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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 06-050044- -00003-0000

Fecha: 2008-06-17 16:36:01
Tra. 11 PATENTEIYII
Act. 538 PETICION

Dep. 2020 NUECREACIONE
Eve: 379 FASENACIONALII
Folios: 2

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Señora Jefe de la
DIVISION DE NUEVAS CREACIONES
Superintendencia de Industria y Come.
E. S. D.

Trámite : 011 Evento : 379 Actuación : 538

Referencia : Expediente No. 06050044
Asunto : Solicitud de patente para VACUNA PARA
ERYSIPELOTHRIX RHUSIOPATHIAE-HAEMOPHILUS
PARASUIS Y METODOS DE USO DE LAS MISMAS
Solicitante : BOEHRINGER INGELHEIM VETMEDICA, INC
Publicada en : La Gaceta No. 583 del 18 de diciembre de 2007

SOLICITUD EXAMEN DE PATENTABILIDAD

1. Yo, Emilio FERRERO, identificado como aparece al pie de mi firma, actuando como apoderado de la sociedad BOEHRINGER INGELHEIM VETMEDICA, INC, atentamente solicito se efectúe el examen de patentabilidad de la solicitud en referencia presentada de acuerdo con las reglas del Capítulo II del PCT y según lo establecido en el artículo 44 de la Decisión 486 de la Comisión de la Comunidad Andina.

Para tal efecto presento junto con este escrito el recibo No. 08-45,899 por valor de \$210.000.00 que corresponde a la tasa legal fijada para el efecto.

2. Por lo anterior, comedidamente solicito se prosiga con el trámite de este asunto.

Señora Jefe,

EMILIO FERRERO
T.P.A. No. 31.929

COLO-15149-841-002-7
SCT\SCT\ID-128549\WPC15149
No. M-2008-128549\SCT

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 08 - 45,899
FECHA : JUNIO 9 DE 2008

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
CAVELIER ABOGADOS	CONSIGNACION	BANCO DE BOGOTA	062754387	2678328	09/06/2008	89,445,000.00

***** CONCEPTO *****

CANT. RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	00005-01-01 SOLICITUDES	
	114 PTC CAP-II EXAMEN DE PATENTIBILID	210,000.00
	TOTAL :	\$ 210,000.00

SON: DOSCIENTOS DIEZ MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

15149-841002-7

DIVISIÓN DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No. 06-50044

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCION**

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **583** DE

FECHA **18 DE DICIEMBRE DE 2007** EL TÉRMINO HÁBIL SEÑALADO POR LA

LEY EXPIRA EL **14 DE MARZO DE 2008**

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI _____

NO X



US006019984A

United States Patent [19][11] **Patent Number:** **6,019,984****MacInnes et al.**[45] **Date of Patent:** **Feb. 1, 2000**[54] **BACTERIAL PREPARATIONS, METHOD FOR PRODUCING SAME, AND THEIR USE AS VACCINES**[75] Inventors: **Janet MacInnes; Paul Ricciatti**, both of Guelph; **Bonnie Mallard**, Ariss; **Soren Rosendal**, deceased, late of Guelph, all of Canada, by Lillian Rosendal, legal representative[73] Assignee: **University of Guelph**, Guelph, Canada[21] Appl. No.: **08/772,270**[22] Filed: **Dec. 23, 1996****Related U.S. Application Data**

[63] Continuation-in-part of application No. 08/396,244, Mar. 1, 1995, abandoned.

[51] Int. Cl.⁷ **A61K 39/102; A61K 39/02; A01N 63/00; C12N 1/00**[52] U.S. Cl. **424/255.1; 424/93.4; 424/93.2; 424/93.48; 424/184.1; 424/278.1; 424/823; 424/824; 424/825; 424/826; 424/827; 424/828; 424/829; 435/243**[58] Field of Search **424/255.1, 93.4, 424/93.2, 93.48, 184.1, 278.1, 823-829; 435/243**[56] **References Cited****FOREIGN PATENT DOCUMENTS**

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Primary Examiner—Nita Minnifield
Attorney, Agent, or Firm—Bereskin & Parr

[57]

ABSTRACT

Novel bacterial preparations containing one or more isolated and purified strain of a microorganism which produces one or more RTX toxins, and which strain has at least one RTX toxin which is substantially cell-associated. Methods of preparing the bacterial preparations and their use as vaccines and to produce antibodies for passive immunization are described.

