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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 06-127739- -00004-0000

Fecha: 2008-06-16 16:23:52 Dep. 2020 NUECREACIONE
Tra. 11 PATENTEIYII Eve: 378 FASENACIONAL
Act. 449 RESPUESOPOSICI Folios: 59

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

Trámite : 011

Evento : 379

Actuación : 449

Referencia : Expediente No. 06.127.739
Asunto : DEFENSA DE LA SOLICITUD DE PATENTE PARA:
"COMPOSICIÓN FARMACÉUTICA QUE CONTIENE
MELOXICAM PARA EL TRATAMIENTO DE
ENFERMEDADES INFLAMATORIAS DOLOROSAS EN
MEDICINA VETERINARIA"
Solicitante : BOEHRINGER INGELHEIM VETMEDICA GMBH
Opositor : PROCAPS S.A.
Fecha de solicitud : 20 de Diciembre de 2006

1. ALEGATOS CONTESTACIÓN OPOSICIÓN

Yo, Emilio FERRERO, en mi condición de apoderado de la sociedad solicitante de la patente de la referencia, por el presente escrito doy respuesta a la oposición formulada por la abogada Laura Catalina SILVA BARRERA, apoderado de la sociedad **PROCAPS S.A.**, opositora en el asunto de la referencia.

2. CONTESTACIÓN DENTRO DEL TÉRMINO LEGAL

El oficio No. 16496, en el cual su Despacho dispuso otorgar a mi representada término para presentar sus argumentaciones a favor de la patentabilidad del invento de que trata el asunto en referencia, me fue notificado por fijación en lista el 21 de diciembre de 2007. Con memorial radicado el 17 de Marzo del 2008, pedí plazo de sesenta días para atender el Oficio No. 16496.

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 06-127739- -00004-0000

Fecha: 2008-08-16 16:23:52 Dep. 2020 NUECREACIONE
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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 890176089-2

RECIBO OFICIAL DE CAJA : 08 - 39,730
FECHA : MAYO 19 DE 2008

**** CONSIGNACION ****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
CAVELIER ABOGADOS	CONSIGNACION	BANCO DE BOGOTA	062754387	1750488	19/05/2008	75,145,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-09	OTROS SERVICIOS DE PROPIEDAD I	21 CERTIFICACIONES
			11,000.00
TOTAL :			\$ 11,000.00

SON: ONCE MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

Bo 04484-264-001-1

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCION DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado
EMILIO FERRERO

ASUNTO Radicación 06-127739
 Trámite 002
 Evento 001
 Actuación 430
 Oficio 0 4 8 6 2
 Folios 5

Patente de Invención ☒ Patente de Modelo de Utilidad ☐
Vía Nacional ☐
Vía PCT (fase nacional) ☒ Cap. I ☒ Cap. II ☐

No. Sol. Internacional PCT/EP2005/006276
Fecha de Presentación Internal. Junio 11 de 2005

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

- 1.1. Descripción: Folios: 49 a 76 como se radicó inicialmente
- 1.2. Reivindicaciones: Folios: 77 a 78 como se radicó inicialmente
 Folios: 176 a 177 modificadas
- 1.3. Prioridad: DE No. 10 2004 0 30409.2 del 23 de Junio de 2004
- 1.4. Oposición: Procaps S.A. Folios: 87 a 158
 Respuesta a Opositor Folios: 166 a 220

2. Objeto de la invención

Título:

“COMPOSICION FARMACEUTICA QUE CONTIENE MELOXICAM PARA EL TRATAMIENTO DE ENFERMEDADES INFLAMATORIAS DOLOROSAS EN MEDICINA VETERINARIA”. Modificado folio 172.

El objeto de la presente solicitud de patente de invención consiste en:

- Reivindicaciones 1 a 10: Composición farmacéutica que contiene Meloxicam o una sal de Meloxicam

El problema planteado en esta solicitud es la necesidad de presentar un nuevo uso de Meloxicam; el cual es para ser usado en mastitis suave y moderada.

Para solucionar este problema técnico la solicitud en estudio presenta una composición farmacéutica que contiene Meloxicam o una sal de Meloxicam.

El objeto de la invención definido anteriormente se clasifico de acuerdo con la IPC: A61K 31/5415

3. De la solicitud de Patente

3.1 Título

Se recomienda ajustar el título, a lo que se desea proteger a través del capítulo reivindicatorio; ya que los métodos de tratamiento de acuerdo a la normatividad vigente, no son patentables.

