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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Re: Expediente No. 06-007-814

Solicitud de patente de invención titulada: "VACUNAS VIRALES SEGURAS QUE CONTIENEN AL MENOS DOS VIRUS MUTANTES DE LA FAMILIA FLAVIVIRIDAE Y MÉTODOS PARA SU PREPARACIÓN"

Solicitante: PFIZER PRODUCTS INC.

N. Ref.: P2006/000013

RESPUESTA AL OFICIO No.08787 NOTIFICADO POR FIJACION EN LISTA No. 242

DE FECHA 09 DE JULIO DE 2010

(PRIMER EXAMEN DE FONDO)

CARLOS R. OLARTE, mayor de edad y vecino de Bogotá, identificado tal como aparece al pie de mi firma, en mi carácter de apoderado de la sociedad solicitante, me permito manifestar lo siguiente ante el auto emanado por ese Despacho, dentro del plazo legal previsto en el artículo 45 de la Decisión 486, en los siguientes términos:

1. En primer lugar, y con el ánimo de brindar mayor claridad a la presente solicitud, me permito adjuntar un **Capítulo Reivindicatorio Modificado**, que reemplaza en su totalidad al Capítulo Reivindicatorio presentado en respuesta al examen de forma, de fecha 16 de mayo de 2006.

Así entonces, en el Capítulo Reivindicatorio Modificado contiene los siguientes cambios:

- Se han eliminado las Reivindicaciones 2, 3, 13 y 14;
- La antigua Reivindicación 4 se renumera como nueva Reivindicación 2;
- Se han limitado las Reivindicaciones 1 y 2 exclusivamente a dos virus de Diarrea Viral Bovina (BVDV) mutantes vivos tipo 1 y 2 (BVDV-1 y BVDV-2);
- Se han introducido las nuevas Reivindicaciones 12 a 14, que encuentra soporte en la Reivindicación 4 (antigua Reivindicación 6) y en la Reivindicación 9 (antigua

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Reivindicación 11), en donde se especifica la región de mutación de los virus BVDV, y que dicha mutación se realiza a través de una inserción.

Valga anotar que las modificaciones efectuadas al Capítulo Reivindicatorio cumplen a cabalidad con lo estipulado en el Artículo 34 de la Decisión 486, ya que éstas de ninguna manera implican una ampliación de la protección correspondiente a la divulgación contenida en la solicitud inicial.

2. a- El Examinador argumenta que la Reivindicación 1 es demasiado amplia con respecto a lo divulgado en el Capítulo Descriptivo, puesto que se divulgan composiciones de vacuna que comprende al menos dos virus mutantes vivos de la misma familia, pero se reclaman una amplitud ilimitada de virus de dicha familia sin estar soportados en el Capítulo Descriptivo. El Examinador establece que solo se encuentra soporte para los virus BVDV-1 mutante y BVDV-2 mutante.

Adicionalmente, el Examinador establece que el método reclamado en las Reivindicaciones 10 a 14 no se encuentra definido por sus características técnicas y no se encuentra sustentado en el Capítulo Descriptivo.

b- Considero superada la anterior objeción, en tanto que la Reivindicación 1 se ha limitado a los virus BVDV-1 mutante y BVDV-2 mutante. Adicionalmente, en las nuevas Reivindicaciones 12 a 14 se especifica que el proceso de mutación ocurre en la región NS2-3 y se efectúa a través de una inserción.

3. a- El Examinador argumenta que la patente CO05-14184 (D1), afecta el nivel inventivo de la presente invención.

El Examinador señala que D1 enseña una composición inmunogénica que comprende 5 virus vivos: virus herpes bovino (BH-1), virus de parainfluenza tipo 3 (PI3), virus sincital respiratorio bovino (BRSV), virus de la diarrea viral bovina tipo 1 (BVDV-1) y virus de la diarrea viral bovina tipo 2 (BVDV-2).

El Examinador establece que la única diferencia entre las composiciones divulgadas en la presente invención y D1 es la mutación en el mismo sitio genómico de los dos virus de la misma familia. Según el Examinador, dichas mutaciones no se encuentran definidas correctamente y no conceden un efecto inesperado a la composición, ya que los componentes inactivos modificados BVDV-1 y BVDV-2 de D1 proporcionarían el mismo efecto técnico y podrían presentar presumiblemente la misma estructura molecular.

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El Examinador concluye que el problema de proporcionar vacunas seguras y efectivas ya se había resuelto en D1 utilizando los mismos ingredientes activos, por lo tanto la presente solicitud carece de nivel inventivo.

b- i) En primera instancia, me permito llamar respetuosamente la atención del Examinador sobre el hecho que el Artículo 16 de la Decisión 486 establece que el contenido de una solicitud de patente en trámite ante la oficina nacional se considerará dentro del estado de la técnica, cuando su fecha de presentación o de prioridad es anterior a la fecha de presentación o de prioridad de la solicitud de patente que se estuviese examinando, sólo para efectos de la determinación de la novedad.

En el presente caso, el Examinador argumenta que D1 afectaría el nivel inventivo de la presente solicitud, a pesar de que la publicación de la solicitud contraparte internacional (WO2004/017990) ocurrió el 10 de marzo de 2005, fecha posterior a la fecha de prioridad de la presente solicitud (26 de agosto de 2002).

Claramente, según el Artículo 16, la solicitud CO05-14184 (D1), que tiene una fecha de presentación internacional anterior a la fecha de prioridad de la presente solicitud, pero una fecha de publicación internacional posterior, no es válida para evaluar el nivel inventivo de la presente solicitud.

ii) Ahora bien, en esta oportunidad deseo resaltar que la presente invención reclama explícitamente una composición de vacuna que comprende dos virus: un virus de Diarrea Viral Bovina Tipo 1 BVDV- 1 mutante y un virus de Diarrea Viral Bovina BVDV- 2 mutante.

Al momento de presentar esta solicitud, se planteaban problemas de seguridad respecto del uso de múltiples cepas de virus BVD en una misma vacuna viva modificada, específicamente relacionados con la posibilidad de recombinación entre las cepas citopáticas de los virus BVD contenidas en dicha composición de vacuna. La preocupación se relacionaba con el hecho que durante el proceso de investigación y producción de una vacuna que comprende dos virus vivos, es alta la posibilidad de que la RNA-polimerasa mezcle información genética de los dos virus en una nueva cadena, proceso denominado recombinación. Dicho nuevo virus recombinado genéticamente puede resultar defectuoso, sin posibilidad de replicarse, o por el contrario, puede resultar un nuevo virus viable, con la posibilidad de replicarse. En algunos casos, el virus viable puede causar citopatogenicidad.

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Así entonces, la contribución de la presente invención al estado del arte, es precisamente que el inventor superó dicha enseñanza y produjo una vacuna que contiene múltiples cepas del virus BVD vivo, demostrando que es segura (como se ha ejemplificado en el Ejemplo 2 del Capítulo Descriptivo) y eficaz (como se ha demostrado con éxito en las ventas de Bovishield®Gold). Adjunto a este memorial –ANEXO 1, se presenta el Boletín Técnico “Bovishield®GOLD circumvents non-cytopathic recombination of vaccine line's cytopathic strains” que describe la presente invención. En este documento se explica el principio de las inserciones, así como la novedad y los efectos sorprendentes e inesperados de la presente invención, específicamente, muestra que Bovi-Shield® GOLD contiene los virus BVD tipo 1 y tipo 2 que contienen insertos celulares en el mismo sitio genómico, de tal forma que los virus mutantes no se pueden recombinar uno con el otro para eliminar las mutaciones. Específicamente, en la presente invención se posibilita la producción de un virus citopático BVDV, a partir de la recombinación de virus BVDV no citopáticos.

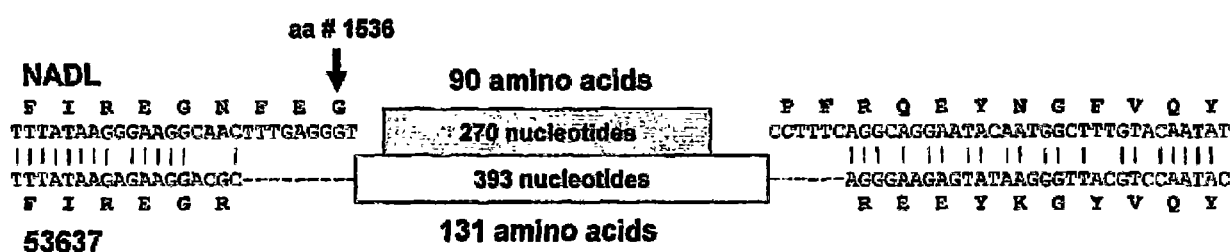
La presente invención es una vacuna combinada, más segura que las vacunas anteriores. En el momento en que esta solicitud fue presentada, las enseñanzas del estado de la técnica alejaban a la persona medianamente versada en la materia a usar dos virus BVD en la misma vacuna. Ridpath, et al, (Virology, 1995, Vol. 212, p. 259-262) –ANEXO 2, divulga la muerte de un animal por enfermedad en las mucosas (MD) luego de que dicho animal fuera vacunado con una cepa del virus BVD-1 vivo modificado. Los autores especularon que la enfermedad de las mucosas fue inducida luego de la recombinación entre BVDV-1-NADL (provenientes de la vacuna) y el virus BVD-2-125 no citopático. Así, este documento enseña que la recombinación puede ocurrir, y que adicionalmente, dicha recombinación tiene como resultado la muerte del animal, alejando, en consecuencia, a los inventores de la presente invención, ya que demuestra que la recombinación de virus tienen como resultado un virus BVD citopático que mata el animal. Lo anterior lleva a la conclusión que la combinación de virus modificados vivos en una vacuna no debe llevarse a cabo, a menos de que se asegure que dicha recombinación no puede ocurrir.

Contrario a las enseñanzas del estado de la técnica, la presente invención proporciona un mecanismo relacionado con una vacuna que comprende virus BVD mutantes vivos, en donde cada virus contiene una mutación en el genoma viral que residen en el mismo sitio genómico, de tal forma que los virus mutantes no pueden recombinarse el uno con el otro con el fin de eliminar las mutaciones. Lo anterior tiene como resultado una vacuna que es más segura que otras vacunas por fuera del alcance de la presente invención, demostrando que la solución al problema de la recombinación y seguridad de la vacuna no es obvia.

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Tal virus citopático BVDV de la invención, puede ser obtenido cuando la hebra negativa del genoma codifica para la inserción de material genético a partir de la célula hospedera infectada (que se denomina “inserto celular”) en la porción NS2-3 de la hebra positiva del genoma. La inserción permite una escisión subsecuente del producto del gen NS2-3 en las proteínas NS2 y NS3. La proteína NS3 resulta ser el marcador de citopatogenicidad del virus BVD. Por lo tanto, la ausencia de la inserción mantiene el gen NS2-3 (ya que la proteína no es escindida) y define el virus BVD como no citopático.

