Ala Ala
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<210> 25
<211> 21
<212> PRT
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<220>
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<400> 25

Lys Asp Pro Asn Ala Pro Lys Arg Pro Pro Ser Ala Phe Phe Leu Phe
1 5 10 15

Cys Ser Glu Phe Arg
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<210> 26
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide: HMGB1 proinflammatory cytokine activity
<400> 26

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Lys Val Asp Ala Gly Lys Lys Val Val Ala Lys Ala Glu Lys Ser Lys
20 25 30

Lys

<210> 27
<211> 215
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> HMGB1

<400> 27

Met Gly Lys Gly Asp Pro Lys Lys Pro Arg Gly Lys Met Ser Ser Tyr
1 5 10 15

Ala Phe Phe Val Gln Thr Cys Arg Glu Glu His Lys Lys Lys His Pro
Page 13

20

25

30

Asp Ala Ser Val Asn Phe Ser Glu Phe Ser Lys Lys Cys Ser Glu Arg
 35 40 45

Trp Lys Thr Met Ser Ala Lys Glu Lys Gly Lys Phe Glu Asp Met Ala
 50 55 60

Lys Ala Asp Lys Ala Arg Tyr Glu Arg Glu Met Lys Thr Tyr Ile Pro
 65 70 75 80

Pro Lys Gly Glu Thr Lys Lys Lys Phe Lys Asp Pro Asn Ala Pro Lys
 85 90 95

Arg Pro Pro Ser Ala Phe Phe Leu Phe Cys Ser Glu Tyr Arg Pro Lys
 100 105 110

Ile Lys Gly Glu His Pro Gly Leu Ser Ile Gly Asp Val Ala Lys Lys
 115 120 125

Leu Gly Glu Met Trp Asn Asn Thr Ala Ala Asp Asp Lys Gln Pro Tyr
 130 135 140

Glu Lys Lys Ala Ala Lys Leu Lys Glu Lys Tyr Glu Lys Asp Ile Ala
 145 150 155 160

Ala Tyr Arg Ala Lys Gly Lys Pro Asp Ala Ala Lys Lys Gly Val Val
 165 170 175

Lys Ala Glu Lys Ser Lys Lys Lys Lys Glu Glu Glu Glu Asp Glu Glu
 180 185 190

Asp Glu Glu Asp Glu Glu Glu Glu Glu Asp Glu Glu Asp Glu Asp Glu
 195 200 205

Glu Glu Asp Asp Asp Asp Glu
 210 215

<210> 28
 <211> 205
 <212> PRT
 <213> Danio rerio

<220>
 <221> misc_feature
 <223> Zebra fish HMGB1

<400> 28

2011-06-09_5658-00102_Sequence_Listing

Met Gly Lys Asp Pro Thr Lys Pro Arg Gly Lys Met Ser Ser Tyr Ala
1 5 10 15

Tyr Phe Val Gln Thr Cys Arg Glu Glu His Lys Lys Lys His Pro Glu
20 25 30

Ala Thr Val Asn Phe Ser Glu Phe Ser Lys Lys Cys Ser Glu Arg Trp
35 40 45

Lys Thr Met Ser Ala Lys Glu Lys Gly Lys Phe Glu Asp Met Ala Lys
50 55 60

Leu Asp Lys Ala Arg Tyr Glu Arg Glu Met Lys Asn Tyr Ile Pro Pro
65 70 75 80

Lys Gly Glu Lys Lys Lys Arg Phe Lys Asp Pro Asn Ala Pro Lys Arg
85 90 95

Pro Pro Ser Ala Phe Phe Ile Phe Cys Ser Glu Phe Arg Pro Lys Val
100 105 110

Lys Glu Glu Thr Pro Gly Leu Ser Ile Gly Asp Val Ala Lys Arg Leu
115 120 125

Gly Glu Met Trp Asn Lys Ile Ser Ser Glu Glu Lys Gln Pro Tyr Glu
130 135 140

Lys Lys Ala Ala Lys Leu Lys Glu Lys Tyr Glu Lys Asp Ile Ala Ala
145 150 155 160

Tyr Arg Ser Lys Gly Lys Val Gly Gly Gly Ala Ala Lys Ala Pro Ser
165 170 175

Lys Pro Asp Lys Ala Asn Asp Glu Asp Glu Asp Asp Asp Glu Glu Glu
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Asp Glu Asp Asp Asp Asp Glu Glu Glu Glu Asp Asp Glu
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<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic primer: 1am-up-f; Loop 9 up

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2011-06-09_5658-00102_Sequence_Listing

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<223> Synthetic primer: lam-up-r; Loop 9 up

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<212> DNA
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<210> 32
<211> 20
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<210> 33
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39

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2011-06-09_5658-00102_Sequence_Listing

<210> 35
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Synthetic primer: Kan4f; Inside Kmr gene

 <400> 35
 caaaagcgct ctgaagttcc 20

 <210> 36
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Synthetic primer: Kan4r; Inside Kmr gene

 <400> 36
 gcgtgagggg atcttgaagt 20

 <210> 37
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Synthetic primer: lam 3f; Outer regions of loop 9

 <400> 37
 gccatctcgc ttggtgataa 20

 <210> 38
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Synthetic primer: lam 3r; Outer regions of loop 9

 <400> 38
 cgctggtatt ttgcgtaca 20

 <210> 39
 <211> 84
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Synthetic primer: Cj0113f; Insert with loop 9 up

 <400> 39
 ttcacggtta cccattcat cacagttacc ttcaacggtg atgctaacac cggaggagga 60
 gttatcgccg tctttgatat agcc 84

 <210> 40

2011-06-09_5658-00102_Sequence_Listing

<211> 100
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic primer: Cj0113r; Insert with loop 9 down

<400> 40
 atgaatgggg taccgatgaa tataaccagg cgtcctcctc ctggatgacc acctcctatg 60
 cgccgacctc ctctcctcc atttcccggt atgccgcagc 100

<210> 41
 <211> 84
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic primer: Cj0420f; Insert with loop 9 up

<400> 41
 atctttacct ttcgcaacac caccgatttc cgcattcaga acgatatctt tggaggagga 60
 gttatcgccg tctttgatat agcc 84

<210> 42
 <211> 100
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic primer: Cj0420r; Insert with loop 9 down

<400> 42
 gtgttgcgaa aggtaaagat ggtaaagaaa aatcctcctc ctggatgacc acctcctatg 60
 cgccgacctc ctctcctcc atttcccggt atgccgcagc 100

<210> 43
 <211> 105
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic primer: Cj0982c-f; Insert with loop 9 up

<400> 43
 ggttttatct ttcagatctt caacgctggt gatgttgcta tctttcgga cgcgaacacc 60
 cagcgcaact ttggaggagg agttatcgcc gtctttgata tagcc 105

<210> 44
 <211> 121
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic primer: Cj0982c-r; Insert with loop 9 down

2011-06-09_5658-00102_Sequence_Listing

<400> 44

aagatctgaa agataaaacc ctgctgctga acaaaggtag caccgcggat gcgtcctcct 60

cctggatgac cacctcctat gcgccgacct cctcctcctc catttcccgt tatgccgcag 120

c 121

LISTADO DE SECUENCIAS

<110> THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ARKANSAS
 <120> VACUNA Y MÉTODOS PARA REDUCIR UNA INFECCIÓN POR *CAMPYLOBACTER*
 <130> 5658-00103
 <150> US 61/353.039
 <151> 2010-06-09
 <160> 44
 <170> PatentIn versión 3.5
 <210> 1
 <211> 165
 <212> PRT
 <213> cjaD de *Campylobacter jejuni*