23 Claims, 45 Drawing Sheets

one or more adjuvants known in the art. Suitable adjuvants include aluminum hydroxide, Freund's adjuvant (complete or incomplete), bacteria such as *Bordetella pertussis* or *E. coli* or bacterium derived matter, immune stimulating complex (iscom), oil, saponin, oligopeptide, emulsified paraffin-Emulsigen™ (MVP Labs, Ralston, Nebr.), L80 adjuvant containing AL(OH)₃ (Reheis, N.J.), Quil A (Superphos), or other adjuvants known to the skilled artisan. Preferably, the adjuvant is L80 adjuvant containing AL(OH)₃ (Reheis, N.J.) and Quil A (Superphos). The vaccine may also contain preservatives such as sodium azide, thimersol, gentamicin, neomycin, and polymyxin.

The vaccine may be a multivalent vaccine and additionally contain other immunogens related to other diseases in a prophylactically or therapeutically effective manner. For example, a vaccine containing a bacterial preparation comprising *A. pleuropneumoniae* strains of serotypes producing RTX toxins which are substantially cell associated, may also contain inactivated cultures of one or more of *Haemophilus parasuis* which causes polyserositis; *Pasteurella multocida* which contributes to the severity of *A. pleuropneumoniae* respiratory clinical signs and pathology; *Bordetella bronchiseptica* which causes atrophic rhinitis; and *Erysipelothrix rhusiopathiae* which causes erysipelas. Additional immunogens may also be added to the vaccine in a number of ways. In one example, the DNA encoding the immunogen may be incorporated into strains in the bacterial preparation using gene transfer techniques. In another example, the immunogen may be added in an immunologically effective amount directly to the preparation.

The vaccines of the invention may be administered in a convenient manner, such as intravenously, intramuscularly, subcutaneously, intraperitoneally, intranasally or orally. Preferably the vaccine is administered intramuscularly or subcutaneously. The vaccine may be administered in a liposome delivery system to allow for the slow sustained release of the immunogens.

The dosage will depend on the nature of the infection, on the desired effect and on the chosen route of administration, and other factors known to persons skilled in the art. For example, in respect of a vaccine containing an *A. pleuropneumoniae* strain which produces an RTX toxin which is substantially cell-associated, about 1 to 5 ml may be administered intramuscularly or subcutaneously to pigs 30 lbs. or over, and 0.5 to 1 ml for pigs under 30 lbs. A second vaccination is generally recommended two to three weeks later. Breeding females are generally given two doses. The initial dose may be administered about five weeks prior to farrowing, and the second dose 2 weeks to three weeks prior to farrowing. Revaccinations are generally given annually.

The bacterial preparations of the invention are also useful for preparing antibodies which may be used as a means of passive immunization. Within the context of the present invention, antibodies are understood to include monoclonal antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, and F(ab')₂ and recombinantly produced binding partners. Antibodies may be prepared using conventional methods.

For example, polyclonal antibodies may be readily generated by one of ordinary skill in the art from a variety of warm-blooded animals such as horses, cows, various fowl, rabbits, mice, or rats. Briefly, the bacterial preparation is utilized to immunize the animal through intraperitoneal, intramuscular, intraocular, or subcutaneous injections, in conjunction with an adjuvant such as Freund complete or incomplete adjuvant. Following several booster immunizations, samples of serum are collected and tested

for reactivity to the bacterial preparations. Particularly preferred polyclonal antisera will give a signal on one of these assays that is at least three times greater than background. Once the titer of the animal has reached a plateau in terms of its reactivity, larger quantities of antisera may be readily obtained either by weekly bleedings, or by exsanguinating the animal.

The following non-limiting examples are illustrative of the present invention:

EXAMPLES

The following materials and methods were utilized in the investigations outlined in the examples:

Bacterial Strains and Growth Conditions

Actinobacillus pleuropneumoniae CM5, a serotype 1 strain, was isolated from a pig with naturally occurring pleuropneumonia (Rosendal et al., 1981) and stored at -70° C. in pleural fluid. *A. pleuropneumoniae* BC181, a serotype 3 strain, was isolated from a case of porcine pleuropneumonia in British Columbia, and stored frozen at -70° C. in skim milk with 20% glycerol (Rosendal et al., 1981). Serotype 1 strain VSB68, was provided as a lyophilized culture by Langford/Cyanamid, Guelph, Ont. For use, cultures were streaked onto trypticase soy agar supplemented with 5% (vol/vol) heated sheep blood (chocolate agar) and 0.01% (wt/vol) nicotinamide adenine dinucleotide (CBA-NAD) and incubated for 24 h at 37° C. with 5% CO₂.