Teniendo en cuenta lo anterior, los investigadores analizaron los genomas de cada virus y gracias a ensayos *in-vitro* determinaron el potencial de que el evento de recombinación resultara en un virus BVD no citopático. Los resultados de la secuenciación de la región del genoma NS2-3 para los virus BVDV-1 y BVDV-2 utilizados en la composición de vacuna de la presente invención, se muestran en la siguiente figura:



Como se evidencia, ambas hebras contienen un inserto, precisamente en el mismo sitio del gen NS2-3, que es la región que los codifica como virus citopáticos en el genoma. Ahora bien, teniendo en cuenta la escisión proteica mencionada anteriormente, y el mismo sitio de inserción para ambos virus, no existe oportunidad para que los virus se recombinen de manera homóloga y se regenere el producto génico NS2-3 intacto, ya que la RNA-polimerasa no tiene otra opción que codificar la inserción en la misma región sin importar qué hebra de qué virus BVD tome como plantilla. Por lo tanto, el producto génico de NS2-3 se escindirá invariablemente, lo que resulta en una hebra citopática.

Específicamente, las mutaciones ejemplificadas se definen claramente en el Ejemplo 1: una inserción de 270 nucleótidos de la secuencia *DnaJ* dentro cepa NADL, sin eliminación o duplicación de la secuencia NADL; una inserción de 393 nucleótidos de la secuencia *DnaJ* dentro de la cepa 53637, con la eliminación de 15 nucleótidos de la secuencia 53637. El capítulo descriptivo, en la página 5, línea 22 a la página 6, línea 5, define claramente que las mutaciones son suficientemente grandes para que la recombinación homóloga no pueda ocurrir, y es

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inherente en la descripción que las mutaciones no son tan grandes como para que se afecte la producción del virus vivo.

Así las cosas, el nivel inventivo de la presente invención recae precisamente en la determinación y solución al problema de combinar los virus BVDV-1 y BVDV-2 en una sola composición de vacuna, teniendo en cuenta la particularidad genética de los dichos virus. La solución encontrada determinó que al encontrarse los virus mutados, en el mismo sitio genómico, la posibilidad de recombinación homologa con el fin de eliminar dicha mutación no existe, proporcionando así una vacuna segura y eficaz.

En cuanto a las combinaciones de diferentes virus, antigua Reivindicación 4, me permito señalar que las combinaciones en vacunas no se pueden evaluar de la misma forma que las combinaciones de fármacos en una composición farmacéutica, puesto que, las combinaciones en vacunas pueden sufrir del fenómeno de supresión inmunológica o interferencia antigénica. Adjunto al presente memorial –ANEXO 3, las etiquetas de los productos: Bovi-Shield GOLD® 4; Bovi-Shield GOLD® FP® 5; Bovi-Shield GOLD® FP® 5 VL5; y Bovi-Shield® GOLD IRB-BVD-BRSV-LP, en donde se demuestra que esta línea de vacunas es segura y eficaz, aún cuando correspondan a combinaciones de diversos virus, puesto que de ser inefectivas o no seguras, no habrían aprobado los exámenes realizados por las entidades regulatorias o no se habría alcanzado éxito en el mercado si fueran.

iii) Ahora bien, si en gracia de discusión se considerara que el documento D1 es válido como arte previo válido para afectar el nivel inventivo de la presente invención, también difiero respetuosamente de la posición del Examinador al establecer que el problema de proporcionar vacunas seguras y eficaces se había resuelto a partir de D1 usando el mismo tipo de compuestos.

Es importante señalar que D1 divulga el desarrollo de vacunas multivalentes, como lo mencionó el Examinador (5 virus vivos: virus herpes bovino (BH-1), virus de parainfluenza tipo 3 (PI3), virus sincital respiratorio bovino (BRSV), virus de la diarrea viral bovina tipo 1 (BVDV-1) y virus de la diarrea viral bovina tipo 2 (BVDV-2)), incluyendo los virus de la diarrea viral bovina tipo 1 y tipo 2. Sin embargo, nada en D1 se refiere o resuelve el problema de la posible recombinación de dos virus en el animal vacunado al eliminar la mutación atenuante. Esto porque los virus BVD usados en D1 se encuentran inactivados y no mutados (ver página 11 de la contraparte de D1 –WO2004/017990), y como es sabido, virus inactivados no pueden recombinarse uno con el otro.

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En conclusión, la persona medianamente versada en la técnica no encontraría nada en D1 que lo condujera a resolver el problema de combinar en una composición de vacuna dos virus vivos de la misma familia, evitando que se recombinen mediante la mutación del mismo sitio genético, y así proporcionar composiciones de vacuna seguras y eficaces.

4. Finalmente, me permito informar que, para el concepto inventivo comprendido en la presente solicitud, ha sido otorgado hasta el momento el privilegio de patente por parte de Oficinas de Patentes en Australia (AU2004268514), Eurasia (EA009391), India (IN239729), Corea del sur (KP10-0810820), México (MX262069), Filipinas (PH1-2006-5002), Rusia (RU009391), Sudáfrica (ZA2006/00585), Ucrania (UA85845), y Estados Unidos (US7,361,357 y US7,754,222).

Si bien es cierto que las concesiones de patentes en oficinas de otros países no son vinculantes en el sentido que obliguen a la SIC a tomar una decisión en el mismo sentido, considero que dichas concesiones sugieren el cumplimiento de los requisitos de patentabilidad de la materia reivindicada, ya que los criterios de evaluación y estudio aplicados para concluir la aceptación de dichas patentes resultan equivalentes a los que la SIC debe emplear.

5. Por todo lo anterior, considero que las objeciones presentadas por el Examinador en su examen de fondo han sido resueltas y por consiguiente solicito respetuosamente que se tengan por cumplidos los requisitos de patentabilidad, y se proceda con la concesión de la patente.

Atentamente,



CARLOS R. OLARTE
C.C. No. 79.782.747 de Bogotá
T.P. 74.295

Anexos:

- Capítulo Reivindicatorio Modificado.
- ANEXO 1:- Pfizer Animal Health Technical Bulletin, Kun *et al*: "*Bovi-Shield®GOLD* circumvents non-cytopathic recombination of vaccine line's cytopathic strains".

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CAPITULO REIVINDICATORIO MODIFICADO
(Primer Examen de Fondo)

1. Una composición de vacuna que comprende al menos dos virus de Diarrea Viral Bovina (BVDV) mutantes vivos, en donde cada virus contiene una mutación en el genoma viral, y las mutaciones en los virus se ubican en el mismo sitio genómico de tal manera que los virus mutantes no pueden recombinarse entre sí para eliminar las mutaciones.
2. La composición de vacuna de la Reivindicación 1, en donde los dos virus mutantes vivos consisten en un virus de Diarrea Viral Bovina Tipo 1 BVDV- 1 mutante y un virus de Diarrea Viral Bovina BVDV- 2 mutante.
3. La composición de vacuna de la Reivindicación 2, en donde los dos virus mutantes vivos consisten de un BVDV- 1 citopático (cp) y un BVDV- 2 cp, y ambos son atenuados.
4. La composición de vacuna de la Reivindicación 3, en donde el BVDV- 1 cp y el BVDV- 2 cp ambos comprenden una mutación en la región NS2- 3 lo cual resulta en un biotipo citopático. CTE.
5. La composición de vacuna de la Reivindicación 4, en donde el BVDV- 1 cp es BVDV- 1 NADL, y el BVDV- 2 cp es BVDV- 2 53637.
6. La composición de vacuna de la Reivindicación 2, que además comprende por lo menos uno entre herpesvirus- 1 bovino, virus sincitial respiratorio bovino, parainfluenza virus- 3, *Campylobacter fetus*, *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira hardjo*, *Leptospira icterohaemorrhagiae*, *Leptospira pomona*, o *Mannhemia haemolytica*.
7. La composición de vacuna de la Reivindicación 1, que además comprende un vehículo veterinariamente aceptable.
8. Un método para la preparación de vacunas virales seguras el cual comprende seleccionar o construir dos virus BVDV mutantes vivos, en donde cada virus contiene una mutación y las mutaciones en los virus se ubican en el mismo sitio genómico de tal manera que los virus mutantes no puedan llevar a cabo recombinación homóloga para eliminar las mutaciones.

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Pfizer Animal Health

Technical Bulletin

106 106

November 2003



***Bovi-Shield® GOLD* circumvents non-cytopathic recombination of vaccine line's cytopathic strains**

Siao-Kun W. Welch, DVM, PhD, Jay G. Calvert, PhD, Xuemi Cao, PhD,
Michael K. O'Hara, PhD
Veterinary Medicine Research and Development-Biologicals
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Key Points

- All respiratory and reproductive vaccines in the *Bovi-Shield® GOLD* product line are formulated with two cytopathic strains of modified-live bovine viral diarrhea virus (BVDV)—BVDV Type 1 and BVDV Type 2.
- Sequencing the genomes of each strain in *Bovi-Shield GOLD* established the improbability of the strains recombining homologously to generate noncytopathic virus: because replication templates could code only for cytopathic RNA genomes, only cytopathic BVD viruses likely could be produced.
- Reverse transcriptase polymerase chain reaction (RT-PCR) assessment of the products of co-culturing the vaccinal BVDV strains confirmed the absence of noncytopathic BVDV, even after 12 co-culture passages.
- Genome sequencing of the cytopathic BVD viruses used in one other commercial modified-live BVD vaccine revealed a potential opportunity for recombination that could yield a noncytopathic BVDV strain.

In November 2003, Pfizer Animal Health began marketing the *Bovi-Shield® GOLD* line of modified-live virus (MLV) vaccines. The *GOLD* descriptor is applied to vaccines whose bovine viral diarrhea (BVD) component is formulated with two cytopathic strains of BVD virus (BVDV)—BVDV Type 1 and BVDV Type 2. All *Bovi-Shield GOLD* vaccines have label claims for use in healthy cattle as an aid in preventing IBR caused by IBR virus, BVD (Type 1 and Type 2) caused by BVDV, and disease

caused by parainfluenza type 3 (PI₃) virus and bovine respiratory syncytial virus (BRSV) as well as an aid in preventing abortion caused by IBR virus and persistently infected calves caused by fetal infection with BVDV Types 1 and 2. Vaccines in the *Bovi-Shield GOLD* line may be administered to pregnant cattle provided they were vaccinated according to label directions with *Bovi-Shield FP 4+ L5*, *Bovi-Shield FP 4 + VL5*, *Bovi-Shield GOLD FP 5 L5*, *Bovi-Shield GOLD FP 5 VL5*, *PregGuard® FP 9*,



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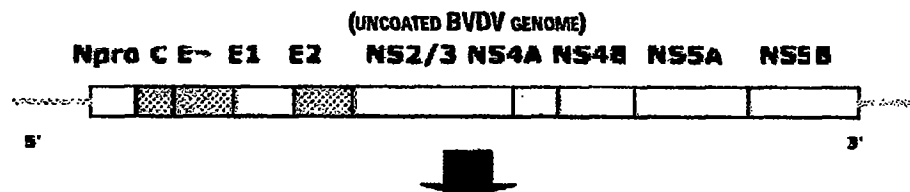
or *PregGuard GOLD FP 10* within the past 12 months.* *Bovi-Shield GOLD* vaccines used for protecting young cattle against respiratory disease may be administered to calves nursing pregnant cows provided their dams were vaccinated within the past 12 months as described above.

Previously, safety concerns have been raised over the possibility of two modified live cytopathic BVD viruses sharing genetic material and recombining to form new strains and/or noncytopathic strains. Recombination of genetic material from modified live BVDV vaccines has been demonstrated when persistently infected cattle were vaccinated.¹⁻⁴ Because *Bovi-Shield GOLD* is formulated with two cytopathic strains of BVDV, Pfizer scientists conducted studies—a genome sequencing study and a co-culturing and reverse transcriptase polymerase chain reaction (RT-PCR) assessment—to evaluate the possibility of the two strains recombining during replication to generate a noncytopathic BVDV strain. Presented in this bulletin is a summary of the investigations confirming the extreme improbability of a noncytopathic BVDV strain emerging from the recombination of the two cytopathic vaccinal strains.