<400> 1

Met Lys Lys Ile Leu Phe Thr Ser Ile Ala Ala Leu Ala Val Val Ile
 1 5 10 15

Ser Gly Cys Ser Thr Lys Ser Thr Ser Val Ser Gly Asp Ser Ser Val
 20 25 30

Asp Ser Asn Arg Gly Ser Gly Gly Ser Asp Gly Trp Asp Ile Asp Ser
 35 40 45

Lys Ile Ser Gln Leu Asn Asp Thr Leu Asn Lys Val Tyr Phe Asp Phe
 50 55 60

Asp Lys Phe Asn Ile Arg Pro Asp Met Gln Asn Val Val Ser Thr Asn
 65 70 75 80

Ala Asn Ile Phe Asn Thr Glu Val Ser Gly Val Ser Ile Thr Val Glu
 85 90 95

Gly Asn Cys Asp Glu Trp Gly Thr Asp Glu Tyr Asn Gln Ala Leu Gly
 100 105 110

Leu Lys Arg Ala Lys Ala Val Lys Glu Ala Leu Ile Ala Lys Gly Val
 115 120 125

Asn Ala Asp Arg Ile Ala Val Lys Ser Tyr Gly Glu Thr Asn Pro Val
 130 135 140

Cys Thr Glu Lys Thr Lys Ala Cys Asp Ala Gln Asn Arg Arg Ala Glu
 145 150 155 160

Phe Lys Leu Ser Arg
 165

<210> 2
 <211> 279
 <212> PRT
 <213> cjaA de *Campylobacter jejuni*

<400> 2

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Met Lys Lys Ile Leu Leu Ser Val Leu Thr Ala Phe Val Ala Val Val
1      5      10
Leu Ala Ala Cys Gly Gly Asn Ser Asp Ser Lys Thr Leu Asn Ser Leu
20      25      30
Asp Lys Ile Lys Gln Asn Gly Val Val Arg Ile Gly Val Phe Gly Asp
35      40      45
Lys Pro Pro Phe Gly Tyr Val Asp Glu Lys Gly Asn Asn Gln Gly Tyr
50      55      60
Asp Ile Ala Leu Ala Lys Arg Ile Ala Lys Glu Leu Phe Gly Asp Glu
65      70      75      80
Asn Lys Val Gln Phe Val Leu Val Glu Ala Ala Asn Arg Val Glu Phe
85      90      95
Leu Lys Ser Asn Lys Val Asp Ile Ile Leu Ala Asn Phe Thr Gln Thr
100     105     110
Pro Gln Arg Ala Glu Gln Val Asp Phe Cys Ser Pro Tyr Met Lys Val
115     120     125
Ala Leu Gly Val Ala Val Pro Lys Asp Ser Asn Ile Thr Ser Val Glu
130     135     140
Asp Leu Lys Asp Lys Thr Leu Leu Leu Asn Lys Gly Thr Thr Ala Asp
145     150     155     160
Ala Tyr Phe Thr Gln Asn Tyr Pro Asn Ile Lys Thr Leu Lys Tyr Asp
165     170     175
Gln Asn Thr Glu Thr Phe Ala Ala Leu Met Asp Lys Arg Gly Asp Ala
180     185     190
Leu Ser His Asp Asn Thr Leu Leu Phe Ala Trp Val Lys Asp His Pro
195     200     205
Asp Phe Lys Met Gly Ile Lys Glu Leu Gly Asn Lys Asp Val Ile Ala
210     215     220
Pro Ala Val Lys Lys Gly Asp Lys Glu Leu Lys Glu Phe Ile Asp Asn
225     230     235     240
Leu Ile Ile Lys Leu Gly Gln Glu Gln Phe Phe His Lys Ala Tyr Asp
245     250     255
Glu Thr Leu Lys Ala His Phe Gly Asp Asp Val Lys Ala Asp Asp Val
260     265     270
Val Ile Glu Gly Gly Lys Ile
275

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<210> 3

<211> 190

<212> PRT

<213> ACE 393 de *Campylobacter jejuni*

<400> 3

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Met Lys Lys Val Leu Leu Ser Ser Leu Val Ala Val Ser Leu Leu Ser
1          5          10          15

Thr Gly Leu Phe Ala Lys Glu Tyr Thr Leu Asp Lys Ala His Thr Asp
20          25          30

Val Gly Phe Lys Ile Lys His Leu Gln Ile Ser Asn Val Lys Gly Asn
35          40          45

Phe Lys Asp Tyr Ser Ala Val Ile Asp Phe Asp Pro Ala Ser Ala Glu
50          55          60

Phe Lys Lys Leu Asp Val Thr Ile Lys Ile Ala Ser Val Asn Thr Glu
65          70          75          80

Asn Gln Thr Arg Asp Asn His Leu Gln Gln Asp Asp Phe Phe Lys Ala
85          90          95

Lys Lys Tyr Pro Asp Met Thr Phe Thr Met Lys Lys Tyr Glu Lys Ile
100         105         110

Asp Asn Glu Lys Gly Lys Met Thr Gly Thr Leu Thr Ile Ala Gly Val
115         120         125

Ser Lys Asp Ile Val Leu Asp Ala Glu Ile Gly Gly Val Ala Lys Gly
130         135         140

Lys Asp Gly Lys Glu Lys Ile Gly Phe Ser Leu Asn Gly Lys Ile Lys
145         150         155         160

Arg Ser Asp Phe Lys Phe Ala Thr Ser Thr Ser Thr Ile Thr Leu Ser
165         170         175

Asp Asp Ile Asn Leu Asn Ile Glu Val Glu Ala Asn Glu Lys
180         185         190