3.2 Reivindicaciones (artículo 30 D 486)

- Reivindicación 1; desea proteger una composición farmacéutica, en la cual no es claro cuando hace referencia a: "vehículos con eficacia analgésica". Hacer la aclaración pertinente.

Por lo anteriormente expuesto, es preciso que señale el alcance de su invención.

4. Análisis de la Oposición: (artículo 43 D. 486):

4.1 OPOSICION DE PROCAPS S.A. (Folios 87 a 158; del 25-10-2007):

El opositor argumenta que a la presente solicitud se debería negar el beneficio patentario; ya que incumple los requisitos de patentabilidad establecidos por la Decisión 486 de 2000 de la Comisión de la Comunidad Andina.

FUNDAMENTOS DE HECHO:

El opositor dice, que la solicitud en referencia refiere al uso de una formulación que contiene Meloxicam o una sal de Meloxicam, la cual se utiliza para tratar una enfermedad inflamatoria, como es la mastitis suave o moderada.

Antes de la fecha de prioridad de esta solicitud, se encuentran y los siguientes documentos que afectan la novedad y altura inventiva; estos son:

1. D1= WO 2004/037264 del 06 de Mayo de 2004, revela:

- Gránulos solubles en agua que contienen Meloxicam, como una sal de meglumina, formando una solución estable; con rangos de dosis de 0,2 a 1,0 mg, para tratar dolor, inflamación y mastitis
- Además del Meloxicam, contiene un aglutinante, azúcar o edulcorante y un vehículo

2. D2= WO 03/049733 del 19 de Junio de 2003, revela:

- Soluciones acuosas que comprenden sales de Meloxicam, para tratar dolor, inflamación y mastitis
- Además del Meloxicam, contiene meglumina, agua, polietilenglicol, copolímero, etanol, glicina, opcionalmente hidróxido de sodio o ácido clorhídrico y EDTA

3. D3= WO 01/197813 del 27 de Diciembre de 2001, revela:

- Solución acuosa que comprende más de 10 mg de una sal de Meloxicam, para tratar dolor, inflamación y mastitis; con rangos de dosis de 0,2 a 1,0 mg
- Además del Meloxicam, contiene meglumina, agua, polietilenglicol, etanol, glicerina, opcionalmente hidróxido de sodio o ácido clorhídrico y EDTA

De acuerdo a lo anterior, la solicitud en estudio:

Carece de Novedad: ya que de acuerdo a lo enseñado en cada uno de los documentos D1, D2 y D3, revelan composiciones de Meloxicam.

4.2 RESPUESTA DEL SOLICITANTE BOEHRINGER INGELHEIM VETMEDICA GMBH (Folios 166 a 220; del 16-06-2008):

El solicitante en su respuesta frente a lo argumentado por el opositor, dice:

Fundamento fáctico de la Oposición:

En primera instancia, refiere a argumentos presentados por la opositora, en lo relacionado a la falta de Novedad y Aplicación Industrial. Y que encontró 3 documentos que afectan la Novedad de la misma, en detalle:

1. D1= WO 2004/037264 del 06 de Mayo de 2004, dice el opositor que enseña:

- Gránulos solubles en agua que contienen Meloxicam, como una sal de meglumina, formando una solución estable; con rangos de dosis de 0,2 a 1,0 mg, para tratar dolor, inflamación y mastitis
- Además del Meloxicam, contiene un aglutinante, azúcar o edulcorante y un vehículo

2. D2= WO 03/049733 del 19 de Junio de 2003, revela:

- Soluciones acuosas que comprenden sales de Meloxicam, para tratar dolor, inflamación y mastitis
- Además del Meloxicam, contiene meglumina, agua, polietilenglicol, copolímero, etanol, glicina, opcionalmente hidróxido de sodio o ácido clorhídrico y EDTA

3. D3= WO 01/197813 del 27 de Diciembre de 2001, revela:

- Solución acuosa que comprende más de 10 mg de una sal de Meloxicam, para tratar dolor, inflamación y mastitis; con rangos de dosis de 0,2 a 1,0 mg

- Además del Meloxicam, contiene meglumina, agua, polietilenglicol, etanol, glicerina, opcionalmente hidróxido de sodio o ácido clorhídrico y EDTA

Respuesta a los Hechos y Alegatos:

El solicitante presenta argumentos que demuestran que los fundamentos de la opositora, carecen de fundamento; de esta manera, por:

Ausencia de Novedad:

Los documentos D1 a D3 revelan composiciones veterinarias que otitis aguda” y contienen Meloxicam en el tratamiento de mastitis aguda; ninguno refiere para tratar la mastitis moderada y suave. Se anexa documento que enseña la diferencia entre la “mastitis aguda” y ”mastitis suave y moderada”.