Essential Scientific Background

To better understand the process of viral recombination and the adverse mutations that could result, it may be helpful to first review the sequence of events occurring on a cellular level when a positive-stranded ribonucleic acid (RNA) virus like BVDV infects a host cell. ("Positive stranded" indicates that the viral RNA can directly bind cellular ribosomes and direct them to manufacture viral proteins.) The viral replication process progresses in the following manner:

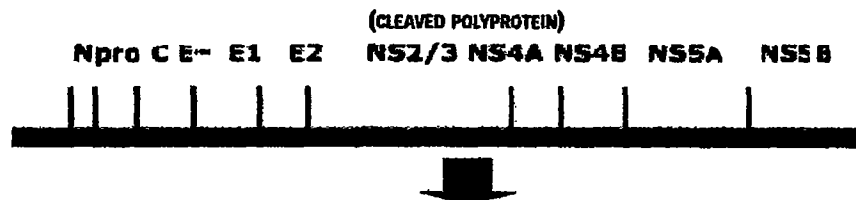
1. After the virus infects the host cell, the viral genome is uncoated.



2. Cellular ribosomes located in the cytoplasm of the infected cells scan the uncoated genome and synthesize a single large protein segment called a polyprotein.

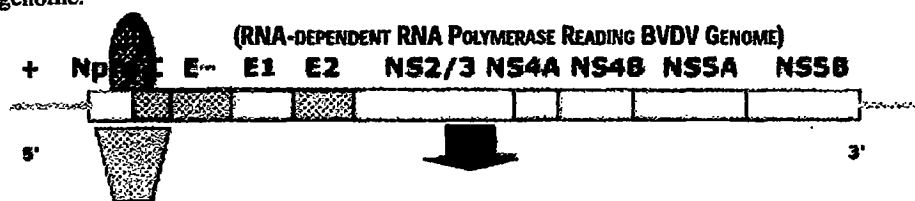


3. An enzyme produced by the infected cell scans the large polyprotein, cleaving it into individual protein segments.



*The *GOLD* designation is also given to vaccines in the *PregGuard*® line that are formulated with the same vaccinal strains of BVDV Types 1 and 2 used in *Bovi-Shield GOLD*.

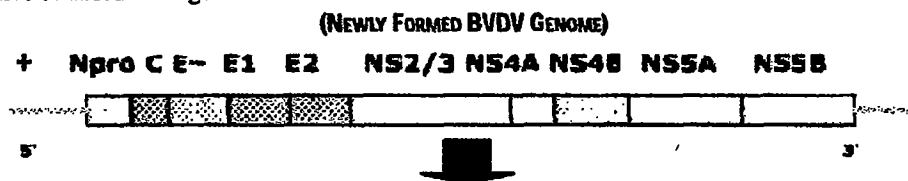
4. One of the individual non-structural proteins (NS5B)—known as RNA-dependent RNA polymerase—returns to the uncoated genome of the infecting virus and reads the entire genome.



5. The RNA-dependent RNA polymerase constructs a full-length negative strand of RNA that mirrors the gene sequence pattern of the infecting virus (i.e., the positive strand serves as a template to guide synthesis of the negative or complementary strand).



6. The negative-strand RNA in turn acts as a template to direct the synthesis of multiple positive-stranded virus genomes.



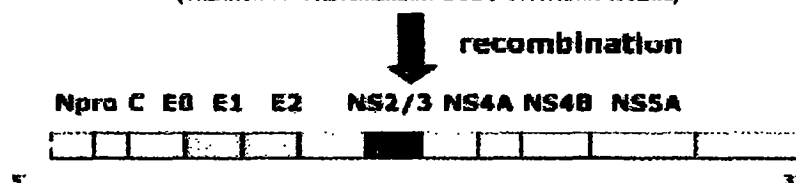
7. The newly synthesized positive-stranded genomes direct the synthesis of new viral proteins, which are used to construct the virion, and are also packaged in the virion.

(NEW BVDV VIRIONS)



In nature, recombination can occur when two strains of BVDV simultaneously infect a host cell. Under these conditions, the RNA-dependent RNA polymerase (Step 4 above) has more than one option when it reads the uncoated genomes of the co-infecting BVD viruses. And because it has options, the polymerase can sometimes switch from reading one virus template to reading the other. The end result of template switching is likely to be production of progeny virus containing genetic material from both infecting strains (recombinant virus). While most recombinant viruses of this sort contain defective genomes and are incapable of replication, rarely viable virus recombinants are produced that can replicate. It has been demonstrated, for example, that cytopathic BVDV can emerge from the recombination of noncytopathic BVDV. As the illustration below shows, cytopathic BVDV can be produced when a negative-strand genome codes for insertion of genetic material from the infected host cell (referred to

(CREATION OF A RECOMBINANT BVDV CYTOPATHIC ISOLATE)



as a "cellular insert") in the NS2/3 portion of the positive-strand genome. Presence of this insert allows for subsequent cleavage of the NS2/3 gene product into NS2 and NS3 proteins. NS3 protein is the marker for BVDV cytopathogenicity. Absence of the cellular insert maintains the integrity of the NS2/3 gene product (since the protein is not cleaved) and defines a BVD virus as being noncytopathic.

Recombination and the Production of BVD Vaccine

It is theoretically possible that a noncytopathic BVDV strain could emerge from the recombination of two cytopathic vaccinal strains. By bypassing the cellular insert sequences associated with vaccinal cytopathic viruses, the RNA-dependent RNA polymerase could conceivably create a replication template in host animal cells that is devoid of genome sequences coding for the production of cytopathic virus. Omission of these sequences "repairs" the BVDV genome and noncytopathic BVDV could be produced.

To assess the possibility of noncytopathic BVDV emerging from the recombination of the cytopathic BVDV strains in *Bovi-Shield GOLD*, Pfizer scientists analyzed the genomes of each vaccinal virus and then by in vitro methods (co-culturing and RT-PCR) assessed the potential of a recombination event yielding noncytopathic BVDV. For comparison purposes, the scientists also assessed whether the cytopathic BVDV strains used in another commercial BVD vaccine could potentially recombine to yield a noncytopathic strain.

Gene Sequencing

Figure 1 presents the results of genome sequencing of the NS2/3 region of the Type 1 and Type 2 BVDV strains in *Bovi-Shield GOLD*. As the figure shows, both strains have cellular inserts at precisely the same site in the NS2/3 gene, which is the region of these genomes that codes the viruses as cytopathic. As was noted above, cytopathic BVDV with a cellular insert in the NS2/3 region is associated with cleavage of the viral protein, whereas noncytopathic BVDV has no cellular insert and the NS2/3 gene product remains intact. Because these insertion sites are the same in the cytopathic BVDV viruses in *Bovi-Shield GOLD*, opportunity does not exist for the vaccinal viruses to recombine homologously and regenerate an intact NS2/3 gene product. The NS2/3 gene product will invariably be cleaved, which in turn yields a cytopathic strain. With the two BVDV strains in *Bovi-Shield GOLD*, the cellular insertion genes occur in the same location; thus, the RNA-dependent RNA polymerase has no choice but to code for the insertion in the same spot regardless of which BVDV template it reads.

In Vitro Evaluation

Having determined by genome sequencing the unlikelihood of generating a recombinant noncytopathic virus with the cytopathic BVDV strains in *Bovi-Shield GOLD*, Pfizer scientists next co-cultured the viruses to further assess whether a noncytopathic virus would emerge following virus replication. A total of 12 x 2 replicates of co-infection culture passages were conducted. To maximize the probability of recombination, the viruses were blind passaged (no virus identification

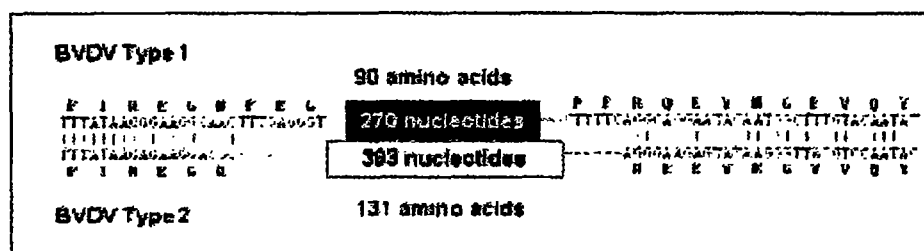


Fig. 1—Gene sequences for the NS2/3 region of the BVDV Type 1 and BVDV Type 2 in *Bovi-Shield GOLD*. Since both strains contain cellular inserts at the same spot in the NS2/3 coding region, the RNA polymerase, which uses the genomes as its blueprint, can only direct synthesis of viruses with cellular inserts at that same position.

between passages) for the first seven passages. Using samples obtained from the middle and final passage, scientists performed RT-PCR analyses to detect any non-cytopathic BVDV that might have emerged during co-culturing. The detection method was shown to be highly sensitive in positive controls where as few as one infectious particle of noncytopathic BVDV could be detected in the presence of 100,000 infectious particles of cytopathic BVDV. Throughout the 12 virus passages, total virus titers remained between 6 and 7 log₁₀ TCID₅₀/mL. The PCR samples were sequenced to determine their identity.

Results

No noncytopathic BVDV recombinants were detected by RT-PCR methods using combinations of Type 1 and Type 2 BVDV specific primers. Thus, the probability of recombination occurring with the two cytopathic BVDV strains in *Bovi-Shield GOLD* to produce noncytopathic virus was shown to approach zero.

RT-PCR amplification and sequencing of the NS2/3 region of the Type 1 and Type 2 BVDV strains used in the production of another commercial modified-live BVD vaccine revealed a potential risk of a recombination event for this vaccine. The Type 2 and Type 1 BVDV strains in this vaccine do not have cellular inserts located in the same region of the NS2/3 gene. This means that during virus replication the RNA-dependent RNA polymerase could potentially jump from reading the uncoated BVDV Type 2 genome to the BVDV Type 1 genome and skip over the genetic instructions coding for a cellular insert in the NS2/3 region. If this were to occur, the polymerase would code for an NS2/3 genome that was devoid of a cellular insertion site. As was noted above, when no cellular insert occurs in the product of the NS2/3 gene, the NS2/3 protein remains intact and noncytopathic virus results. Because the NS2/3 sequence in the Type 2 BVDV strain could be "repaired" by the sequence in the Type 1 BVDV strain, a risk exists with this second vaccine for the generation of a noncytopathic BVDV strain.

Conclusion

Genome sequencing and co-culturing and RT-PCR assessments of the cytopathic Type 1 and Type 2 BVDV strains in *Bovi-Shield GOLD* established that generation of noncytopathic BVD virus from the homologous recombination of the two vaccinal strains is virtually impossible. Sequencing first indicated the improbability of the event. Only replication templates coding for cellular inserts in the same NS2/3 region of the BVDV genome could be built from the RNA of the two vaccinal viruses, so all new viruses produced by these templates would also be cytopathic. Co-culturing and RT-PCR assessment subsequently provided *in vitro* confirmation of the insights gained from the sequencing study. Even multiple co-passaging of the two vaccinal BVDV strains under conditions considered conducive to generation of recombinants did not result in the emergence of a noncytopathic BVDV recombinant strain.