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<210> 4

<211> 120

<212> ADN

<213> cjaD de *Campylobacter jejuni*

<400> 4

tcctcctccg gtgtagcat caccgttgaa ggtaactgtg atgaatgggg taccgatgaa 60

tataaccagg cgtcctcctc ctggatgacc acctcctatg cgccgacctc ctctcctcc 120

<210> 5

<211> 162

<212> ADN

<213> cjaA de *Campylobacter jejuni*

<400> 5

tcctcctcca aagttgcgct ggggtgttgcg gttccgaaag atagcaacat caccagcggt 60

gaagatctga aagataaaac cctgctgctg aacaaaggta ccaccgcgga tgcgtcctcc 120

tcctggatga ccacctccta tgcgccgacc tcctcctcct cc 162

<210> 6

<211> 120

<212> ADN

<213> ACE 393 de *Campylobacter jejuni*

<400> 6

tcctcctcca aagatatcgt tctggatgcg gaaatcggtg gtgttgcgaa aggtaaagat 60

ggtaaagaaa aatcctcctc ctggatgacc acctcctatg cgccgacctc ctcctcctcc 120

<210> 7

<211> 21

<212> PRT

<213> Cj0113 de *Campylobacter jejuni*

<400> 7

Gly Val Ser Ile Thr Val Glu Gly Asn Cys Asp Glu Trp Gly Thr Asp
1 5 10 15

Glu Tyr Asn Gln Ala
20

<210> 8

<211> 21

<212> PRT

<213> Cj0982 de *Campylobacter jejuni*

<400> 8

Lys Asp Ile Val Leu Asp Ala Glu Ile Gly Gly Val Ala Lys Gly Lys
1 5 10 15

Asp Gly Lys Glu Lys
20

<210> 9

<211> 35

<212> PRT

<213> Cj0420 de *Campylobacter jejuni*

<400> 9

Lys Val Ala Leu Gly Val Ala Val Pro Lys Asp Ser Asn Ile Thr Ser
1 5 10 15

Val Glu Asp Leu Lys Asp Lys Thr Leu Leu Leu Asn Lys Gly Thr Thr
20 25 30

Ala Asp Ala
35

<210> 10

<211> 31

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético: Cj0113-CD154

<400> 10

Gly Val Ser Ile Thr Val Glu Gly Asn Cys Asp Glu Trp Gly Thr Asp
1 5 10 15

Glu Tyr Asn Gln Ala Trp Met Thr Thr Ser Tyr Ala Pro Thr Ser
20 25 30

<210> 11

<211> 31

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético: Cj0982-CD154

<400> 11

Lys Asp Ile Val Leu Asp Ala Glu Ile Gly Gly Val Ala Lys Gly Lys
1 5 10 15

Asp Gly Lys Glu Lys Trp Met Thr Thr Ser Tyr Ala Pro Thr Ser
20 25 30

<210> 12

<211> 45

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético: Cj0420-CD154

<400> 12

Lys Val Ala Leu Gly Val Ala Val Pro Lys Asp Ser Asn Ile Thr Ser
1 5 10 15

Val Glu Asp Leu Lys Asp Lys Thr Leu Leu Leu Asn Lys Gly Thr Thr
20 25 30

Ala Asp Ala Trp Met Thr Thr Ser Tyr Ala Pro Thr Ser
35 40 45

<210> 13

<211> 272

<212> PRT

<213> *Gallus gallus*

<220>

<221> caract_misc

<223> CD154 de pollo

<400> 13

Met Asn Glu Ala Tyr Ser Pro Ala Ala Pro Arg Pro Met Gly Ser Thr
 1 5 10 15
 Ser Pro Ser Thr Met Lys Met Phe Met Cys Phe Leu Ser Val Phe Met
 20 25 30
 Val Val Gln Thr Ile Gly Thr Val Leu Phe Cys Leu Tyr Leu His Met
 35 40 45
 Lys Met Asp Lys Met Glu Glu Val Leu Ser Leu Asn Glu Asp Tyr Ile
 50 55 60
 Phe Leu Arg Lys Val Gln Lys Cys Gln Thr Gly Glu Asp Gln Lys Ser
 65 70 75 80
 Thr Leu Leu Asp Cys Glu Lys Val Leu Lys Gly Phe Gln Asp Leu Gln
 85 90 95
 Cys Lys Asp Arg Thr Ala Ser Glu Glu Leu Pro Lys Phe Glu Met His
 100 105 110
 Arg Gly His Glu His Pro His Leu Lys Ser Arg Asn Glu Thr Ser Val
 115 120 125
 Ala Glu Glu Lys Arg Gln Pro Ile Ala Thr His Leu Ala Gly Val Lys
 130 135 140
 Ser Asn Thr Thr Val Arg Val Leu Lys Trp Met Thr Thr Ser Tyr Ala
 145 150 155 160
 Pro Thr Ser Ser Leu Ile Ser Tyr His Glu Gly Lys Leu Lys Val Glu
 165 170 175
 Lys Ala Gly Leu Tyr Tyr Ile Tyr Ser Gln Val Ser Phe Cys Thr Lys
 180 185 190
 Ala Ala Ala Ser Ala Pro Phe Thr Leu Tyr Ile Tyr Leu Tyr Leu Pro
 195 200 205
 Met Glu Glu Asp Arg Leu Leu Met Lys Gly Leu Asp Thr His Ser Thr
 210 215 220
 Ser Thr Ala Leu Cys Glu Leu Gln Ser Ile Arg Glu Gly Gly Val Phe
 225 230 235 240
 Glu Leu Arg Gln Gly Asp Met Val Phe Val Asn Val Thr Asp Ser Thr
 245 250 255
 Ala Val Asn Val Asn Pro Gly Asn Thr Tyr Phe Gly Met Phe Lys Leu
 260 265 270

<210> 14
 <211> 261
 <212> PRT
 <213> *Homo sapiens*

<220>
 <221> caract_misc
 <223> CD154 humano

<400> 14

Met Ile Glu Thr Tyr Asn Gln Thr Ser Pro Arg Ser Ala Ala Thr Gly
 1 5 10 15
 Leu Pro Ile Ser Met Lys Ile Phe Met Tyr Leu Leu Thr Val Phe Leu
 20 25 30
 Ile Thr Gln Met Ile Gly Ser Ala Leu Phe Ala Val Tyr Leu His Arg
 35 40 45
 Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp Phe Val
 50 55 60
 Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser Leu Ser
 65 70 75 80
 Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe Val Lys
 85 90 95
 Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser Phe Glu
 100 105 110
 Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser
 115 120 125
 Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly
 130 135 140
 Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys Gln
 145 150 155 160
 Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val Thr
 165 170 175
 Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile Ala Ser
 180 185 190
 Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu Arg Ala
 195 200 205
 Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser Ile His
 210 215 220
 Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe Val Asn
 225 230 235 240
 Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr Ser Phe
 245 250 255
 Gly Leu Leu Lys Leu
 260

<210> 15

<211> 11

<212> PRT

<213> *Homo sapiens*

<220>
 <221> caract_misc
 <223> CD154 humano parcial

<400> 15

Trp Ala Glu Lys Gly Tyr Tyr Thr Met Ser Cys
 1 5 10

<210> 16
 <211> 11
 <212> PRT
 <213> *Gallus gallus*

<220>
 <221> caract_misc
 <223> péptido CD154 de pollo

<400> 16

Trp Met Thr Thr Ser Tyr Ala Pro Thr Ser Ser
 1 5 10

<210> 17
 <211> 10
 <212> PRT
 <213> *Anas* sp.

<220>
 <221> caract_misc
 <223> péptido CD154 de pato

<400> 17

Trp Asn Lys Thr Ser Tyr Ala Pro Met Asn
 1 5 10

<210> 18
 <211> 10
 <212> PRT
 <213> *Mus* sp.