Cultures used in preparation of antigens for polyacrylamide gel electrophoresis (PAGE) and Western blotting (WB) experiments were grown in several different media under different conditions. A well defined commercial medium, RPMI (Table 3) supplemented with 10% fetal calf serum (FCS) was used. Tryptone yeast extract broth (TYE) (Table 3; O'Reilly, and Niven, 1985) was used to provide enriched growth conditions.

To provide oxygen-limited conditions, screw-capped 500 ml glass bottles (Corning) were filled to the top with medium prior to autoclaving and the caps tightened to limit dissolved oxygen after autoclaving. Cultures were incubated without agitation. Aerobic conditions were achieved by incubating cultures in 500 ml bottles partially filled with medium at 200 rpm on a rotary shaker.

Strains of *A. pleuropneumoniae* serotypes 1, 2, 5, 7, and 9 from a commercial bacterin were provided as individual, formalin-inactivated suspensions by Langford/Cyanamid.

Inactivation

Cultures were inactivated using one of three techniques. Six h cultures were heated to 6° C. for 2 h and then cooled to 4° C. or β-propiolactone (β-Prone, Grand Laboratories Inc., Larchwood, Iowa.) was added at the rate of 1 ml/1000 ml and the cultures incubated at 37° C. and 100 rpm overnight. Alternatively, 0.1 M binary-ethylenimine (BEI) was added at the rate of 100 ml/1000 ml, and the cultures incubated at 37° C., and 100 rpm overnight. The BEI was neutralized using 100 ml of 1 M sodium thiosulfate. Inactivation of the cultures was confirmed by plating multiple 1 ml samples onto CBA-NAD, and incubating the plates for 48 h at 37° C. with 5% CO₂. From each inactivated culture, 500 ml was stored at 4 to 7° C. The remaining 500 ml from each culture was centrifuged at 15,000xg for 15 min at 4° C. The supernatant was recovered and stored at 4° C. The cell pellet was washed once in PBS. The cells were resuspended in 10 ml of PBS and stored frozen at -20° C.



US006342231B1

(12) **United States Patent**
Burkhardt et al.

(10) **Patent No.:** **US 6,342,231 B1**
(45) **Date of Patent:** ***Jan. 29, 2002**

(54) **HAEMOPHILUS PARASUIS VACCINE AND DIAGNOSTIC**

(75) **Inventors:** **Douglas T. Burkhardt, Waukeg; Karen L. Lenz, West Des Moines, both of IA (US)**

(73) **Assignee:** **Akzo Nobel N.V., Arnhem (NL)**

(*) **Notice:** This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) **Appl. No.:** **09/108,438**

(22) **Filed:** **Jul. 1, 1998**

(51) **Int. Cl.⁷** **A61K 39/102; A61K 39/02; A61K 39/00; A61K 38/00; C07K 1/00**

(52) **U.S. Cl.** **424/256.1; 424/234.1; 424/236.1; 424/184.1; 514/2; 530/350; 530/825; 530/806; 435/975; 435/7.2**

(58) **Field of Search** **424/256.1, 234.1, 424/186.1, 823, 824, 825, 826, 236.1; 514/2; 435/7.1, 975, 820, 961, 259, 261; 530/412, 350, 806, 825, 820**

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Primary Examiner—S. Devi

(74) **Attorney, Agent, or Firm**—Michael G. Sullivan

(57)

ABSTRACT

A cellfree extract of *Haemophilus parasuis* that exhibits toxic activity is described. The cellfree extract is useful as a vaccine or diagnostic reagent.

8 Claims, 1 Drawing Sheet

1

HAEMOPHILUS PARASUIS VACCINE AND DIAGNOSTIC

FIELD OF THE INVENTION

The invention relates to a cellfree extract preparation of *Haemophilus parasuis*, which has toxic activity. The preparation is useful as a vaccine and as a diagnostic agent for *Haemophilus parasuis*.

BACKGROUND OF THE INVENTION

Glässer observed in 1910 a small Gram-negative organism associated with a fibrinous serositis and polyarthritis in swine, which was eventually identified as *H. parasuis* and distinguished taxonomically from *H. suis*. It is now accepted that *H. parasuis* is the infectious agent of porcine polyserositis and arthritis (or Glasser's disease).