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SHORT COMMUNICATION

Delayed Onset Postvaccinal Mucosal Disease as a Result of Genetic Recombination between Genotype 1 and Genotype 2 BVDV¹JULIA F. RIDPATH² and STEVEN R. BOLIN

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Bovine viral diarrhoea viruses (BVDV) are segregated into two genotypes, BVDV 1 and BVDV 2. Viruses within both genotypes may exist as one of two biotypes, cytopathic or noncytopathic. A highly fatal form of BVDV termed mucosal disease (MD) occurs when an animal persistently infected with noncytopathic BVDV becomes superinfected with cytopathic BVDV. In this study, we characterized a noncytopathic (BVDV2-125nc)/cytopathic (BVDV2-125c) viral pair isolated from an animal that died of MD 9 months after vaccination with modified-live BVDV1-NADL. In comparison to BVDV2-125nc, BVDV2-125c contained a 389-nucleotide insertion. The insertion was in the correct reading frame for the large open reading frame of the BVDV genome and occurred in the portion of the genome that codes for the p125 viral polypeptide. There was a 99% identity between the inserted sequences found in BVDV2-125c and sequences from the vaccine virus BVDV1-NADL. These data suggest that MD was induced after a recombination between noncytopathic BVDV2-125nc and cytopathic vaccine virus BVDV1-NADL created the cytopathic virus BVDV2-125c.

Bovine viral diarrhoea virus (BVDV), an ubiquitous pathogen of cattle, is classified as a member of the *Pestivirus* genus within the family *Flaviviridae* (1). The BVDV genome is a single positive-sense RNA strand approximately 12.5 kb in length. It contains one large open reading frame (ORF) preceded by a highly conserved 380- to 390-nucleotide untranslated region (5' UTR) (2). The large ORF codes for a polyprotein that is co- and posttranslationally cleaved by cellular and viral proteases (3-5).

Previously we have used phylogenetic analysis, based on sequences from the 5' UTR, to segregate BVDV into two genotypes (6). These genotypes will be referred to hereafter as BVDV 1 and BVDV 2. Viruses from either genotype may exist as one of two biotypes, cytopathic or noncytopathic (6-9). Cytopathic BVDV induce cytoplasmic vacuolization in cultured cells (8) and produce a polypeptide, p80, which is not produced by noncytopathic BVDV (10, 11). The genomic sequences coding for p80 are collinear to sequences coding for the carboxy-terminal two-thirds of the viral polypeptide p125. Both cytopathic and noncytopathic BVDV produce p125. Genomic insertions in the portion of the genome coding for the p125 polypeptide occur in some cytopathic BVDV (12-

18). The insertions reported to date are the result of recombination between viral and host RNA or of recombination of viral RNA with itself. Such insertions may result in the production of p80 and trigger the switch from a noncytopathic to a cytopathic viral biotype. Hypothetically, this results from the introduction of a cleavage site into the p125 polypeptide or from expression of an inserted copy of the gene encoding the p80 (12, 13). In this report, we characterize an insertion in a cytopathic BVDV 2 virus that apparently resulted from a recombination between a noncytopathic BVDV 2 virus and a cytopathic BVDV 1 vaccine virus.

A noncytopathic/cytopathic pair of viruses from the BVDV 2 genotype (BVDV2-125nc/BVDV2-125c, respectively) was isolated from an animal that died 3 months after vaccination with a federally licensed modified-live BVDV1-NADL vaccine, marketed in the United States. The postmortem isolation of a noncytopathic and a cytopathic BVDV, clinical history, and gross and microscopic lesions were consistent with death due to mucosal disease (for review see 19). Mucosal disease (MD) is a highly fatal form of BVDV infection that occurs when an animal persistently infected with noncytopathic BVDV is superinfected with cytopathic BVDV. Isolation and propagation of viruses were performed as described earlier (20). Viruses were cloned at limiting dilution and genotyped based on polymerase chain reaction (PCR) amplification and sequence comparison of the 5' UTR (6). Based on activity in cell culture (7-9) and production of p80 (10, 11), BVDV2-125c was characterized as a cytopathic virus

¹ Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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TABLE 1

Comparison of Viruses by Mab Binding

Mab	BVDV1-NADL	BVDV2-125c	BVDV2-125no
CA-1	+	-	+
N2	+	+	+
CA-3	+	-	-
CA-24	+	+	+
CA-38	+	+	+
CA-72	+	+	+
CA-80	+	-	-
CA-82	+	-	-
BZ-2	+	-	-
BZ-3	+	-	-
BZ-4	+	-	-
BZ-14	+	-	-
BZ-24	+	-	-
BZ-26	+	+	+
BZ-29	+	+	-
BZ-32	+	+	+
BZ-33	+	+	+
BZ-35	+	-	-
BZ-38	+	-	-

and BVDV2-125no was characterized as a noncytopathic virus. Monoclonal antibody (Mab) binding results (6) for BVDV1-NADL, BVDV2-125no, and BVDV2-125c are shown in Table 1. All Mabs used recognized neutralizing epitopes on the viral polypeptide gp53. For genomic anal-

TABLE 2

Primers Used for PCR Amplification and Sequencing

	Equivalent position in BVDV1-NADL
Viral sense primers	
GGA-CTC-CAT-GGT-ACA-AG	2579
CAG-ATG-GTT-TGG-TGA-GG	4748
GTG-GAA-TGA-CAO-TCG-CA	4820
Viral anti-sense primers	
GTC-TGC-GAG-TGT-CAT-TC	4823
CTG-GCA-CTC-TTA-GGT-TO	5042
GGC-ACT-TCT-CAT-GGT-TG	5449
CCT-GAG-GCA-GTG-ACA-CA	5852

ysis, RNA from bovine turbinate cells infected with either BVDV2-125no or BVDV2-125c was prepared, amplified by PCR, and sequenced as described (6). The sequence and location of PCR primers are shown in Table 2.

Previously, it was reported that BVDV1-NADL contains an insertion in the region of the genome coding for the p125 (15). In comparison to the noncytopathic virus BVDV1-SD-1' (21), this insertion codes for the 90 amino acids (aa) beginning after residue 1637. While insertions have been reported for other cytopathic BVDV 1 viruses, the location and sequence of the BVDV1-NADL insert

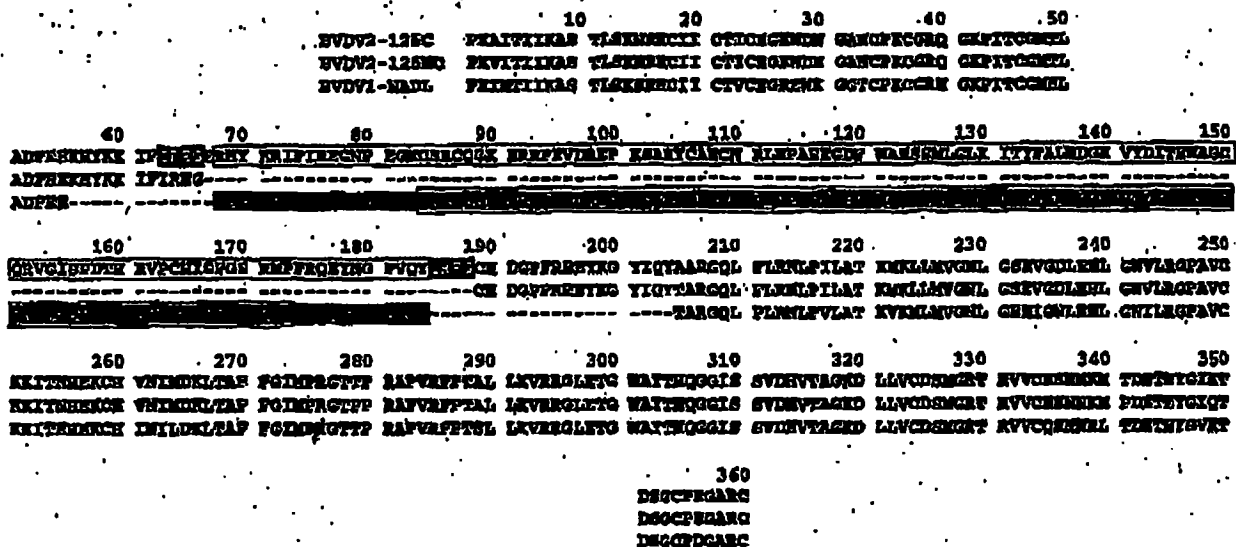


FIG. 1. Comparison of predicted aa sequences from BVDV2-125c, BVDV2-125no, and BVDV1-NADL. Portions of the genomic region coding for the p125 polypeptide were amplified from BVDV2-125c and BVDV2-125no and sequenced. To conserve space, sequences are shown as the derived aa sequences. These sequences were then aligned with the aa sequence derived from the published genomic sequence of BVDV1-NADL (2). Aligned sequences correspond to aa residues 1487 to 1795, numbering from the first ATG of the large open reading frame of BVDV1-NADL. In comparison to BVDV2-125no, BVDV2-125c has a 122-aa insertion. This insertion is preceded by and ends with the 4-aa sequence IREG (boxed in black). Included in this insertion is a 117-aa sequence that has a high homology to BVDV1-NADL. The homologous BVDV2-125c sequences are boxed in white. The homologous BVDV1-NADL sequences are highlighted in gray. In comparison to noncytopathic BVDV 1 viruses, BVDV1-NADL contains a 90-aa insertion (boxed in gray). As shown in this figure, the BVDV2-125c insertion contains the BVDV1-NADL insertion, the 18-aa residues preceding the BVDV1-NADL insertion, and the 11-aa residues following the BVDV1-NADL insertion.

Flanking Sequence

5' Insert Terminus

TTT-ATA-AGA-GAA-GGA-GAA-AGA-CAC-TAT-AAA

3' Insert Terminus

Flanking Sequence

TAT-ATA-AGA-GAA-GGA-TGC-CAC-GAC-GGA-CCT

FIG. 2. Nucleic acid sequences flanking the BVDV2-125c insertion. Homologous bases between the sequence flanking the 5' start of the insertion and the sequence found at the 3' end of the insertion are shown in boldface type. The start of the insertion is indicated by a Δ . The end of the insertion is indicated by a $\dagger$.

are unique (12-18). The 90-aa sequence making up the BVDV1-NADL insertion is homologous with a bovine mRNA that codes for a protein of unknown function (15). All other reported cytopathic BVDV 1 insertions consist of ubiquitin monomers and/or duplicated BVDV viral sequences. The RNA of BVDV2-125c contained an extra 366 nucleotides in the portion of the genome coding for the p126 polypeptide that were absent in BVDV2-125nc (Fig. 1). For the purposes of this discussion, this 366-nucleotide sequence shall be referred to as an insertion. The insertion was in the correct reading-frame for the ORF and coded for 122 aa residues. In reference to the published sequence of BVDV1-NADL, the beginning of the insertion corresponds to residue 1533 of the predicted aa sequence of the large ORF. It is preceded by and ends with the 4-residue aa sequence IREG. The nucleotide sequences flanking the beginning and occurring at the end of the insertion are shown in Fig. 2. Within the 122-aa insertion is a sequence of 117 aa that has a greater than 99% identity to aa residues 1522 through 1639 of BVDV1-NADL (Fig. 1). This 117-residue sequence is homologous to the 90-aa BVDV1-NADL insertion plus the 16-aa sequence that precedes and the 11 aa that follow the BVDV1-NADL insertion. Outside of this 117-residue sequence the identity at the aa level (for the 200 residues flanking either side of the insertion) between BVDV2-125c and BVDV1-NADL is 80%. The aa

sequence identity between BVDV2-125nc and the BVDV2-125c aa sequences preceding and following the BVDV2-125c insertion, shown in Fig. 1, is 96%. While we cannot examine the virus from the exact dose of vaccine the animal received we were able to sequence the master stock from which the vaccine was derived. The sequence corresponding to the BVDV2-125c insertion from the master stock virus is shown in Fig. 3. Interestingly, the 1-aa substitution observed between the published sequence for BVDV1-NADL and BVDV2-125c is also observed between the sequence of the master stock and the published sequence of BVDV1-NADL.