<220>
 <221> caract_misc
 <223> péptido CD154 de ratón

<400> 18

Trp Ala Lys Lys Gly Tyr Tyr Thr Met Lys
 1 5 10

<210> 19
 <211> 10
 <212> PRT
 <213> *Bos taurus*

<220>
 <221> caract_misc
 <223> péptido CD154 de vaca

<400> 19

Trp Ala Pro Lys Gly Tyr Tyr Thr Leu Ser
1 5 10

<210> 20

<211> 190

<212> PRT

<213> *Gallus gallus*

<220>

<221> caract_misc

<223> aminoácido HMGB1 de pollo

<400> 20

Met Gly Lys Gly Asp Pro Lys Lys Pro Arg Gly Lys Met Ser Ser Tyr
1 5 10 15

Ala Phe Phe Val Gln Thr Cys Arg Glu Glu His Lys Lys Lys His Pro
20 25 30

Asp Ala Ser Val Asn Phe Ser Glu Phe Ser Lys Lys Cys Ser Glu Arg
35 40 45

Trp Lys Thr Met Ser Ser Lys Glu Lys Gly Lys Phe Glu Asp Met Ala
50 55 60

Lys Ala Asp Lys Leu Arg Tyr Glu Lys Glu Met Lys Asn Tyr Val Pro
65 70 75 80

Pro Lys Gly Glu Thr Lys Lys Lys Phe Lys Asp Pro Asn Ala Pro Lys
85 90 95

Arg Pro Pro Ser Ala Phe Phe Leu Phe Cys Ser Glu Phe Arg Pro Lys
100 105 110

Ile Lys Gly Glu His Pro Gly Leu Ser Ile Gly Asp Val Ala Lys Lys
115 120 125

Leu Gly Glu Met Trp Asn Asn Thr Ala Ala Asp Asp Lys Gln Pro Tyr
130 135 140

Glu Lys Lys Ala Ala Lys Leu Lys Glu Lys Tyr Glu Lys Asp Ile Ala
145 150 155 160

Ala Tyr Arg Ala Lys Gly Lys Val Asp Ala Gly Lys Lys Val Val Ala
165 170 175

Lys Ala Glu Lys Ser Lys Lys Lys Lys Glu Glu Glu Glu Asp
180 185 190

<210> 21

<211> 85

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético: caja HMGB1 a1

<400> 21

Met	Gly	Lys	Gly	Asp	Pro	Lys	Lys	Pro	Arg	Gly	Lys	Met	Ser	Ser	Tyr
1				5					10					15	
Ala	Phe	Phe	Val	Gln	Thr	Cys	Arg	Glu	Glu	His	Lys	Lys	Lys	His	Pro
			20					25					30		
Asp	Ala	Ser	Val	Asn	Phe	Ser	Glu	Phe	Ser	Lys	Lys	Cys	Ser	Glu	Arg
		35					40					45			
Trp	Lys	Thr	Met	Ser	Ser	Lys	Glu	Lys	Gly	Lys	Phe	Glu	Asp	Met	Ala
	50					55					60				
Lys	Ala	Asp	Lys	Leu	Arg	Tyr	Glu	Lys	Glu	Met	Lys	Asn	Tyr	Val	Pro
65					70					75					80
Pro	Lys	Gly	Glu	Thr											
				85											

<210> 22

<211> 54

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético: caja HMGB1 a2

<400> 22

Pro	Asp	Ala	Ser	Val	Asn	Phe	Ser	Glu	Phe	Ser	Lys	Lys	Cys	Ser	Glu
1				5					10					15	
Arg	Trp	Lys	Thr	Met	Ser	Ser	Lys	Glu	Lys	Gly	Lys	Phe	Glu	Asp	Met
			20					25					30		
Ala	Lys	Ala	Asp	Lys	Leu	Arg	Tyr	Glu	Lys	Glu	Met	Lys	Asn	Tyr	Val
		35					40					45			
Pro	Pro	Lys	Gly	Glu	Thr										
		50													

<210> 23

<211> 73

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético: caja HMGB1 b1

<400> 23

Lys	Asp	Pro	Asn	Ala	Pro	Lys	Arg	Pro	Pro	Ser	Ala	Phe	Phe	Leu	Phe
1				5					10					15	
Cys	Ser	Glu	Phe	Arg	Pro	Lys	Ile	Lys	Gly	Glu	His	Pro	Gly	Leu	Ser
			20					25					30		

Ile Gly Asp Val Ala Lys Lys Leu Gly Glu Met Trp Asn Asn Thr Ala
 35 40 45

Ala Asp Asp Lys Gln Pro Tyr Glu Lys Lys Ala Ala Lys Leu Lys Glu
 50 55 60

Lys Tyr Glu Lys Asp Ile Ala Ala Tyr
 65 70

<210> 24

<211> 69

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético: caja HMGB1 b2

<400> 24

Asn Ala Pro Lys Arg Pro Pro Ser Ala Phe Phe Leu Phe Cys Ser Glu
 1 5 10 15

Phe Arg Pro Lys Ile Lys Gly Glu His Pro Gly Leu Ser Ile Gly Asp
 20 25 30

Val Ala Lys Lys Leu Gly Glu Met Trp Asn Asn Thr Ala Ala Asp Asp
 35 40 45

Lys Gln Pro Tyr Glu Lys Lys Ala Ala Lys Leu Lys Glu Lys Tyr Glu
 50 55 60

Lys Asp Ile Ala Ala
 65

<210> 25

<211> 21

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético: dominio de unión HMGB1 RAGE

<400> 25

Lys Asp Pro Asn Ala Pro Lys Arg Pro Pro Ser Ala Phe Phe Leu Phe
 1 5 10 15

Cys Ser Glu Phe Arg
 20

<210> 26

<211> 33

<212> PRT

<213> Secuencia artificial

<220>

<223> Péptido sintético: actividad de citoquina proinflamatoria HMGB1

<400> 26

Leu Lys Glu Lys Tyr Glu Lys Asp Ile Ala Ala Tyr Arg Ala Lys Gly
 1 5 10 15

Lys Val Asp Ala Gly Lys Lys Val Val Ala Lys Ala Glu Lys Ser Lys
 20 25 30

Lys

<210> 27

<211> 215

<212> PRT

<213> *Homo sapiens*

<220>

<221> caract_misc

<223> HMGB1

<400> 27

Met Gly Lys Gly Asp Pro Lys Lys Pro Arg Gly Lys Met Ser Ser Tyr
 1 5 10 15

Ala Phe Phe Val Gln Thr Cys Arg Glu Glu His Lys Lys Lys His Pro
 20 25 30

Asp Ala Ser Val Asn Phe Ser Glu Phe Ser Lys Lys Cys Ser Glu Arg
 35 40 45

Trp Lys Thr Met Ser Ala Lys Glu Lys Gly Lys Phe Glu Asp Met Ala
 50 55 60

Lys Ala Asp Lys Ala Arg Tyr Glu Arg Glu Met Lys Thr Tyr Ile Pro
 65 70 75 80

Pro Lys Gly Glu Thr Lys Lys Lys Phe Lys Asp Pro Asn Ala Pro Lys
 85 90 95

Arg Pro Pro Ser Ala Phe Phe Leu Phe Cys Ser Glu Tyr Arg Pro Lys
 100 105 110

Ile Lys Gly Glu His Pro Gly Leu Ser Ile Gly Asp Val Ala Lys Lys
 115 120 125

Leu Gly Glu Met Trp Asn Asn Thr Ala Ala Asp Asp Lys Gln Pro Tyr
 130 135 140

Glu Lys Lys Ala Ala Lys Leu Lys Glu Lys Tyr Glu Lys Asp Ile Ala
 145 150 155 160

Ala Tyr Arg Ala Lys Gly Lys Pro Asp Ala Ala Lys Lys Gly Val Val
 165 170 175

Lys Ala Glu Lys Ser Lys Lys Lys Lys Glu Glu Glu Glu Asp Glu Glu
 180 185 190

Asp Glu Glu Asp Glu Glu Glu Glu Glu Asp Glu Glu Asp Glu Asp Glu

195 200 205
 Glu Glu Asp Asp Asp Asp Glu
 210 215

 <210> 28
 <211> 205
 <212> PRT
 <213> *Danio rerio*

 <220>
 <221> caract_misc
 <223> HMGB1 de pez zebra

 <400> 28

 Met Gly Lys Asp Pro Thr Lys Pro Arg Gly Lys Met Ser Ser Tyr Ala
 1 5 10 15

 Tyr Phe Val Gln Thr Cys Arg Glu Glu His Lys Lys Lys His Pro Glu
 20 25 30

 Ala Thr Val Asn Phe Ser Glu Phe Ser Lys Lys Cys Ser Glu Arg Trp
 35 40 45

 Lys Thr Met Ser Ala Lys Glu Lys Gly Lys Phe Glu Asp Met Ala Lys
 50 55 60

 Leu Asp Lys Ala Arg Tyr Glu Arg Glu Met Lys Asn Tyr Ile Pro Pro
 65 70 75 80

 Lys Gly Glu Lys Lys Lys Arg Phe Lys Asp Pro Asn Ala Pro Lys Arg
 85 90 95

 Pro Pro Ser Ala Phe Phe Ile Phe Cys Ser Glu Phe Arg Pro Lys Val
 100 105 110

 Lys Glu Glu Thr Pro Gly Leu Ser Ile Gly Asp Val Ala Lys Arg Leu
 115 120 125

 Gly Glu Met Trp Asn Lys Ile Ser Ser Glu Glu Lys Gln Pro Tyr Glu
 130 135 140

 Lys Lys Ala Ala Lys Leu Lys Glu Lys Tyr Glu Lys Asp Ile Ala Ala
 145 150 155 160

 Tyr Arg Ser Lys Gly Lys Val Gly Gly Gly Ala Ala Lys Ala Pro Ser
 165 170 175

 Lys Pro Asp Lys Ala Asn Asp Glu Asp Glu Asp Asp Asp Glu Glu Glu
 180 185 190

 Asp Glu Asp Asp Asp Asp Glu Glu Glu Glu Asp Asp Glu
 195 200 205

 <210> 29
 <211> 20
 <212> ADN
 <213> Secuencia artificial

<220>
 <223> Cebador sintético: lam-up-f; Bucle 9 5'