H. parasuis (Hps) is a small pleomorphic Gram-negative rod, varying from a coccobacillary form to slender filaments. Growth is supported only on media containing nicotinamide-adenine dinucleotide (NAD) (heated blood agar, Levinthal agar) or on blood agar in the vicinity of a streak of a *Staphylococcus* strain (satellitism). Visible growth occurs generally after 36–48 hours incubation.

Porcine polyserositis-arthritis is found worldwide and was historically considered to be a sporadic stress-associated disease of young pigs. More recently, introduction of the pathogen into large populations of specific pathogen free (SPF) herds has shown a devastating effect: infection may spread as a contagious disease of high morbidity, affecting pigs of all ages without obvious associated stress factors. The disease most often affects young pigs (2 weeks to 4 months), principally after the weaning period (5–8 weeks). Mortality may reach 50%.

Serological studies using extracts from autoclaved cells with an agar-gel precipitation test have shown at least fifteen distinct serotypes that differ in their pathogenicity (Kielstein, *J. Clin. Micro.* 30:4:862, (1992)).

Vaccines for *H. parasuis* are commercially available, which are made of inactivated bacteria, or bacterins. A disadvantage of bacterin vaccines is that they elicit antibodies against primarily (lipo)polysaccharides that are only specific for a certain serotype of *H. parasuis* and hence are not protective against other *H. parasuis* serotypes. The degree of protection against field infection afforded by bacterin vaccines may also be lower than desired.

Even though not currently available, live attenuated *H. parasuis* vaccines would suffer from the drawbacks normally associated with live vaccines, including the risk of inoculating animals with inadequately attenuated pathogens and the possibility that the attenuated bacteria may revert to a pathogenic state resulting in disease in the inoculated animal and spread of the pathogens to other animals.

Thus, there is a need for a *H. parasuis* vaccine which is safe, effective and provides heterologous protection (serotype independent). To this end, the present inventors endeavored to develop an alternative vaccine.

The rapid onset of disease and severity of internal damage suggest that the clinical effects may be caused by the expression of one or more Hps toxin(s). It has been suggested that Hps may induce functional and structural damage to the nasal mucosa by initially associating with the mucous layer and inducing damage to the underlying mucosa by release of one or more toxic compounds (Vahle, J. L., Ph.D. thesis, AAI9626072, 960902, Iowa State University (1996)). The chemical nature of any such toxins and how they exerted their effects was not elucidated.

2

Gram-negative endotoxin has been studied as a possible virulence factor of *H. parasuis*. Endotoxin is a lipopolysaccharide (LPS) component of the outer membrane of Gram-negative bacteria and is released with bacterial lysis. The study of Amano et al (*J. Vet. Med. Sci.* Vol. 59, No. 6, 451–455 (1997)) reported that Gram-negative endotoxin levels increased in the blood of pigs with acute septicemia caused by *H. parasuis*, and that *H. parasuis* antigen was found associated with pathological lesions. However, one cannot conclude on the basis of the findings of Amano et al. that it is the endotoxin that is causing the clinical and pathological manifestations of the disease, and the identity of the factor(s) causing the pathological effects of *H. parasuis* infection remains unclear.

It is an object of the present invention to find one or more toxins that contribute to the pathology of *H. parasuis* infection. It is also an object of the present invention to develop vaccines containing the one or more toxins. Finally, it is an object of this invention to use this antigenic material as a reagent in a diagnostic test.

SUMMARY OF THE INVENTION

The present invention provides a novel cellfree extract of *Haemophilus parasuis*, which has toxic activity. This extract causes pathological lesions characteristic of *H. parasuis* infection when administered intraperitoneally to pigs. Thus, the extract appears to contain at least one toxin, which is termed herein HpTx. It is also contemplated that there may be more than one toxin in this extract.

Also provided are vaccines containing the extract, which are effective in providing protection against homologous and heterologous challenge. It is contemplated that vaccines containing the isolated toxin or toxins as the immunogen(s) will provide this same type of protection.

Finally, the extract is useful as a diagnostic tool, and can be used to raise antibodies that can be used as diagnostic reagents to detect *H. parasuis* antigen, or could be used itself as a diagnostic reagent to detect antibodies to *H. parasuis*. Kits for diagnostic tests using these materials are also considered part of the present invention.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows the results of SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of a cell free extract prepared in Example 1 (Preparation C) along with purified outer membrane proteins (OMPs) of serotypes 4 and 5.