The mechanism by which cytopathic and noncytopathic BVDV interact to cause MD is unknown. Initial characterization of BVDV, isolated from persistently infected cattle that died of MD, showed antigenic similarity between cytopathic and noncytopathic BVDV (22, 23). This observation led to the hypothesis that antigenic similarity between viruses is necessary to trigger MD and that the persistent noncytopathic BVDV is the source of the cytopathic BVDV. This hypothesis fails to account for several reports of naturally occurring and experimentally induced MD, in which the viruses involved were clearly antigenically distinct from each other and likely of different origin (24-28). In the present study, the noncytopathic/cytopathic viral pair BVDV2-125nc/BVDV2-125c was antigenically similar by monoclonal antibody panel. However, the presence of sequences with an extremely high sequence identity to BVDV1-NADL within the BVDV2-125c insert, coupled with a history of vaccination with a modified-live BVDV1-NADL, suggests that MD was induced after a recombination between BVDV1-NADL and BVDV2-125nc. The prolonged time between vaccination and development of disease may reflect the time required for a single viral particle, generated by recombination, to replicate to a concentration sufficient to induce MD. While it is likely that many outbreaks of MD are the result of a spontaneous mutation involving only the endogenous noncytopathic virus present in the persistently infected animal, the data presented here

	10	20	30	40	50
BVDV1-NADL	RHYKRIFIRE	GNFEGMCSC	QGKHRRFEND	REPXSARTCA	ECNLEHPAEK
BVDV2-125c					
MASTER STOCK					
	60	70	80	90	100
	GDVWAESSML	GLKITYPALM	DGRVYDILVN	AGGQVVGIS?	DTNRVPCGIS
					
					
	110				
	FGSRMPFRQE	INGFVQY			
					
					

FIG. 3. Comparison of sequences corresponding to the BVDV2-125c insertion. The sequence corresponding to the BVDV2-125c insertion was derived from the BVDV1-NADL master stock used to make the federally licensed modified-live vaccine with which the animal was inoculated with 3 months prior to death. Please note that both the BVDV2-125c insertion (BVDV2-125c) and the master stock sequence (master stock) have the same single-aa substitution in comparison to the published sequence of BVDV1-NADL (BVDV1-NADL).

suggest that MD may also result from recombination between the persistent noncytopathic virus and an exogenous virus. The possibility that the exogenous virus may be introduced by vaccination impacts upon the design of vaccines.

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BOVI-SHIELD GOLD® 4

Pfizer Animal Health

Bovine Rhinotracheitis-Virus Diarrhea-Parainfluenza₃ Vaccine

Modified Live Virus

PRODUCT DESCRIPTION: Bovi-Shield GOLD 4 is for vaccination of healthy cattle as an aid in preventing infectious bovine rhinotracheitis caused by infectious bovine rhinotracheitis (IBR) virus, bovine viral diarrhea caused by bovine viral diarrhea (BVD) virus Type 1 and 2, and disease caused by parainfluenza₃ (PI₃) virus. Bovi-Shield GOLD 4 may be administered to pregnant cattle provided they were vaccinated, according to label directions, with any Bovi-Shield GOLD FP® or PregGuard® GOLD FP vaccine within the past 12 months. Bovi-Shield GOLD 4 may also be administered to calves nursing pregnant cows provided their dams were vaccinated within the past 12 months as described above. Bovi-Shield GOLD 4 is a freeze-dried preparation of modified live virus (MLV) strains of IBR, BVD (Types 1 and 2), and PI₃ viruses, plus a sterile diluent used to rehydrate the freeze-dried vaccine. Viral antigens are propagated on established cell lines.

DISEASE DESCRIPTION: IBR, BVD, and PI₃ viruses are commonly associated with respiratory disease and/or reproductive failure in cattle. IBR virus infection is characterized by high temperature, excessive nasal discharge, conjunctivitis and ocular discharge, inflamed nose ("red nose"), increased rate of respiration, coughing, loss of appetite, and depression. Cattle infected during pregnancy may abort.

BVD virus may be transmitted in nasal secretions, saliva, blood, feces, and/or urine, and by direct contact with contaminated objects; it invades through the nose and mouth and replicates systemically. Infection during pregnancy may result in abortion, fetal resorption, or congenital malformation of the fetus. Moreover, if susceptible cows are infected with noncytopathic BVD virus during the first trimester of pregnancy, their calves may be born persistently infected with the virus. Exposure of those calves to certain virulent cytopathic BVD virus strains may precipitate BVD-mucosal disease. Both BVD Types 1 and 2 can show a variety of clinical signs. The signs may be mild and not readily apparent. Clinical signs may include severe immune suppression, diarrhea, anorexia, depression, fever and respiratory disease. If infected with some Type 2 strains of BVD, severe thrombocytopenia may occur and hemorrhaging may be seen.

PI₃ virus usually localizes in the upper respiratory tract, causing elevated temperature and moderate nasal and ocular discharge. Although clinical signs typically are mild, PI₃ infection weakens respiratory tissues. Invasion and replication of other pathogens, particularly *Pasteurella* spp., is thereby facilitated and may result in pneumonia.

SAFETY AND EFFICACY: The cell lines on which the modified live virus fractions are produced have been extensively tested to ensure freedom from adventitious agents. Two studies were conducted demonstrating the safety of the BVD Type 2 fraction of Bovi-Shield GOLD 4. In the first study 14- to 15-month-old crossbred beef calves negative for BVD virus and BVD neutralizing antibodies, and with no history of BVD vaccination, were given a field dose of the BVD Type 2 strain intramuscularly. Susceptible contact control animals maintained in this study did not seroconvert to BVD. In the second study, 4- to 8-week-old, colostrum-deprived crossbred beef calves, negative for BVD virus and BVD neutralizing antibodies, were given a field dose of the BVD Type 2 strain intranasally. In both studies, no clinical signs attributable to BVD infection were observed and attempts to re-isolate the vaccine virus from nasal secretions were unsuccessful.

The safety of the fractions of Bovi-Shield GOLD 4 was demonstrated in 2 field safety studies, utilizing a total of 404 animals. Seven- to 8-month old beef calves received a single dose of Bovi-Shield GOLD 4 administered either subcutaneously or intramuscularly, followed by a single dose of Bovi-Shield® BRSV 3 weeks later. No

injection site, serious systemic reactions or adverse events attributable to vaccination were observed. Three field studies demonstrated the safety of Bovi-Shield GOLD 4 when administered to pregnant cows previously vaccinated with one of the Bovi-Shield FP or PregGuard FP products. In all three studies, cows were vaccinated with either Bovi-Shield FP 4+L5 or PregGuard FP 9 between 12 and 17 months prior to vaccination with either Bovi-Shield GOLD FP 5 VL5 or placebo during the first, second, or third trimester of pregnancy. The pregnancy status of cows vaccinated during the first trimester of pregnancy was determined approximately 3 months postvaccination. Pregnancy was confirmed in 197 of 198 (99.5%) placebo-vaccinated cows and 198 of 200 (99%) cows vaccinated with Bovi-Shield GOLD FP 5 VL5. Cows vaccinated during the second or third trimester of pregnancy were observed through calving, and the health status of their newborn calves was determined. Second trimester vaccination resulted in the delivery of healthy calves in 149 of 150 (99.3%) placebo vaccinates, and in 162 of 164 (98.8%) cows vaccinated with Bovi-Shield GOLD FP 5 VL5. One hundred percent of cows vaccinated with placebo (138 of 138) and Bovi-Shield (184 of 184) during the third trimester of pregnancy delivered healthy calves.

Efficacy of each fraction of Bovi-Shield GOLD 4 was demonstrated in challenge-of-immunity studies. Cattle vaccinated with any fraction of Bovi-Shield GOLD 4, followed by challenge with a disease-causing strain of that fraction, showed no signs or had significantly fewer clinical signs than nonvaccinated control cattle. Serologic studies demonstrated no immunologic interference among the fractions of Bovi-Shield GOLD 4.

The effectiveness of Bovi-Shield GOLD 4 in providing protection from a virulent BVDV Type 2 challenge was demonstrated in 2- to 3-month-old, colostrum-deprived beef calves. Vaccinated calves, along with a group of nonvaccinated controls, were challenged intranasally with virulent, noncytopathic BVDV Type 2 at 28 days postvaccination. Results are summarized in the following table:

Group	No. of Calves	% Viremic	% Morbidity*	% Mortality
Controls	10	100 ^a	100 ^a	70 ^a
Bovi-Shield GOLD 5	20	0 ^b	10 ^b	0 ^b

*Observed ill for ≥ 1 day.

^{a,b}Numbers within the same column with different lower-case superscripts are significantly different ($P \leq 0.05$).

This study demonstrated that vaccination with Bovi-Shield GOLD 4, containing immunogenicity levels of BVDV-1 and BVDV-2, was effective in protecting young calves from severe BVDV-2 disease as evidenced by significant differences between vaccinates and controls in mortality, morbidity, viremia, rectal temperatures, white blood cell counts, and platelet counts.

DIRECTIONS:

General Directions: Vaccination of healthy cattle is recommended. Aseptically rehydrate the freeze-dried vaccine with the sterile diluent provided, shake well, and administer 2 mL subcutaneously or intramuscularly. In accordance with Beef Quality Assurance guidelines, this product should be administered SC or IM in the neck region.

Primary Vaccination: Administer a single 2-mL dose to healthy cattle.

Revaccination: Annual revaccination with a single dose is recommended.

Good animal husbandry and herd health management practices should be employed.

PRECAUTIONS:

Do not use in pregnant cows (abortions can result) unless they were vaccinated, according to label directions, with any Bovi-Shield GOLD FP or PregGuard GOLD FP vaccine within the past 12 months. Do not use in calves nursing pregnant cows unless their dams were vaccinated within the past 12 months as described above.

Consistent with good vaccination practices, it is recommended that heifers receive at least 2 doses with the second dose administered approximately 30 days prebreeding.

Store at 2°-7°C. Prolonged exposure to higher temperatures and/or direct sunlight may adversely affect potency. Do not freeze.

Use entire contents when first opened.

Sterilized syringes and needles should be used to administer this vaccine. Do not sterilize with chemicals because traces of disinfectant may inactivate the vaccine.

Burn containers and all unused contents.

Do not vaccinate within 21 days before slaughter.

Contains gentamicin as preservative.

As with many vaccines, anaphylaxis may occur after use. Initial antidote of epinephrine is recommended and should be followed with appropriate supportive therapy.