 <400> 29
 tgtacaagtg gacgccaatc 20

 <210> 30
 <211> 24
 <212> ADN
 <213> Secuencia artificial

 <220>
 <223> Cebador sintético: lam-up-r; Bucle 9 5'

 <400> 30
 gttatcgccg tctttgatat agcc 24

 <210> 31
 <211> 20
 <212> ADN
 <213> Secuencia artificial

 <220>
 <223> Cebador sintético: lam-dn-f; Bucle 9 3'

 <400> 31
 atttcccgtt atgccgcagc 20

 <210> 32
 <211> 20
 <212> ADN
 <213> Secuencia artificial

 <220>
 <223> Cebador sintético: lam-dn-r; Bucle 9 3'

 <400> 32
 gttaaacaga gggcgacgag 20

 <210> 33
 <211> 68
 <212> ADN
 <213> Secuencia artificial

 <220>
 <223> Cebador sintético: Km-f; gen I-SceI/Kmr

 <400> 33
 gctatatcaa agacggcgat aactaactat aacggctcta aggtagcgaa tttccgggga 60

 tccgtcga 68

 <210> 34
 <211> 39
 <212> ADN
 <213> Secuencia artificial

 <220>

<223> Cebador sintético: Km-r; gen I-SceI/Kmr

<400> 34
gctgcggcat aacgggaaat gtaggctgga gctgcttcg 39

<210> 35
<211> 20
<212> ADN
<213> Secuencia artificial

<220>
<223> Cebador sintético: Kan4f; interior del gen Kmr

<400> 35
caaaagcgct ctgaagttcc 20

<210> 36
<211> 20
<212> ADN
<213> Secuencia artificial

<220>
<223> Cebador sintético: Kan4r; interior del gen Kmr

<400> 36
gcgtgagggg atcttgaagt 20

<210> 37
<211> 20
<212> ADN
<213> Secuencia artificial

<220>
<223> Cebador sintético: lam 3f; regiones externas del bucle 9

<400> 37
gccatctcgc ttggtgataa 20

<210> 38
<211> 20
<212> ADN
<213> Secuencia artificial

<220>
<223> Cebador sintético: lam 3r; regiones externas del bucle 9

<400> 38
cgctgggtatt ttgcggtaca 20

<210> 39
<211> 84
<212> ADN
<213> Secuencia artificial

<220>
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gttatcgccg tctttgatat agcc 84

<210> 40
 <211> 100
 <212> ADN
 <213> Secuencia artificial

<220>
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<400> 40
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<210> 41
 <211> 84
 <212> ADN
 <213> Secuencia artificial

<220>
 <223> Cebador sintético: Cj0420f; Inserto con el bucle 9 5'

<400> 41
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 gttatcgccg tctttgatat agcc 84

<210> 42
 <211> 100
 <212> ADN
 <213> Secuencia artificial

<220>
 <223> Cebador sintético: Cj0420r; Inserto con el bucle 9 3'

<400> 42
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 cgccgacctc ctcctcctcc atttcccgtt atgccgcagc 100

<210> 43
 <211> 105
 <212> ADN
 <213> Secuencia artificial

<220>
 <223> Cebador sintético: Cj0982c-f; Inserto con el bucle 9 5'

<400> 43
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 cagcgcaact ttggaggagg agttatcgcc gtctttgata tagcc 105

<210> 44
 <211> 121
 <212> ADN
 <213> Secuencia artificial

<220>

<223> Cebador sintético: Cj0982c-r; Inserto con el bucle 9 3'

<400> 44

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cctggatgac cacctcctat gcgccgacct cctcctcctc catttcccgt tatgccgcag 120

c 121

From the INTERNATIONAL BUREAU

PCTNOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

To:

VANHEYNINGEN, Tambryn K.
Andrus, Sceales, Starke & Sawall, LLP
100 East Wisconsin Avenue, Suite 1100
Milwaukee, WI 53202
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year)

25 August 2011 (25.08.2011)

Applicant's or agent's file reference

5658-00102

IMPORTANT NOTIFICATION

International application No.

PCT/US2011/039832

International filing date (day/month/year)

09 June 2011 (09.06.2011)

1. The following indications appeared on record concerning:

☒ the applicant ☒ the inventor ☐ the agent ☐ the common representative

Name and Address

HARGIS, Billy
3329 Oakland Zion Road
Fayetteville, AR 72703
United States of America

State of Nationality

US

State of Residence

US

Telephone No.

Facsimile No.

E-mail address

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address

HARGIS, Billy
3329 Oakland Zion Road
Fayetteville, AR 72703
United States of America

State of Nationality

US

State of Residence

US

Telephone No.

Facsimile No.

E-mail address

☐ Notifications by e-mail authorized

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office
☒ the International Searching Authority
☐ the Authority(ies) specified for supplementary search☐ the International Preliminary Examining Authority
☐ the designated Offices concerned
☐ the elected Offices concerned
☐ other:The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

Chahine Leila

e-mail pt06.pct@wipo.int

Telephone No. +41 22 338 74 06

Facsimile No. +41 22 338 89 70

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

TAMBRYN K. VANHEYNINGEN
ANDRUS, SCEALES, STARKE & SAWALL, LLP
100 EAST WISCONSIN AVENUE, SUITE 1100
MILWAUKEE, WI 53202

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT AND
THE WRITTEN OPINION OF THE INTERNATIONAL
SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing
(day/month/year)

23 NOV 2011

Applicant's or agent's file reference

5658-00102

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.

PCT/US 11/39832

International filing date
(day/month/year)

09 June 2011 (09.06.2011)

Applicant THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ARKANSAS

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes
1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

For more detailed instructions, see PCT Applicant's Guide, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Reminders

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90bis.1 and 90bis.3).

Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide, National Chapters*.

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer

Lee W. Young

PCT Helpdesk: 571-272-4300

Telephone No. PCT OSP: 571-272-7774

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

TAMBRYN K. VANHEYNINGEN
ANDRUS, SCEALES, STARKE & SAWALL, LLP
100 EAST WISCONSIN AVENUE, SUITE 1100
MILWAUKEE, WI 53202

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT AND
THE WRITTEN OPINION OF THE INTERNATIONAL
SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing
(day/month/year)

23 NOV 2011

Applicant's or agent's file reference

5658-00102

FOR FURTHER ACTION See paragraphs 1 and 4 below

International application No.

PCT/US 11/39832

International filing date
(day/month/year)

09 June 2011 (09.06.2011)

Applicant THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ARKANSAS

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:

The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes
1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

For more detailed instructions, see PCT Applicant's Guide, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. Reminders

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90bis.1 and 90bis.3).

Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide, National Chapters*.

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer

Lee W. Young

PCT Helpdesk: 571-272-4300

Telephone No. PCT OSP: 571-272-7774

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 5658-00102	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, item 5 below.	
International application No. PCT/US 11/39832	International filing date (<i>day/month/year</i>) 09 June 2011 (09.06.2011)	(Earliest) Priority Date (<i>day/month/year</i>) 09 June 2010 (09.06.2010)
Applicant THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ARKANSAS		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 5 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☒ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/39832

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

a. (means)

☐

on paper

☒

in electronic form

b. (time)

☐

in the international application as filed

☒

together with the international application in electronic form

☐

subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/39832

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 7-32
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/39832

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/02; C12N 1/20; C12N 15/00 (2011.01)

USPC - 424/200.1, 424/234.1, 424/190.1; 435/252.3; 435/320.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A61K 39/02; C12N 01/20; C12N 15/00 (2011.01)

USPC ? 424/200.1, 424/234.1, 424/190.1; 435/252.3; 435/320.1; 514/44R; 435/69.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

IPC(8) - A61K 39/02; C12N 01/20; C12N 15/00 (2011.01) - see keyword below

USPC ? 424/200.1, 424/234.1, 424/190.1; 435/252.3; 435/320.1; 514/44R; 435/69.3 - see keyword below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWEST(USPT,PGPB,EPAB,JPAB); Medline, Google: Campylobacter jejuni, C. jejuni, cjaD, cjaA; ACE 393, cj0982c, cj0113, cj0420, vector, express, encoding, polynucleotide, polypeptide, vaccine, attenuated, bacteria, Salmonella, Escherichia, Bacillus, Lactobacillus, immunostimulatory, CD154, CD40L, HMGB1, genome, chromosome, integrated, insert, intro

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2007/0128183 A1 (MEINKE et al.) 07 June 2007 (07.06.2007), Abstract, para [0001], [0046], [0047], [0054], [0057], [0098], [0099], [0110], [0122], [0147], [0148], [0149], [0200], [0209], [0218], [0301], and SEQ ID NO: 1209	1 ----- 2-6
Y	US 2010/0112002 A1 (LIEN et al.) 06 May 2010 (06.05.2010), para [0012], [0031], [0051], [0060], [0061], [0064], and [0078]	2-6
A	Skerra, Use of the tetracycline promoter for the tightly regulated production of a murine antibody fragment in Escherichia coli. Gene. 1994, Vol. 151, p.131?135. Abstract [online]. [Retrieved on 2011.11.01]. Retrieved from the Internet: <URL: http://www.ncbi.nlm.nih.gov/pubmed/7828861> Abstract	1-6
A	WYSZYNSKA et al. Oral immunization of chickens with avirulent Salmonella vaccine strain carrying C. jejuni 72Dz/92 cjaA gene elicits specific humoral immune response associated with protection against challenge with wild-type Campylobacter. Vaccine. 2004, Vol. 22, p. 1379?1389. Entire documentation, especially Abstract; pg 1382, col 1, para 2; pg 1383, col 1, lower para, col 2, lower para, and Fig 1; and pg 1385, col 1	1-6
A	US 2007/0249553 A1 (NEWELL et al.) 25 October 2007 (25.10.2007), para [0001], and SEQ ID NO: 14, which is 100% identical to the claimed SEQ ID NO: 3, and comprises a region between amino acid residues 130-150, that is 100% identical to the claimed SEQ ID NO: 8	1-6
A	UniProt_A3ZI25, CjaA protein, Last modified April 3, 2007 [online]. [Retrieved on 2011.11.01]. Retrieved from the Internet: <URL: http://www.uniprot.org/uniprot/A3ZI25> the sequence is 100% identical to the claimed SEQ ID NO: 2, and comprises a region between amino acid residues 127-161, that is 100% identical to the claimed SEQ ID NO: 9.	1-6

☒ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

01 November 2011 (01.11.2011)

Date of mailing of the international search report

23 NOV 2011

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/39832

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	LAYTON et al. Evaluation of Salmonella-Vectored Campylobacter Peptide Epitopes for Reduction of Campylobacter jejuni in Broiler Chickens. Clin Vaccine Immunol. 2011, Vol. 18(3), p. 449-54. Epub 2010 Dec 22. Entire documentation, especially Abstract; pg 450, col 1, para 2, and col 2, para 1 and para 3	1-6

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: TAMBRYN K. VANHEYNINGEN
ANDRUS, SCEALES, STARKE & SAWALL, LLP
100 EAST WISCONSIN AVENUE, SUITE 1100
MILWAUKEE, WI 53202

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing
(day/month/year)

23 NOV 2011

Applicant's or agent's file reference
5658-00102

FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US 11/39832

International filing date (day/month/year)

09 June 2011 (09.06.2011)

Priority date (day/month/year)

09 June 2010 (09.06.2010)

International Patent Classification (IPC) or both national classification and IPC

IPC(8) - A61K 39/02; C12N 01/20; C12N 15/00 (2011.01)

USPC - 424/200.1, 424/234.1, 424/190.1; 435/252.3; 435/320.1

Applicant THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ARKANSAS

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Date of completion of this opinion

01 November 2011 (01.11.2011)

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/39832

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
☒ the international application in the language in which it was filed.
☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:
 - a. (means)
☐ on paper
☒ in electronic form
 - b. (time)
☐ in the international application as filed
☒ together with the international application in electronic form
☐ subsequently to this Authority for the purposes of search
4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
5. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 11/39832

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 7-32

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search (*specify*):

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 7-32 are so unclear that no meaningful opinion could be formed (*specify*):

Claims 7-32 are not drafted in accordance with the second and third sentences of Rule 6.4 (a). These claims are improper multiple dependent claims.

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

☒ no international search report has been established for said claims Nos. 7-32

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b).

☐ See Supplemental Box for further details.

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-6	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-6	NO
Industrial applicability (IA)	Claims	1-6	YES
	Claims	None	NO

2. Citations and explanations:

Claim 1 lacks an inventive step under PCT Article 33(3) as being obvious over US 2007/0128183 A1 to MEINKE et al. (hereinafter 'Meinke').

Regarding claim 1, Meinke discloses a vector (para [0047] - 'the vector is adapted for recombinant expression of the hyperimmune serum reactive antigens'; Abstract - 'hyperimmune serum reactive antigens or fragments thereof from ...C. jejuni') --comprising a first polynucleotide sequence encoding an antigenic polypeptide that is a fragment of SEQ ID NO: 1 (cjaD; cj0113) (para [0046] - 'a vector comprising a nucleic acid molecule according to any of the present invention is provided'; para [0054] - 'expressing one or more of the nucleic acid molecules'; para [0147] - 'the vector is adapted for recombinant expression of the hyperimmune serum reactive antigens'; para [0148] - 'hyperimmune serum-reactive antigens ...selected...Seq ID No ... 1203-1242...and fragments thereof', wherein SEQ ID NO: 1209 is 100% identical to the claimed SEQ ID NO: 1). Although Meinke does not expressly disclose the first polynucleotide sequence not natively associated with the vector, Meinke discloses the vector is a recombinant vector adapted for expressing antigens (para [0147] - 'the vector is adapted for recombinant expression of the hyperimmune serum reactive antigens', para [0310] - 'The vector pMAL4.31 was constructed on a pASK-IBA backbone {Skerra, A., 1994} with the beta-lactamase (bla) gene exchanged with the Kanamycin resistance gene'; please also see Skerra, Abstract - 'A generic vector, pASK75, was developed for the synthesis of foreign proteins'), which indicates 'the first polynucleotide sequence not natively associated with the vector'.