Por lo anterior los documentos citados por el opositor no se consideran relevantes en el estado del arte para que afecten la novedad y e la aplicación industrial de la presente solicitud. Además el capítulo reivindicatorio ha sido modificado, ya que no refiere usos sino composiciones farmacéuticas.

4.3 RESPUESTA DEL EXAMINADOR TECNICO A OPOSICION DE PROCAPS S.A.:

Esta oficina considera que la Oposición presentada por PROCAPS S.A., tiene fundamento y que para la evaluación de patentabilidad se tendrán en cuenta los documentos por éste presentados.

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 la solicitud prioritaria: Junio 23 de 2004

Teniendo en cuenta la fecha indicada se consultaron las bases de datos y los archivos con que cuenta la entidad con fecha de publicación anterior y se encontró el siguiente documento que anticipa el objeto de esta solicitud:

Nº	Documento	Título	Fecha
D1	WO 01/197813	HIGHLY CONCENTRATED STABLE MELOXICAM SOLUTIONS	Diciembre 27 de 2001 Publicación

6. Análisis de patentabilidad (artículo 14 D486)

6.1 Novedad (artículo 16 D486)

- Reivindicación 1: Composición farmacéutica que contiene Meloxicam o una sal de Meloxicam

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CARACTERISTICAS ESENCIALES DE LA SOLICITUD COLOMBIANA No. 06-127739	D1 = WO 01/197813
Composición farmacéutica que contiene: - Meloxicam o una sal de Meloxicam - Con base orgánica e inorgánica - Y vehículos	Composición farmacéutica que contiene: - Meloxicam o una sal de Meloxicam - Con base orgánica e inorgánica - Y coadyuvantes adecuados Folio 157

Teniendo en cuenta el objeto de la invención y la materia descrita en la anterioridad D1, esta dependencia se permite señalar que para la reivindicación 1y sus dependientes; no es nueva la composición farmacéutica, que contiene Meloxicam o una sal de Meloxicam.

7. Conclusión:

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

N°	Documento N°	Reivindicaciones afectadas	Requisito que afecta
D1	WO 01/197813	1 a 10	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 sesenta 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 (5), del título primero de la circular única N° 10 de 2001.


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

El anterior requerimiento fue notificado por fijación en lista, de fecha
09 ABR. 2010

CONCEPTO TÉCNICO No. 835	EXAMINADOR TÉCNICO Código No. 202080
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3. RESPUESTA A LAS PETICIONES

Me opongo a las peticiones de la sociedad opositora y en consecuencia, solicito a ese Despacho que con fundamento en las consideraciones de hecho y de derecho que se presentan en este escrito se declare sin fundamento la oposición presentada por PROCAPS S.A.

En consecuencia, se declare que el invento titulado **COMPOSICIÓN FARMACÉUTICA QUE CONTIENE MELOXICAM PARA EL TRATAMIENTO DE ENFERMEDADES INFLAMATORIAS DOLOROSAS EN MEDICINA VETERINARIA** cumple con los requisitos exigidos en el artículo 14 de la Decisión 486 de la Comisión de la Comunidad Andina y demás normas concordantes. Por tanto, solicito se sirva otorgar a mi representada la patente solicitada.

4. FUNDAMENTO FÁCTICO DE LA OPOSICIÓN

A continuación me referiré a los argumentos de fondo presentados por la opositora para concluir que no son aplicables a la solicitud de mi representada y que por tanto, no constituyen fundamento válido para señalar que el invento de que trata la solicitud de la referencia no cumple con los requisitos establecidos en los artículos 14 y 20 de la Decisión 486 de la Comisión de la Comunidad Andina. Lo anterior por cuanto el señalamiento que hace la opositora de la falta de novedad y aplicación industrial para el invento para el cual pretende mi representada la concesión de la patente es errado, carece de sustento fáctico y de derecho para que prosperen en contra de la solicitud de mi representada. Adicionalmente a que, de acuerdo con el nuevo capítulo reivindicatorio que aquí presento, el objeto de la presente solicitud se encuentra comprendido dentro de las categorías de invenciones consideradas como patentables en Colombia, por lo tanto merece se le otorgue dicho derecho.