This product has been shown to be efficacious in healthy animals. A protective immune response may not be elicited if animals are incubating an infectious disease, are malnourished or parasitized, are stressed due to

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BOVI-SHIELD GOLD® FP® 5

Pfizer Animal Health

Bovine Rhinotracheitis-Virus Diarrhea-Parainfluenza₃-Respiratory Syncytial Virus Vaccine

Modified Live Virus

PRODUCT DESCRIPTION: Bovi-Shield GOLD FP 5 is for vaccination of healthy cows and heifers prior to breeding to prevent persistently infected calves caused by bovine virus diarrhea (BVD) virus Types 1 and 2 and as an aid in preventing abortion caused by infectious bovine rhinotracheitis (IBR, bovine herpesvirus Type 1) virus; respiratory disease caused by IBR, BVD Types 1 and 2, parainfluenza₃ (PI₃) and bovine respiratory syncytial virus (BRSV); and BVD Type 2 testicular infection. A 12-month duration of immunity has been demonstrated against IBR-induced abortion and persistently infected calves caused by BVD Types 1 and 2. Bovi-Shield GOLD FP 5 may be administered to pregnant cattle provided they were vaccinated

according to label directions with any Bovi-Shield GOLD FP or PregGuard® GOLD FP vaccine within the past 12 months. Bovi-Shield GOLD FP 5 may also be administered to calves nursing pregnant cows provided their dams were vaccinated within the past 12 months as described above. The freeze-dried vaccine is a preparation of modified live virus (MLV) strains of IBR, BVD (Types 1 and 2), PI₃, and BRSV propagated on an established cell line. An adjuvanted sterile diluent is provided to rehydrate the freeze-dried component.

DISEASE DESCRIPTION: IBR, BVD, PI₃, and BRSV viruses are commonly associated with respiratory disease and/or reproductive failure in cattle. IBR virus infection is characterized by high temperature, excessive nasal discharge, conjunctivitis and ocular discharge, inflamed nose ("red nose"), increased rate of respiration, coughing, loss of appetite, and depression. Cattle infected during pregnancy may abort.

BVD virus may be transmitted in nasal secretions, saliva, blood, feces, and/or urine, and by direct contact with contaminated objects; it invades through the nose and mouth and replicates systemically. BVD virus in the semen of persistently and acutely infected postpubertal bulls is capable of infecting susceptible inseminated cows.

Infection during pregnancy may result in abortion, fetal resorption, or congenital malformation of the fetus.

Moreover, if susceptible cows are infected with noncytopathic BVD virus during the first trimester of pregnancy, their calves may be born persistently infected with the virus. Exposure of those calves to certain virulent cytopathic BVD virus strains may precipitate BVD-mucosal disease. Both BVD Types 1 and 2 can show a variety of clinical signs. The signs may be mild and not readily apparent. Clinical signs may include severe immune suppression, diarrhea, anorexia, depression, fever and respiratory disease. If infected with some Type 2 strains of BVD, severe thrombocytopenia may occur and hemorrhaging may be seen.

PI₃ virus usually localizes in the upper respiratory tract, causing elevated temperature and moderate nasal and ocular discharge. Although clinical signs typically are mild, PI₃ infection weakens respiratory tissues. Invasion and replication of other pathogens, particularly *Pasteurella* spp., is thereby facilitated and may result in pneumonia. BRSV is the etiologic agent of a specific viral respiratory disease of cattle of all ages, including nursing calves. Infection is characterized by rapid breathing, coughing, loss of appetite, discharge from the nose and eyes, fever, and swelling around the throat and neck. In an acute outbreak, deaths may follow within 48 hours after onset of signs.

Clinically, BRSV infection may be indistinguishable from other viral infections associated with the bovine respiratory disease complex. BRSV infection, like PI₃, facilitates invasion and replication of other respiratory pathogens. Exacerbation of clinical signs has been documented when concurrent BRSV and BVD or IBR infection exists.

SAFETY AND EFFICACY: The cell lines on which the modified live virus fractions are produced have been extensively tested to ensure freedom from adventitious agents. Two studies were conducted demonstrating the safety of the BVD Type 2 fraction of Bovi-Shield GOLD FP 5. In the first study, 14- to 15-month-old crossbred beef calves negative for BVD virus and BVD neutralizing antibodies, and with no history of BVD vaccination, were given a field dose of the BVD Type 2 strain intramuscularly. Susceptible contact control animals maintained in this study did not seroconvert to BVD. In the second study, 4- to 8-week-old, colostrum-deprived crossbred beef calves, negative for BVD virus and BVD neutralizing antibodies, were given a field dose of the BVD Type 2 strain intranasally. In both studies, no clinical signs attributable to BVD infection were observed, and attempts to re-isolate the vaccine virus from nasal secretions were unsuccessful.

The safety of the fractions of Bovi-Shield GOLD FP 5 was demonstrated in 3 field safety studies, each at different geographic locations, utilizing a total of 605 animals. Safety was evaluated in 6- to 8-month-old beef calves in a feedlot environment, in 3- to 7-month-old beef calves prior to weaning, and in 1- to 4-week old dairy calves. No injection site, serious systemic or allergic reactions or clinical abnormalities attributable to vaccination were observed.

Three additional field studies demonstrated the safety of Bovi-Shield GOLD FP 5 when administered to pregnant cows previously vaccinated with one of the Bovi-Shield FP or PregGuard FP products. In all three studies, cows were vaccinated with either Bovi-Shield FP 4+L5 or PregGuard FP 9 between 12 and 17 months prior to vaccination with either Bovi-Shield GOLD FP 5 VL5 or placebo during the first, second, or third trimester of pregnancy. The pregnancy status of cows vaccinated during the first trimester of pregnancy was determined approximately 3 months postvaccination. Pregnancy was confirmed in 197 of 198 (99.5%) placebo-vaccinated cows and 198 of 200 (99%) cows vaccinated with Bovi-Shield GOLD FP 5 VL5.

Cows vaccinated during the second or third trimester of pregnancy were observed through calving, and the health status of their newborn calves was determined. Second trimester vaccination resulted in the delivery of healthy calves in 149 of 150 (99.3%) placebo vaccinates, and in 162 of 164 (98.8%) cows vaccinated with Bovi-Shield GOLD FP 5 VL5. One hundred percent of cows vaccinated with placebo (138 of 138) and Bovi-Shield (184 of 184) during the third trimester of pregnancy delivered healthy calves.

The effectiveness of the IBR and BVD fractions of Bovi-Shield GOLD FP 5 as an aid in preventing abortion caused by IBR and persistently infected calves caused by BVD (Types 1 & 2) was demonstrated in multiple IBR, BVD Type 1, and BVD Type 2 challenge-of-immunity studies, including studies demonstrating that Bovi-Shield GOLD FP 5 protected pregnant cows and their fetuses for one year following vaccination. Heifers were challenged with BHV-1, BVD Type 1 or BVD Type 2 viruses at least 365 days following vaccination. Following challenge with BHV-1 virus, 100% of placebo-vaccinated control cows aborted, compared to 15.8% of vaccinates. Following challenge with BVD Type 1 virus, 100% of fetuses born to vaccinated cows were protected from persistent BVD infection, compared to 12% of calves born to placebo-vaccinated control cows. Similarly, following BVD Type 2 challenge, 90% of calves from vaccinated heifers were protected, while 0% calves from placebo-vaccinated control cows were protected.

The efficacy of Bovi-Shield GOLD FP 5 in preventing BVD Type 2 testicular infection was demonstrated in a vaccination-challenge study. Following challenge with BVD Type 2 (28 days postvaccination), 84.7% of testicular samples from nonvaccinated controls were positive, while none of vaccinated calves' samples were BVD positive.

DIRECTIONS:

General Directions: Vaccination of healthy cattle is recommended. Aseptically rehydrate the freeze-dried vaccine with the sterile diluent provided, shake well, and administer 2 mL intramuscularly. In accordance with Beef Quality Assurance guidelines, this product should be administered in the muscular region of the neck.

Primary Vaccination: Administer a single 2-mL dose to all breeding cows and heifers approximately 1 month prior to breeding or being added to the herd, followed by a single dose of Bovi-Shield BRSV 3-4 weeks later.

Revaccination: Annual revaccination with a single dose of Bovi-Shield GOLD FP 5 is recommended.

Good animal husbandry and herd health management practices should be employed.

PRECAUTIONS:

Do not use in pregnant cows (abortions can result) unless they were vaccinated, according to label directions, with any Bovi-Shield FP or PregGuard FP vaccine within the past 12 months. Do not use in calves nursing pregnant cows unless their dams were vaccinated within the past 12 months as described above.

Consistent with good vaccination practices, it is recommended that heifers receive at least 2 doses with the second dose administered approximately 30 days prebreeding.

Store at 2°-7°C. Prolonged exposure to higher temperatures and/or direct sunlight may adversely affect potency. Do not freeze.

Use entire contents when first opened.

Sterilized syringes and needles should be used to administer this vaccine. Do not sterilize with chemicals because traces of disinfectant may inactivate the vaccine.

Burn containers and all unused contents.

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BOVI-SHIELD GOLD® FP® 5 VL5

Pfizer Animal Health

Bovine Rhinotracheitis-Virus Diarrhea-Parainfluenza₃-Respiratory Syncytial Virus Vaccine

Modified Live Virus

Campylobacter Fetus-Leptospira Canicola-Grippotyphosa-Hardjo-Icterohaemorrhagiae-Pomona Bacterin

PRODUCT DESCRIPTION: Bovi-Shield GOLD FP 5 VL5 is for vaccination of healthy cows and heifers prior to breeding to prevent persistently infected calves caused by bovine virus diarrhea (BVD) virus Types 1 and 2 and as an aid in preventing abortion caused by infectious bovine rhinotracheitis (IBR, bovine herpesvirus Type 1) virus; respiratory disease caused by IBR, BVD Types 1 and 2, parainfluenza₃ (PI₃) and bovine respiratory syncytial virus (BRSV); BVD Type 2 testicular infection; campylobacteriosis (vibriosis) caused by *Campylobacter fetus*; and leptospirosis caused by *Leptospira canicola*, *L. grippotyphosa*, *L. hardjo*, *L. icterohaemorrhagiae*, and *L. pomona*. A 12-month duration of immunity has been demonstrated against IBR-induced abortion and persistently infected calves caused by BVD Types 1 and 2. Bovi-Shield GOLD FP 5 VL5 may be administered to pregnant cattle provided they were vaccinated, according to label directions, with any Bovi-Shield GOLD FP or PregGuard® GOLD FP vaccine within the past 12 months. Bovi-Shield GOLD FP 5 VL5 may also be administered to calves nursing pregnant cows provided their dams were vaccinated within the past 12 months as described above. The freeze-dried vaccine is a preparation of modified live virus (MLV) strains of IBR, BVD (Types 1 and 2), PI₃, and BRSV propagated on an established cell line. The *Campylobacter* bacterin is an inactivated suspension of *C. fetus*. It is combined with an inactivated *Leptospira* bacterin prepared from whole cultures of the agents indicated. The *Campylobacter*-*Leptospira* bacterin is supplied as a diluent for the IBR-BVD-PI₃-BRSV vaccine.

DISEASE DESCRIPTION: IBR, BVD, PI₃, and BRSV viruses are commonly associated with respiratory disease and/or reproductive failure in cattle. IBR virus infection is characterized by high temperature, excessive nasal discharge, conjunctivitis and ocular discharge, inflamed nose ("red nose"), increased rate of respiration, coughing, loss of appetite, and depression. Cattle infected during pregnancy may abort.