Although Meinke does not specifically teach wherein the first polynucleotide sequence encoding an fragment of SEQ ID NO: 1 is an antigenic polypeptide of SEQ ID NO: 7 (cjaD; cj0113) or a fragment thereof, one of ordinary skill in the art at the time the invention was made would have known to obtain such fragments, because Meinke further discloses a method for screening and identifying antigens against a pathogen (para [0098] - 'identifying a comprehensive set of antigens ... bacterial surface expression libraries from each pathogen are screened'; para [0099] - 'The expression libraries ... allow expression of all potential antigens'), and a method of using a polynucleotide sequence encoding a fragment of antigenic polypeptides of at least 4 amino acids to more than 20 amino acids including the claimed SEQ ID NO: 7 and the fragment thereof (para [0122] - 'nucleic acids ... consist of at least 20 ...50 contiguous bases ...nucleic acid ...a contiguous 15 base portion ...predicted immunogenic amino acid sequences'; para [0149] - 'The hyperimmune serum reactive antigens and fragments... include portions...at least 4 amino acids.. 8...10, 20, 30... amino acids'; para [0110] - 'nucleic acids, which encode hyperimmune serum reactive antigens or fragments...the Seq ID No ...1203-1336', wherein SEQ ID NO: 1209 comprises a region between amino acid residues 90-110, that is 100% identical to the claimed SEQ ID NO: 7) for treating infections by *Campylobacter jejuni* (para [0001] - 'isolated nucleic acid molecules, which encode antigens ... for the prevention and treatment of bacterial infections caused by ... *Campylobacter jejuni*'; Sequence list SEQ ID NO: 1209 'organism: *Campylobacter jejuni*').

It would have been obvious to one of ordinary skill in the art at the time the invention was made to obtain a vector comprising a first polynucleotide sequence encoding an antigenic polypeptide of SEQ ID NO: 7 (cjaD; cj0113) or a fragment thereof, based on the teaching of Meinke, in order to express the smallest antigenic portion of an antigenic polypeptide that is specific to *Campylobacter jejuni* for effectively induce immune response against campylobacter jejuni infection for preventing and treating campylobacter jejuni infection with a reduced undesired immune response because of non-specific antigenic portion of an expressed peptide.

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Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Note: claim 1 directed to SEQ ID NO: 8 (cjaA; cj0982) or SEQ ID NO: 9 (ACE 393; cj0420). However these are not supported by the sequence search results.

US 2007/0249553 A1 to NEWELL et al. Discloses a sequence (SEQ ID NO: 14), which is 100% identical to the claimed SEQ ID NO: 3 (ACE393; cj0420, as in claim 3), and comprises a region between amino acid residues 130-150, that is 100% identical to the claimed SEQ ID NO: 8.

UniProt_A3ZI25, discloses a CjaA protein sequence <URL: <http://www.uniprot.org/uniprot/A3ZI25>>, wherein the amino acid sequence is 100% identical to the claimed SEQ ID NO: 2 (cjaA; cj0982, as in claim 3), and comprises a region between amino acid residues 127-161, that is 100% identical to the claimed SEQ ID NO: 9.

Supplemental Box

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Continuation of:
Box V No 2 (citations and explanations)

Claims 2-6 lack an inventive step under PCT Article 33(3) as being obvious over Meinke, as above, in view of US 2010/0112002 A1 to LIEN et al. (hereinafter 'Lien').

Regarding claim 2, Meinke obviates a vector comprising a first polynucleotide sequence encoding an antigenic polypeptide of SEQ ID NO: 7 (cjaD; cj0113) or a fragment thereof, the first polynucleotide sequence not natively associated with the vector, as discussed in the discussion of claim 1. Meinke further discloses using the vector for delivering antigens for inducing therapeutic immune response (para [0200] - 'inducing an immunological response in an individual ... through gene therapy ... delivering a nucleic acid ... for expressing the hyperimmune serum reactive antigens and fragments thereof), wherein the method further comprising co-administering an immunostimulatory peptide or an immunostimulatory nucleotide for further stimulating the immune response (para [0057] - 'the pharmaceutical composition further comprises an immunostimulatory substance ... selected from ... peptides containing at least two LysLeuLys motifs'; para [0209] - 'A preferable carrier...is an immunostimulatory compound for further stimulating the immune response... the immunostimulatory compound...selected from ... immunostimulatory nucleic acids molecules'; para [0218]).

Meinke does not specifically teach the vector further comprising a second polynucleotide sequence encoding an immunostimulatory polypeptide not natively associated with the vector. Lien discloses a method for delivering an antigen to a subject by using a vector comprising an antigen specific to a pathogen and a polynucleotide sequence encoding an immunostimulatory polypeptide (para [0012] - 'delivering to a subject...a therapeutically effective amount of a microorganism modified to express an immunostimulatory molecule, wherein the microorganism further comprises an antigen.....from a pathogen', wherein 'microorganism modified' is 'a vector', and wherein 'an antigen.....from a pathogen' is the first antigen and 'express an immunostimulatory molecule' is 'the vector further comprising a second polynucleotide sequence encoding an immunostimulatory polypeptide not natively associated with the vector'; para [0064] - 'introducing into the microorganism a nucleic acid encoding an immunostimulatory protein') for immunizing a subject against associated pathogen infection with a strong adjuvant activity and antigen presentation by inducing an increased desired immune response (para [0064] - 'making a vaccine for immunizing a subject population against infection by a pathogen. ...providing the pathogen in a modified form ... introducing into the microorganism a nucleic acid encoding an immunostimulatory protein'; para [0078] - 'The modified microorganisms ... used as ...vaccine delivery systems....the modified microorganisms ...will provide strong adjuvant activity and antigen presentation... a microorganism modified to express an immunostimulatory molecule is further modified to ...express one or more... heterologous macromolecules, against which an immune response is desired. A "heterologous molecule" in a microorganism...not naturally present...a pathogenic antigen').

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Meinke and Lien, to obtain a vector comprising a first polynucleotide sequence encoding an antigenic polypeptide of SEQ ID NO: 7 (cjaD; cj0113) or a fragment thereof, the first polynucleotide sequence not natively associated with the vector, based on the teaching of Meinke, wherein the vector further comprising a second polynucleotide sequence encoding an immunostimulatory polypeptide not natively associated with the vector, based on the combination Lien and Meinke, in order to prepare a delivery vector for simultaneously delivering a specific antigen to a pathogen and an immunostimulatory polypeptide with a strong adjuvant activity and antigen presentation for inducing an increased desired immune response against the pathogen targeted.