DETAILED DESCRIPTION OF THE INVENTION

The cellfree extract of the present invention is essentially prepared by lysing the bacterial cells, pelleting leftover cells and large cell debris, and collecting the supernatant. The bacterial cells may be lysed by any conventional method; preferably, high pressure is used, such as passing through a microfluidizer. Preferably, the bacterial culture is concentrated prior to lysing, by centrifugation and resuspension of the cell pellet in a smaller volume of fluid than originally present.

Any serotype or strain of *H. parasuis* may be used to prepare the cellfree extract of the present invention. Preferred are serotypes 4 and 5; most preferred is serotype 4.

Preferably, the supernatant containing the cellfree extract is filtered to remove any remaining cells or debris, and this is preferably done using a 0.2 micron filter. The supernatant may be frozen for future use. It is also possible to freeze-dry

the cellfree extract and reconstitute the lyophilized material just prior to use.

For parenteral administration, the supernatant material itself may be used as a vaccine formulation or may be admixed with an appropriate pharmaceutical carrier as desired. Lyophilized material is reconstituted in a pharmaceutically suitable carrier, such as an aqueous medium or a water-containing suspension. These carriers are often mixed with other constituents, for example, in order to increase the immunizing activity or shelf life. These constituents may be salts, pH buffers, stabilizers, emulsifiers, and adjuvants to improve the immune response. Examples of adjuvants, without intending to be limiting, include oil-in-water and water-in-oil emulsions, Vitamin E and other tocopherols, mineral oils, metabolizable organic oils, aluminum compounds, muramyl dipeptide, saponins, polyanions, amphipathic compounds, metal salts, and block (co)polymers. The use of adjuvants is not necessary, however, to provide immunogenic activity to the compositions of the present invention.

The immunogenic preparations of the invention are preferentially administered to swine parenterally, generally by intramuscular or subcutaneous injection. The vaccine is most effective if administered in a series of at least two doses separated by two to three week intervals, but other immunization schedules may be efficacious and may be determined by conventional means.

The amount of cellfree extract preparation used is the amount that is capable of inducing immunity in swine against challenge by *H. parasuis*. Immunity is defined as the induction of a significant level of protection in a population of swine after vaccination compared with an unvaccinated group. On a per dose basis, the quantity of the cellfree extract in a vaccine dose can range from 10 micrograms of protein to about 10 milligrams of protein per pig. A preferable range is from about 1 milligram to about 5 milligrams per pig. One skilled in the art can readily determine the appropriate dose, usually according to the age of the pig.

The vaccines of the present invention may also be formulated into multivalent vaccines comprising other immunogenic materials from one or more other pathogens. For example, the vaccine may contain material from one or more of the following pathogens: pseudorabies virus, transmissible gastroenteritis virus, porcine parvovirus, swine influenza virus, *Mycoplasma hyopneumoniae*, *Escherichia coli*, *Erysipelothrix rhusiopathiae*, *Bordetella bronchiseptica*, *Pasteurella multocida*, and *Actinobacillus pleuropneumonia*.

In a further embodiment of the present invention, the cellfree extract of the invention may be used as a diagnostic tool to detect infection by *H. parasuis*. For instance, the cellfree extract may be used as antigen in an immunological test for *H. parasuis* antibodies in a test sample (tissue or body fluid, e.g. blood, plasma, serum, etc.). Alternatively, the cellfree extract can be used to raise antibodies (monoclonal or polyclonal) to the antigenic material, and the antibody or antibodies can in turn be used in an immunological test for antigen in the test sample. Any immunological test format is contemplated, such as ELISA, Western blot, sandwich assay, etc., which are well known to those skilled in the art. Diagnostic test kits are also embodied in the present invention, and may comprise one or both of cellfree extract and antibodies thereto, optionally bound to a solid support and optionally containing the various reagents (buffers, etc.) used in the test. Detectable labels for the antigen and antibody are well known in the art.

The compositions and methods of the present invention are illustrated by the following non-limiting examples.

EXAMPLES

Example 1

Method of Preparation of Cellfree Extract Culture Conditions

H. parasuis serotype 4, strain SW124, was grown in Tryptic Soy Broth containing 0.1% NAD and 1% yeast extract (H-TSB) with 5% horse serum in 3000 ml total volume. The culture was grown at 37° C. for 5 hours, to about 1×10^8 – 1×10^9 CFU/ml.

Preparation A

After the above incubation, 250 ml of the culture was harvested by centrifugation at 6000 rpm for 15 minutes to separate out the cells. The supernatant was filtered with a 0.2 μ filter (Nalgene). This is a supernatant preparation that would contain any exotoxin(s) and other secreted materials, and is used for comparison purposes.