BVD virus may be transmitted in nasal secretions, saliva, blood, feces, and/or urine, and by direct contact with contaminated objects; it invades through the nose and mouth and replicates systemically. Infection during pregnancy may result in abortion, fetal resorption, or congenital malformation of the fetus. Moreover, if susceptible cows are infected with noncytopathic BVD virus during the first trimester of pregnancy, their calves may be born persistently infected with the virus. Exposure of those calves to certain virulent cytopathic BVD virus strains may precipitate BVD-mucosal disease. Both BVD Types 1 and 2 can show a variety of clinical signs. The signs may be mild and not readily apparent. Clinical signs may include severe immune suppression, diarrhea, anorexia, depression, fever and respiratory disease. If infected with some Type 2 strains of BVD, severe thrombocytopenia may occur and hemorrhaging may be seen.

PI₃ virus usually localizes in the upper respiratory tract, causing elevated temperature and moderate nasal and ocular discharge. Although clinical signs typically are mild, PI₃ infection weakens respiratory tissues. Invasion and replication of other pathogens, particularly *Pasteurella* spp., is thereby facilitated and may result in pneumonia. BRSV is the etiologic agent of a specific viral respiratory disease of cattle of all ages, including nursing calves. Infection is characterized by rapid breathing, coughing, loss of appetite, discharge from the nose and eyes, fever, and swelling around the throat and neck. In an acute outbreak, deaths may follow within 48 hours after onset of signs. Clinically, BRSV infection may be indistinguishable from other viral infections associated with the bovine

respiratory disease complex. BRSV infection, like PI₃, facilitates invasion and replication of other respiratory pathogens. Exacerbation of clinical signs has been documented when concurrent BRSV and BVD or IBR infection exists.

Campylobacteriosis (vibriosis) is an insidious venereal disease of cattle. The *Campylobacter* organism infects the cow's genital tract causing early embryonic death. The disease is characterized by infertility, repeat breeding, and a prolonged calving season.

Leptospirosis may be caused by several serovars of *Leptospira*, of which *L. canicola*, *L. grippityphosa*, *L. hardjo*, *L. icterohaemorrhagiae*, and *L. pomona* are the most common affecting cattle. *Leptospira* localize in the kidneys, are shed in the urine, and cause anemia, bloody urine, fever, loss of appetite, and prostration in calves. Signs are usually subclinical in adult cattle. Infected pregnant cows, however, often abort, and dairy cows may exhibit a marked decrease in milk production. *Leptospira* spp. are known zoonotic pathogens.

SAFETY AND EFFICACY: The cell lines on which the modified live virus fractions are produced have been extensively tested to ensure freedom from adventitious agents. Two studies were conducted demonstrating the safety of the BVD Type 2 fraction of Bovi-Shield GOLD FP 5 VL5. In the first study 14- to 15-month-old crossbred beef calves negative for BVD virus and BVD neutralizing antibodies, and with no history of BVD vaccination, were given a field dose of the BVD Type 2 strain intramuscularly. Susceptible contact control animals maintained in this study did not seroconvert to BVD. In the second study, 4- to 8-week-old, colostrum-deprived crossbred beef calves, negative for BVD virus and BVD neutralizing antibodies, were given a field dose of the BVD Type 2 strain intranasally. In both studies, no clinical signs attributable to BVD infection were observed, and attempts to re-isolate the vaccine virus from nasal secretions were unsuccessful.

The safety of the fractions of Bovi-Shield GOLD FP 5 VL5 was demonstrated in 2 field safety studies, each at different geographic locations, utilizing a total of 399 animals. Safety was evaluated in 6- to 8-month-old beef calves in a feedlot environment, and in 3- to 7-month-old beef calves prior to weaning. No injection site, serious systemic or allergic reactions or clinical abnormalities attributable to vaccination were observed.

Three additional field studies demonstrated the safety of Bovi-Shield GOLD FP 5 VL5 when administered to pregnant cows previously vaccinated with one of the Bovi-Shield FP or PregGuard FP products. In all three studies, cows were vaccinated with either Bovi-Shield FP 4+L5 or PregGuard FP 9 between 12 and 17 months prior to vaccination with either Bovi-Shield GOLD FP 5 VL5 or placebo during the first, second, or third trimester of pregnancy. The pregnancy status of cows vaccinated during the first trimester of pregnancy was determined approximately 3 months postvaccination. Pregnancy was confirmed in 197 of 198 (99.5%) placebo-vaccinated cows and 198 of 200 (99%) cows vaccinated with Bovi-Shield GOLD FP 5 VL5.

Cows vaccinated during the second or third trimester of pregnancy were observed through calving, and the health status of their newborn calves was determined. Second trimester vaccination resulted in the delivery of healthy calves in 149 of 150 (99.3%) placebo vaccinates, and in 162 of 164 (98.8%) cows vaccinated with Bovi-Shield GOLD FP 5 VL5. One hundred percent of cows vaccinated with placebo (138 of 138) and Bovi-Shield (184 of 184) during the third trimester of pregnancy delivered healthy calves.

The effectiveness of the IBR and BVD fractions of Bovi-Shield GOLD FP 5 VL5 as an aid in preventing abortion caused by IBR and persistently infected calves caused by BVD (Types 1 & 2) was demonstrated in multiple IBR, BVD Type 1, and BVD Type 2 challenge-of-immunity studies, including studies demonstrating that Bovi-Shield GOLD FP 5 VL5 protected pregnant cows and their fetuses for one year following vaccination. Heifers were challenged with BHV-1, BVD Type 1 or BVD Type 2 viruses at least 365 days following vaccination. Following challenge with BHV-1 virus, 100% of placebo-vaccinated control cows aborted, compared to 15.8% of vaccinates. Following challenge with BVD Type 1 virus, 100% of fetuses born to vaccinated cows were protected from persistent BVD infection, compared to 12% of calves born to placebo-vaccinated control cows. Similarly, following BVD Type 2 challenge, 90% of calves from vaccinated heifers were protected, while 0% of calves from placebo-vaccinated control cows were protected.

The efficacy of Bovi-Shield GOLD FP 5 VL5 in preventing BVD Type 2 testicular infection was demonstrated in a vaccination-challenge study. Following challenge with BVD Type 2 (28 days postvaccination), 84.7% of testicular samples from nonvaccinated controls were positive, while none of vaccinated calves' samples were BVD positive.

DIRECTIONS:

General Directions: Vaccination of healthy cattle is recommended. Aseptically rehydrate the freeze-dried vaccine (Bovi-Shield GOLD FP 5) with the liquid bacterin provided (Vibrio/Leptoform-5™), shake well, and administer 2 mL intramuscularly. In accordance with Beef Quality Assurance guidelines, this product should be administered in the muscular region of the neck.

Primary Vaccination: Administer a single 2-mL dose to all breeding cows and heifers approximately 1 month prior to breeding or being added to the herd, followed by single doses of Vibrio/Leptoform-5 and Bovi-Shield BRSV 3-4 weeks later.

Revaccination: Annual revaccination with a single dose of Bovi-Shield GOLD FP 5 VL5 is recommended. Good animal husbandry and herd health management practices should be employed.

PRECAUTIONS:

Do not use in pregnant cows (abortions can result) unless they were vaccinated, according to label directions, with any Bovi-Shield FP or PregGuard FP vaccine within the past 12 months. Do not use in calves nursing pregnant cows unless their dams were vaccinated within the past 12 months as described above. Do not vaccinate calves under 3 months of age.

Consistent with good vaccination practices, it is recommended that heifers receive at least 2 doses with the second dose administered approximately 30 days prebreeding.

Store at 2°-7°C. Prolonged exposure to higher temperatures and/or direct sunlight may adversely affect potency. Do not freeze.

Use entire contents when first opened.

Sterilized syringes and needles should be used to administer this vaccine. Do not sterilize with chemicals because traces of disinfectant may inactivate the vaccine.

Burn containers and all unused contents.

Do not vaccinate within 21 days before slaughter.

Contains gentamicin as preservative.

Vaccination of stressed animals should be delayed.

Occasional hypersensitivity reactions may occur up to 18 hours postvaccination. Owners should be advised to observe animals during this period. While this event appears to be rare overall, dairy cattle may be affected more frequently than other cattle. Animals affected may display excessive salivation, incoordination, and/or dyspnea. Animals displaying such signs should be treated immediately with epinephrine or equivalent. In nonresponsive animals, other modes of treatment should be considered.

As with many vaccines, anaphylaxis may occur after use. Initial antidote of epinephrine is recommended and should be followed with appropriate supportive therapy.

This product has been shown to be efficacious in healthy animals. A protective immune response may not be elicited if animals are incubating an infectious disease, are malnourished or parasitized, are stressed due to shipment or environmental conditions, are otherwise immunocompromised, or the vaccine is not administered in accordance with label directions.

Technical inquiries should be directed to Pfizer Animal Health Veterinary Services, (800) 366-5288 (USA), (800) 461-0917 (Canada).

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75-0557-02

Presentation: 10 and 50 dose vials.

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BOVI-SHIELD® GOLD IBR-BVD-BRSV-LP

Pfizer Animal Health

Bovine Rhinotracheitis-Virus Diarrhea-Respiratory Syncytial Virus Vaccine

Modified Live Virus

Leptospira Pomona Bacterin

PRODUCT DESCRIPTION: Bovi-Shield GOLD IBR-BVD-BRSV-LP is for vaccination of healthy cattle as an aid in preventing infectious bovine rhinotracheitis caused by infectious bovine rhinotracheitis (IBR) virus, bovine viral diarrhea (Type 1 and Type 2) caused by bovine viral diarrhea (BVD) virus, disease caused by bovine respiratory syncytial virus (BRSV), and leptospirosis caused by *Leptospira pomona*. Bovi-Shield GOLD IBR-BVD-BRSV-LP may be administered to pregnant cattle provided they were vaccinated, according to label directions, with Bovi-Shield FP™ 4+L5, Bovi-Shield FP 4+VL5, Bovi-Shield GOLD FP 5 L5, Bovi-Shield GOLD FP 5 VL5, PregGuard® FP 9 or PregGuard GOLD FP 10 within the past 12 months. Bovi-Shield GOLD IBR-BVD-BRSV-LP may also be administered to calves nursing pregnant cows provided their dams were vaccinated within the past 12 months as described above. Bovi-Shield GOLD IBR-BVD-BRSV-LP is a freeze-dried preparation of modified live virus (MLV) strains of IBR, BVD (Types 1 and 2), and BRSV viruses, plus a liquid bacterin containing *L. pomona*. The liquid bacterin is used to rehydrate the freeze-dried vaccine. Viral antigens are propagated on established cell lines.

DISEASE DESCRIPTION: IBR, BVD, and BRSV viruses are commonly associated with respiratory disease and/or reproductive failure in cattle. IBR virus infection is characterized by high temperature, excessive nasal discharge, conjunctivitis and ocular discharge, inflamed nose ("red nose"), increased rate of respiration, coughing, loss of appetite, and depression. Cattle infected during pregnancy may abort.

BVD virus may be transmitted in nasal secretions, saliva, blood, feces, and/or urine, and by direct contact with contaminated objects; it invades through the nose and mouth and replicates systemically. Infection during pregnancy may result in abortion, fetal resorption, or congenital malformation of the fetus. Moreover, if susceptible cows are infected with noncytopathic BVD virus during the first trimester of pregnancy, their calves may be born persistently infected with the virus. Exposure of those calves to certain virulent cytopathic BVD virus strains may precipitate BVD-mucosal disease. Both BVD Types 1 and 2 can show a variety of clinical signs. The signs may be mild and not readily apparent. Clinical signs may include severe immune suppression, diarrhea, anorexia, depression, fever and respiratory disease. If infected with some Type 2 strains of BVD, severe thrombocytopenia may occur and hemorrhaging may be seen.