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The previous supplemental box - Box V No 2 (citations and explanations)

Regarding claim 3, Meinke discloses a vector (para [0047] - 'the vector is adapted for recombinant expression of the hyperimmune serum reactive antigens'; Abstract - 'hyperimmune serum reactive antigens or fragments thereof from ...C. jejuni') - comprising a first polynucleotide sequence encoding an antigenic polypeptide (para [0046] - 'a vector comprising a nucleic acid molecule according to any of the present invention is provided'; para [0054] - 'expressing one or more of the nucleic acid molecules'; para [0147] - 'the vector is adapted for recombinant expression of the hyperimmune serum reactive antigens'), - wherein the antigenic polypeptide is a fragment of SEQ ID NO: 1 (cjd; cj0113) (para [0147] - 'the vector is adapted for recombinant expression of the hyperimmune serum reactive antigens'; para [0148] - 'hyperimmune serum-reactive antigens ...selected...Seq ID No ... 1203-1242...and fragments thereof', wherein SEQ ID NO: 1209 is 100% identical to the claimed SEQ ID NO: 1). Although Meinke does not expressly disclose the first polynucleotide sequence not natively associated with the vector, Meinke discloses the vector is a recombinant vector adapted for expressing antigens (para [0147] - 'the vector is adapted for recombinant expression of the hyperimmune serum reactive antigens', para [0310] - 'The vector pMAL4.31 was constructed on a pASK-IBA backbone {Skerra, A., 1994} with the beta-lactamase (bla) gene exchanged with the Kanamycin resistance gene'; please also see Skerra, Abstract - 'A generic vector, pASK75, was developed for the synthesis of foreign proteins'), which indicates 'the first polynucleotide sequence not natively associated with the vector'. Meinke further discloses using the vector for delivering antigens for inducing therapeutic immune response (para [0200] - 'inducing an immunological response in an individual ... through gene therapy ... delivering a nucleic acid ... for expressing the hyperimmune serum reactive antigens and fragments thereof', wherein the method further comprising co-administering an immunostimulatory peptide or an immunostimulatory nucleotide for further stimulating the immune response (para [0057] - 'the pharmaceutical composition further comprises an immunostimulatory substance ... selected from ... peptides containing at least two LysLeuLys motifs'; para [0209] - 'A preferable carrier...is an immunostimulatory compound for further stimulating the immune response... the immunostimulatory compound...selected from ... immunostimulatory nucleic acids molecules'; para [0218]).

Meinke does not specifically teach wherein the vector comprising a second polynucleotide sequence encoding an immunostimulatory polypeptide. Lien discloses a method for delivering an antigen to a subject by using a vector comprising an antigen specific to a pathogen and a polynucleotide sequence encoding an immunostimulatory polypeptide (para [0012] - 'delivering to a subject...a therapeutically effective amount of a microorganism modified to express an immunostimulatory molecule, wherein the microorganism further comprises an antigen...from a pathogen', wherein 'microorganism modified' is 'a vector', and wherein 'an antigen...from a pathogen' is the first antigen and 'express an immunostimulatory molecule' is 'the vector further comprising a second polynucleotide sequence encoding an immunostimulatory polypeptide not natively associated with the vector'; para [0064] - 'introducing into the microorganism a nucleic acid encoding an immunostimulatory protein') for immunizing a subject against associated pathogen infection with a strong adjuvant activity and antigen presentation by inducing an increased desired immune response (para [0064] - 'making a vaccine for immunizing a subject population against infection by a pathogen. ...providing the pathogen in a modified form ... Introducing into the microorganism a nucleic acid encoding an immunostimulatory protein'; para [0078] - 'The modified microorganisms ... used as ...vaccine delivery systems...the modified microorganisms ...will provide strong adjuvant activity and antigen presentation... a microorganism modified to express an immunostimulatory molecule is further modified to ...express one or more... heterologous macromolecules, against which an immune response is desired. A "heterologous molecule" in a microorganism...not naturally present...a pathogenic antigen').

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Meinke and Lien, to obtain a vector comprising a first polynucleotide sequence encoding an antigenic polypeptide not natively associated with the vector, wherein the antigenic polypeptide is a fragment of SEQ ID NO: 1 (cjd; cj0113), based on the teaching of Meinke, wherein the vector comprising a second polynucleotide sequence encoding an immunostimulatory polypeptide, based on the combination of Lien and Meinke, in order to prepare a delivery vector for simultaneously delivering a specific antigen to a pathogen and an immunostimulatory polypeptide with a strong adjuvant activity and antigen presentation for inducing an increased desired immune response against the pathogen targeted.

Regarding claim 4/(1), Meinke obviates a vector comprising a first polynucleotide sequence encoding an antigenic polypeptide of SEQ ID NO: 7 (cjd; cj0113) or a fragment thereof, the first polynucleotide sequence not natively associated with the vector, as discussed in the discussion of claim 1. Meinke further discloses using the vector for delivering antigens for inducing therapeutic immune response (para [0200] - 'inducing an immunological response in an individual ... through gene therapy ... delivering a nucleic acid ... for expressing the hyperimmune serum reactive antigens and fragments thereof', and expressing an antigen using a bacterium for screening and identifying antigens against a pathogen (para [0164] - 'expression vectors can be used to express a hyperimmune serum reactive antigen or fragment thereof ...to express a polypeptide in a host ... expressed in ... bacteria'; para [0098] - 'identifying a comprehensive set of antigens ... bacterial surface expression libraries from each pathogen are screened'; para [0099] - 'The expression libraries ... allow expression of all potential antigens').

Meinke does not specifically teach wherein the vector is a bacterium. Lien discloses a method for delivering an antigen to a subject by using a genetically modified bacterium comprising an antigen specific to a pathogen (para [0012] - 'delivering to a subject...a therapeutically effective amount of a microorganism modified ... the microorganism... comprises an antigen...from a pathogen'; para [0031] - 'Preferred microorganisms to be modified ... A microorganism may be a prokaryotic ...Prokaryotic microorganisms include bacteria') for immunizing a subject against associated pathogen infection with a strong adjuvant activity and antigen presentation by inducing an increased desired immune response (para [0078] - 'The modified microorganisms ... used as ...vaccine delivery systems...the modified microorganisms ...will provide strong adjuvant activity and antigen presentation... a microorganism modified to ...express one or more... heterologous macromolecules, against which an immune response is desired. A "heterologous molecule" in a microorganism...not naturally present...a pathogenic antigen'; para [0031]; para [0060]).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Meinke and Lien, to obtain a vector comprising a first polynucleotide sequence encoding an antigenic polypeptide of SEQ ID NO: 7 (cjd; cj0113) or a fragment thereof, the first polynucleotide sequence not natively associated with the vector, based on the teaching of Meinke, wherein the vector is a bacterium, based on the teaching of Lien, in order to use a modified bacterium for delivering a specific antigen to a pathogen with a strong adjuvant activity and antigen presentation for inducing an increased desired immune response against the pathogen targeted.

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The previous supplemental box - Box V No 2 (citations and explanations)

Regarding claim 4/(2-3), the combination of Meinke and Lien discloses the vector of anyone of claims 2-3, and Lien further discloses wherein the vector is a bacterium (para [0031] - 'Preferred microorganisms to be modified as described herein are pathogenic microorganisms ... A microorganism may be a prokaryotic ...Prokaryotic microorganisms include bacteria'; para [0060] - 'A modified microorganism, especially when used as a live vaccine, may further be attenuated, ...making and using live attenuated strains of bacteria that are suitable for vaccines or immunogenic compositions').

Regarding claim 5, the combination of Meinke and Lien discloses the vector of claim 4, and Lien further discloses wherein the bacterium is from a genus selected from Salmonella or Escherichia (para [0031] - 'Preferred microorganisms to be modified...Exemplary gram-negative bacteria, include members of... Salmonella ... Escherichia; para [0061] - 'attenuating gram-negative bacteria.... A Salmonella species... An Escherichia species').

Regarding claim 6, the combination of Meinke and Lien discloses the vector of claim 4, and Lien further discloses wherein the first polynucleotide sequence and the second polynucleotide sequence are integrated into the genome of the bacterium (para [0051] - 'Nucleic acids that are introduced into a microorganism may become part of the chromosome of the microorganism', which indicates ' the first polynucleotide sequence and the second polynucleotide sequence are integrated into the genome of the bacterium'; para [0078] - 'a microorganism modified to express an immunostimulatory molecule is further modified to ...express one or more... heterologous macromolecules, against which an immune response is desired.....macromolecule is a protein, such as a pathogenic antigen'; para [0031], [0060]).

Claims 1-6 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.