Preparation B

The remaining portion of the culture (2500 ml) was centrifuged at 6000 rpm for 15 minutes. For this preparation the cells were concentrated by discarding the supernatant and resuspending the pellet with saline to a volume of approximately 300 ml.

The concentrated cells were then lysed with a single pass through a microfluidizer (Microfluidics, Newton, Mass.) at 16,000 psi. The lysed cells were then centrifuged at 8000 rpm for 20 minutes to remove cell debris. The supernatant of this run was then collected and filtered with a 0.2 μ filter to remove any remaining cells or large cellular debris. This is a cellfree extract preparation that has toxic activity.

Preparation C

In a preferred method of preparing the cellfree extract, *Haemophilus parasuis* serotype 4, strain SW124, is grown at 37° C. in H-TSB for 3–5 hours to approximately 30% T (1×10^8 – 1×10^9 CFU/ml). The culture is centrifuged at 8500 $\times$ g for 20 minutes. The cell pellet is resuspended in sufficient saline equivalent to a 10-fold concentration of the cells originally present. The cell concentrate is microfluidized at approximately 16,000 psi. Large cell debris is removed by centrifugation at 15,000 $\times$ g for 20 minutes. The supernatant is filtered through a 0.2 μ m filter. This filtrate can be used as a fresh preparation, but may also be frozen or lyophilized for future use. Storage is at about –70° C.

Example 2

Testing of Biological Activity in Pigs

The preparations of Example 1 were then used to assess biological activity in pigs. Three pigs were inoculated intraperitoneally with 10 ml of preparation A, three with 10 ml of preparation B (3 mg/ml protein), and two served as controls. The controls were not inoculated at all. All animals were kept in the same room and same pen during the study. The feed was removed 16–24 hours prior to the administration of the cellular fractions (A and B).

Within 24 hours of inoculation one of the pigs inoculated with preparation B (no. 1681) was dead. Necropsy showed fibrin strings and fluid accumulation in the peritoneal cavity. The thoracic cavity showed fibrin strings and pleuritis.

Several of the remaining pigs (see TABLE 1) were showing respiratory signs 24 hours post inoculation, and all remaining pigs were then sacrificed and necropsied. The results are shown on TABLE 1.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES

Abogado
Emilio Ferrero

ASUNTO	Radicación	06-50044
	Trámite	011
	Evento	001
	Actuación	430
	Oficio	006
	Folios	006

06-50044

Patente de Invención	☐	Patente de Modelo de Utilidad	☐
Vía Nacional	☐		
Vía PCT (fase nacional)	☐	Cap. I	☐
		Cap. II	☐
No. Sol. Internacional	PCT/US2004/036336		
Publicación Internacional	WO 2005/041890		

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

<u>Descripción:</u>	Folios: 30 a 50 como se radicó inicialmente.
<u>Reivindicaciones:</u>	Folios: 51 a 57 como se radicó inicialmente. Folios: 80 a 87 modificadas fase Internacional
<u>Prioridad:</u>	30/10/2003, Estados Unidos, US 60/515,860 28/10/2004, Estados Unidos, US 10/975,702

2. Objeto de la invención

Título de la solicitud: “Vacuna para *Erysipelothrix rhusiopathiae* – *Haemophilus paraseis* y métodos de uso de las mismas”.

El objeto de la presente solicitud de patente de invención es suministrar una vacuna para conferir inmunidad contra *Erysipelothrix rhusiopathiae* de dosis simple y suministrar una vacuna combinada de *Erysipelothrix rhusiopathiae* - *Haemophilus paraseis* de dosis única y métodos para elaborar las mismas.

El problema planteado en esta solicitud es que las vacunas existente para estos dos organismos, son vacunas separadas que requieren dosis múltiples durante el intervalo de vida.

Para solucionar este problema técnico la solicitud en estudio proporciona una vacuna para conferir inmunidad contra *Erysipelothrix rhusiopathiae* que se administra en dosis única y que confiere una inmunidad de al menos 4 meses. Adicionalmente, la solicitud proporciona una vacuna combinada *Erysipelothrix rhusiopathiae* - *Haemophilus paraseis* que puede ser administrada en una dosis única y con una duración de inmunidad (DOI) equivalente a 4-6 meses.

Clasificación Internacional Patentes (IPC): A61K 39/085, 39/102, 39/02, 38/00, 39/12, 39/00; A01N 63/00; C12N 1/00; C07K 1/00; A61P 37/04.