BRSV is the etiologic agent of a specific viral respiratory disease of cattle of all ages, including nursing calves. Infection is characterized by rapid breathing, coughing, loss of appetite, discharge from the nose and eyes, fever, and swelling around the throat and neck. In an acute outbreak, deaths may follow within 48 hours after onset of signs. Clinically, BRSV infection may be indistinguishable from other viral infections associated with the bovine respiratory disease complex. BRSV infection facilitates invasion and replication of other respiratory pathogens. Exacerbation of clinical signs has been documented when concurrent BRSV and BVD or IBR infection exists. Leptospirosis may be caused by several serovars of *Leptospira*, of which *L. pomona* is one of the most common affecting cattle. *Leptospira* localize in the kidneys, are shed in the urine, and cause anemia, bloody urine, fever, loss of appetite, and prostration in calves. Signs are usually subclinical in adult cattle. Infected pregnant cows, however, often abort, and dairy cows may exhibit a marked decrease in milk production. *Leptospira* spp. are known zoonotic pathogens.

SAFETY AND EFFICACY: The cell lines on which the modified live virus fractions are produced have been extensively tested to ensure freedom from adventitious agents. Two studies were conducted demonstrating the safety of the BVD Type 2 fraction of Bovi-Shield GOLD IBR-BVD-BRSV-LP. In the first study 14- to 15-month-old

crossbred beef calves negative for BVD virus and BVD neutralizing antibodies, and with no history of BVD vaccination, were given a field dose of the BVD Type 2 strain intramuscularly. Susceptible contact control animals maintained in this study did not seroconvert to BVD. In the second study, 4- to 8-week-old, colostrum-deprived crossbred beef calves, negative for BVD virus and BVD neutralizing antibodies, were given a field dose of the BVD Type 2 strain intranasally. In both studies, no clinical signs attributable to BVD infection were observed and attempts to re-isolate the vaccine virus from nasal secretions were unsuccessful.

The safety of the fractions of Bovi-Shield GOLD IBR-BVD-BRSV-LP was demonstrated in 3 field safety studies, each at different geographic locations, utilizing a total of 600 animals. Safety was evaluated in 6- to 8-month-old beef calves in a feedlot environment, in 3- to 7-month-old beef calves prior to weaning, and in 1- to 4-week-old dairy calves. No injection site, serious systemic or allergic reactions or clinical abnormalities attributable to vaccination were observed.

Three field studies demonstrated the safety of Bovi-Shield GOLD IBR-BVD-BRSV-LP when administered to pregnant cows previously vaccinated with one of the Bovi-Shield FP or PregGuard FP products. In all three studies, cows were vaccinated with either Bovi-Shield FP 4+L5 or PregGuard FP 9 between 12 and 17 months prior to vaccination with either Bovi-Shield GOLD FP 5 VL5 or placebo during the first, second, or third trimester of pregnancy. The pregnancy status of cows vaccinated during the first trimester of pregnancy was determined approximately 3 months postvaccination. Pregnancy was confirmed in 197 of 198 (99.5%) placebo-vaccinated cows and 198 of 200 (99%) cows vaccinated with Bovi-Shield GOLD FP 5 VL5.

Cows vaccinated during the second or third trimester of pregnancy were observed through calving, and the health status of their newborn calves was determined. Second trimester vaccination resulted in the delivery of healthy calves in 149 of 150 (99.3%) placebo vaccinates, and in 162 of 164 (98.8%) cows vaccinated with Bovi-Shield GOLD FP 5 VL5. One hundred percent of cows vaccinated with placebo (138 of 138) and Bovi-Shield (184 of 184) during the third trimester of pregnancy delivered healthy calves.

Efficacy of each fraction of Bovi-Shield GOLD IBR-BVD-BRSV-LP was demonstrated in challenge-of-immunity studies. Cattle vaccinated with any fraction of Bovi-Shield GOLD IBR-BVD-BRSV-LP, followed by challenge with a disease-causing strain of that fraction, showed no signs or had significantly fewer clinical signs than nonvaccinated control cattle. Serologic studies demonstrated no immunologic interference among the fractions of Bovi-Shield GOLD IBR-BVD-BRSV-LP.

The effectiveness of Bovi-Shield GOLD IBR-BVD-BRSV-LP in providing protection from a virulent BVDV Type 2 challenge was demonstrated in 2- to 3-month-old, colostrum-deprived beef calves. Vaccinated calves, along with a group of nonvaccinated controls, were challenged intranasally with virulent, noncytopathic BVDV Type 2 at 28 days postvaccination. Results are summarized in the following table:

Group	No. of Calves	% Viremic	% Morbidity*	% Mortality
Controls	10	100 ^a	100 ^a	70 ^a
Bovi-Shield GOLD 5	20	0 ^b	10 ^b	0 ^b

*Observed ill for ≥ 1 day.

^{a,b}Numbers within the same column with different lower-case superscripts are significantly different ($P \leq 0.05$).

This study demonstrated that vaccination with Bovi-Shield GOLD IBR-BVD-BRSV-LP, containing immunogenicity levels of BVDV-1 and BVDV-2, was effective in protecting young calves from severe BVDV-2 disease as evidenced by significant differences between vaccinates and controls in mortality, morbidity, viremia, rectal temperatures, white blood cell counts, and platelet counts.

DIRECTIONS:

- General Directions:** Vaccination of healthy cattle is recommended. Aseptically rehydrate the freeze-dried vaccine with the liquid bacterin provided, shake well, and administer 2 mL intramuscularly. In accordance with Beef Quality Assurance guidelines, this product should be administered in the muscular region of the neck.
- Primary Vaccination:** Administer a single 2-mL dose to healthy cattle, followed by a second dose of Bovi-Shield BRSV 3-4 weeks later.
- Revaccination:** Annual revaccination with a single dose is recommended.
- Good animal husbandry and herd health management practices should be employed.

PRECAUTIONS:

- Do not use in pregnant cows (abortions can result) unless they were vaccinated, according to label directions, with Bovi-Shield FP 4+L5, Bovi-Shield FP 4+VL5, Bovi-Shield GOLD FP 5 L5, Bovi-Shield GOLD FP 5 VL5, PregGuard FP 9 or PregGuard GOLD FP 10 within the past 12 months. Do not use in calves nursing pregnant cows unless their dams were vaccinated within the past 12 months as described above.
- Store at 2°-7°C. Prolonged exposure to higher temperatures and/or direct sunlight may adversely affect potency. Do not freeze.
- Use entire contents when first opened.

2. Objeto de la invención

- Título de la solicitud (folio 1): "Vacunas virales seguras que contienen al menos dos virus mutantes de la familia flaviviridae y métodos para su preparación".
- La presente solicitud se refiere a vacunas adecuadas para la administración a animales frente a infecciones víricas.
- El problema planteado en esta solicitud es la necesidad de proporcionar vacunas seguras y eficaces que protejan a los animales frente a infecciones víricas.
- Para solucionar este problema técnico la solicitud en estudio proporciona una composición de vacuna que comprende al menos dos virus mutantes vivos de la misma familia, en donde cada virus contiene una mutación en el genoma viral, y las mutaciones en los virus se ubican en el mismo sitio genómico de tal manera que los virus mutantes no pueden recombinarse entre si para eliminar las mutaciones.
- La Clasificación Internacional de Patentes (IPC) es: A61K 39/12, 39/15, 39/187.

3. De la solicitud de Patente

Reivindicaciones (artículo 30 D 486)

El capítulo reivindicatorio de la solicitud en estudio presenta los siguientes defectos:

- La composición y el método no se encuentran correctamente caracterizados por cuanto no se especifica la estructura o el sitio donde se realiza la mutación (Reivindicaciones 1 – 3 y 8 – 11). Si bien en el anterior concepto técnico no se había requerido mayor claridad a este particular, la respuesta del solicitante ha mostrado claramente una característica técnica esencial en la mutación de la región NS2 – 3 que resuelve el problema técnico objetivo y permite definir las características estructurales de la composición y el método empleado.

Por lo anterior, el capítulo reivindicatorio NO cumple con el requisito de definición, claridad, concisión o sustento por la descripción de acuerdo al art. 30 de la Decisión 486 y/o art. 22 Tratado PCT.

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4. 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 la solicitud prioritaria: 29/07/2003.

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:

Nº	Documento	Título	Fecha *
D1	CO 05-14184	Vacunas para enfermedades reproductoras del ganado	26/08/2002

*Fecha de prioridad

5. Análisis de patentabilidad (artículo 14 D486)

Novedad (artículo 18 D486)

- La reivindicación 1 a 3 divulgan una composición de vacuna que comprende al menos dos virus de Diarrea Viral Bovina (BVDV) mutantes vivos, en donde cada virus contiene una mutación en el genoma viral, y las mutaciones en los virus se ubican en el mismo sitio genómico de tal manera que los virus mutantes no pueden recombinarse entre si para eliminar las mutaciones.

El documento del Estado de la Técnica D1 (folio 91) enseña una composición inmunogénica que comprende: 1) un Virus Herpes Bovino (BH-1) vivo modificado; 2) un Virus de parainfluenza vivo modificado Tipo 3 (PI3); 3) un Virus Sincital Respiratorio Bovino (BRSV); 4) Un Virus de la Diarrea Viral Bovina Tipo 1 (BVDV-1) y 5) un Virus de la Diarrea Viral Bovina Tipo 2 (BVDV-2).

Debido a que las reivindicaciones no presentan las características estructurales que permiten una diferenciación con la vacuna descrita en D1 con referencia a la mutación realizada, la invención esta anticipada por D1 presentando el mismo efecto y estructura al ser los componentes BVDV1 y BVDV2 modificados la característica técnica esencial de la invención.

- El método divulgado en las reivindicaciones 8 a 11, presentan la preparación de vacunas que comprende seleccionar o construir dos virus BVDV mutantes vivos. Dichas reivindicaciones se encuentran anticipadas por D1 en tanto que la composición de la anterioridad también presenta una modificación en estos dos genotipos.

La anterior comparación se realiza debido a que tanto las composiciones como el método implican su realización a partir de la selección de los virus mutantes, y dado que no se establecen las características técnicas estructurales de la invención en las reivindicaciones objetadas, el documento citado antecede la invención.

De esta forma se establece que las reivindicaciones 1 a 3 y 8 a 11 no cumplen con el requisito de Novedad.

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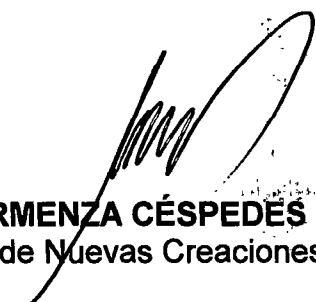
6. Conclusión:

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

N°	Documento N°	Reivindicaciones afectadas	Requisito que afecta
D1	CO 05-14184	1 – 3 y 8.- 11	Novedad

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.


ALIX CARMENZA CÉSPEDÉS DE VERGEL
Directora de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha
_____ **26 NOV. 2010**

CONCEPTO TÉCNICO No. 4081	EXAMINADOR TÉCNICO Código No. 202089
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