3. De la solicitud de Patente

3.1. Reivindicaciones (artículo 30 D 486)

El capítulo reivindicatorio presentado carece de concisión y claridad por cuánto:

- 1) La reivindicación 1 y 9, ambas independientes, no definen de manera clara los componentes de la composición y vacuna, respectivamente. Debe indicarse cual es la cantidad de antígeno y el adyuvante, teniendo en cuenta la materia sustentada en la descripción. Por ejemplo el único adyuvante sustentado es la base de aceite mineral ISA206;
- 2) Falta sustento en la descripción para la vacuna de *E.rhusiopathiae* reclamada en la reivindicación 9, si bien se menciona la posibilidad de una composición que proteja contra infección de este organismo a lo largo de la descripción, los ejemplos aportados prueban dos tipos de formulaciones: composición combinada *E.rhusiopathiae* –*H.parasuis* (ejemplos 1 y 3) y *H.parasuis* (ejemplo 2).
- 3) Se utilizan términos ambiguos (en la mayoría de reivindicaciones independientes incluso) que no permiten definir la invención, tales como: “una cantidad de antígeno” (RV 1, 9,37);
- 4) Hay reivindicaciones que repiten las mismas características técnicas, entre reivindicaciones independientes e incluso entre las dependientes, lo que hace que el capítulo reivindicatorio carezca de concisión (RV 19 y dependientes y RV 28 y dependientes). Estas reivindicaciones parecen ser usos diferentes del mismo proceso;
- 5) La indicación de cantidad efectiva de antígeno dada en volumen no indica la concentración del mismo (RV 3,5,11,13,22,30,31);
- 6) La reivindicación 2 no da ninguna característica técnica que permita establecer la cantidad de antígeno empleada;
- 7) La reivindicación 38 carece de sustento puesto que la única inactivación realizada para las bacterias ensayadas es inactivación por formalina;
- 8) La reivindicación 41 no define una característica técnica puesto que no es claro ni objetivo el término “estandarizar dicha composición para suministrar cantidades efectivas conocidas de antígeno”;
- 9) Las reivindicaciones 1,4 y 47 presentan errores tipográficos;

De esta manera, el capítulo reivindicatorio presentado no cumple con los requisitos establecidos en el art. 30 de la Decisión 486.

4. Excepción a la patentabilidad (artículo 20 D486 literal d)

El método para reducir el riesgo a un animal de desarrollar signos clínicos de una determinada patología, donde este método consiste en administrar un fármaco y monitorear dicho animal, tal como es mencionado en las reivindicaciones 19 -36, constituye un método de tratamiento aplicado a animales; según la norma comunitaria los métodos de diagnóstico y tratamiento están excluidos de patentabilidad, por tanto, la materia reclamada en estas cláusulas no podrá ser patentada.

De esta manera y de acuerdo a lo establecido en el artículo 20 de la Decisión 486 el contenido de las reivindicaciones 19 a 36 deberá excluirse.

5. Unidad de invención (artículo 25, D486)

A partir del capítulo reivindicatorio se identifican dos grupos de invenciones:

- Composición que comprende antígeno basado en célula de *Erysipelothrix rhusiopathiae* y antígeno basado en célula de *Haemophilus parasuis* (Reivindicación 1 y dependientes). Esta invención se encuentra sustentada con la información aportada en la descripción;

- Vacuna de *Erysipelothrix rhusiopathiae* (Reivindicación 9 y dependientes). Como se mencionó en el numeral 3.1. de este concepto técnico esta invención no estaría totalmente sustentada en la descripción;

Se sugiere al solicitante revisar la totalidad del capítulo reivindicatorio y presentar las reivindicaciones independientes con todas las características técnicas que conforman su invención.

De acuerdo con lo anterior, el capítulo reivindicatorio en general no cumple con el requisito de unidad de invención establecido en el art. 25 de la Decisión 486.

5. Determinación del Estado de la Técnica

La fecha que se ha tenido en cuenta para determinar el Estado de la Técnica es:

Fecha de presentación de las solicitudes prioritarias: 30/10/2003 y 28/10/2004

Teniendo en cuenta la fecha indicada se realizó la búsqueda en el estado de la técnica de documentos relacionados con la solicitud encontrándose los siguientes documentos más relevantes:

La búsqueda se realizó sobre el primer grupo inventivo de la solicitud, es decir sobre la composición que comprende antígeno basado en célula de *Erysipelothrix rhusiopathiae* y antígeno basado en célula de *Haemophilus parasuis* (Reivindicación 1 y dependientes).

Nº	Documento	Título	Fecha de publicación
D1	US 6,019,984	"Bacterial preparations, method for producing same, and their use as vaccines"	01/02/2000
D2	US 6,342,231	" <i>Haemophilus parasuis</i> vaccine and diagnostic"	29/01/2002

Resumen de los documentos citados:

D1: Divulga una nueva preparación bacteriana que contiene una o más cepas aisladas y purificadas de un microorganismo el cual produce una o más toxinas RTX. Métodos de preparar la preparación bacteriana y su uso como vacunas es descrito.

D2: Un extracto libre de células de *Haemophilus paraseis* que exhibe actividad tóxica es descrito. El extracto libre de células es útil como una vacuna o reactivo de diagnóstico.

6. Análisis de patentabilidad (artículo 14 D486)

6.1. Novedad (artículo 16 D486)

La solicitud reclama una composición que comprende:

- una cantidad de antígeno basado en célula de *Erysipelothrix rhusiopathiae* ;
- una cantidad de antígeno basado en célula de *Haemophilus paraseis*; y
- un adyuvante

(Reivindicación 1 y dependientes).

En el estado del arte se encuentra el documento US 6,342,231 (D2) el cual divulga un extracto de *H. paraseis* preparado a partir de lisado de células bacteriales, el cual tiene actividad tóxica (columna 2, líneas 52-59). Esta composición de vacuna contiene también adyuvante (columna 3, líneas 12-16) y puede ser una vacuna multivalente que contiene antígeno de *Erysipelothrix rhusiopathiae* (columna 3, línea 37-46). De esta manera el documento D2 contiene todas las características esenciales de la invención reclamada en la solicitud.

Otro documento relevante es US 6,019,984 (D1) el cual divulga composiciones y vacunas bacteriales, comprendiendo una cantidad de antígeno *Erysipelothrix rhusiopathiae* y una cantidad de antígeno de *Haemophilus paraseis* y un adyuvante (columna 15, reivindicaciones, resumen, ejemplos). Este documento también divulga métodos de preparar composiciones de vacunas y métodos de inmunización de animales (columna 15 y ejemplos).

De lo anterior se evidencia que este documento también contiene todas las características técnicas de la invención. Por tanto, la invención reclamada carece de novedad frente a D1 ó D2 e incumple el artículo 16 de la D486.

6.2. Nivel inventivo (artículo 18 D486)

Además de las características técnicas de la reivindicación principal, el documento D2 también menciona otras características que coinciden con el desarrollo realizado en la solicitud en estudio. Entre estas tenemos:

- El serotipo o cepa empleado para *Haemophilus paraseis* puesto que se menciona que puede ser empleado cualquier serotipo, de manera preferida los serotipos 4 y 5 (columna 2, línea 60-63). La solicitud por su parte utiliza la cepa Z-1517, la cual corresponde a una denominación particular y no se

puede identificar dentro de un serotipo. Sin embargo, no parece aportar características diferentes a la invención;

- El tipo de adyuvante empleado, puesto que D2 menciona entre otros emulsiones aceite en agua y agua en aceite, aceites minerales, aceites orgánicos metabolizables, saponinas, copolímeros de bloque (columna 3, líneas 12-16). La solicitud por su parte, reclama adyuvantes con base de aceite mineral;
- El tipo de material antigénico empleado, puesto que en D2 utiliza lisado celular de *Haemophilus paraseis* el cual también es un antígeno basado en célula como el que reclama la solicitud. Lo mismo sucede con el antígeno inmunógeno que proviene de *Erysipelothrix rhusiopathiae*, por ser indeterminado también se puede considerar un antígeno basado en célula como el reclamado por la solicitud.

De lo anterior se evidencia, que a partir de la información divulgada en D2 un técnico medio hubiese podido realizar la invención tal como es reclamada en la solicitud. Por tanto, frente a D2 el desarrollo reclamado carece de actividad inventiva e incumple el artículo 18 de la D486.

7. Conclusión:

Los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

N°	Documento N°	Reivindicaciones afectadas	Requisito que afecta
D1	US 6,019,984	1-8, 19-51	Novedad
D2	US 6,342,231	1-8, 19-51 1-51	Novedad Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de 60 días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.


JOSE LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista, de fecha
_____ 11 FEB. 2011

CONCEPTO TÉCNICO No. 274	EXAMINADOR TÉCNICO Código No.202052
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