4.1. En efecto, en el escrito de oposición señala la apoderada de la opositora, que la solicitud de mi representada no cumple con los requisitos del artículo 20 de la Decisión 486, debido a que las

reivindicaciones se refieren al uso y al método para el tratamiento de enfermedades inflamatorias y no a la composición farmacológica o a un método de fabricación mediante la cual se obtenga un producto tangible.

4.2. Adicionalmente, el apoderado de la oposición indica que la solicitud de mi representada no cumple con uno de los tres requisitos de patentabilidad establecido en el artículo 14 de la Decisión 486.

4.2.1. El artículo 14 de la Decisión 486 indica que las invenciones objeto de patente deben ser nuevas y tener nivel inventivo. Así pues, el opositor encontró lo siguientes documentos que, en su opinión, afectan la novedad de la presente solicitud:

4.2.1.1. D1 "WO2004037264". "En este documento se revelan gránulos solubles en agua que contienen meloxicam, preferiblemente como una sal meglumina, rápidamente disueltos para formar una solución estable. Así mismo, revela rangos de dosis entre 0,2 a 1,0mg de sustancia activa por kilogramo de peso, para formas de dosis orales para el tratamiento del dolor, inflamación y mastitis en animales. Al contener meloxicam, un aglutinante, un azúcar o edulcorante, un vehiculo, un formador de sales que forma la sal de meglumina, sodio potasio o amonio del meloxicam, eventualmente un aromatizante y otros coadyuvantes, se revelan composiciones de meloxicam o una sal de la misma y uno o más vehículos para el tratamiento del dolor o inflamaciones, incluida la mastitis. Por lo tanto, la opositora expresa que la presente solicitud no aporta ningún efecto significativo relevante respecto al arte anterior, pues lo revelado ya se encontraba en el estado de la técnica, afectando su novedad".

4.2.1.2. D2: "WO03049733". "Por su parte este documento revela soluciones acuosas que comprenden sales de meloxicam para el tratamiento del dolor, inflamación, mastitis. Revela particularmente un compuesto que comprende meloxicam, meglumina, agua, un polietilenglicol, un copolímero polietilen-polioxipropilen, etanol, glicina y opcionalmente

hidróxido de sodio o un ácido hidroclicorico y EDTA disódico, para dosis en el rango de 0,2 a 1mg de sustancia por cada kilogramo de peso corporal. Por lo tanto, este documento también revela combinaciones del activo meloxicam con vehículos, por lo que encuentra la opositora que la presente invención no aporta tampoco ningún efecto significativo relevante respecto al arte anterior, afectando su novedad".

4.2.1.3. D3: "WO0197813". "En cuanto a este documento, por su parte revela una solución acuosa que comprende más de 10mg por ml de una sal de meloxicam para el tratamiento del dolor, la inflamación y mastitis. Así mismo, revela dosis de administraciones orales o parenterales que comprende meloxicam, meglumina, agua, un polietilenglico, un copolímero polietilen-polioxipropilen, etanol, glicerina y opcionalmente hidróxido de sodio o ácido hidroclicorico y EDTA disódica. Según dicha anterioridad D3, se revelan dosis en el rango entre 0,2 y 1mg de sustancia por cada kilogramo de peso corporal. Por lo tanto la opositora encuentra a D3 como un documento que también afecta la novedad de la presente solicitud. Así las cosas, la opositora llega a la conclusión que a partir de los documentos D1, D2 y D3 en combinación, es posible revelar combinaciones del activo de meloxicam con vehículos sobre el cual busca protección en el capítulo reivindicatorio".

En consecuencia, la opositora solicita a su Despacho negar la concesión del privilegio de patente solicitada por mi representada.

5. RESPUESTA A LOS HECHOS Y ALEGATOS

A continuación presento a su Despacho las razones que demuestran que contrario a lo señalado por la apoderada de la sociedad opositora, el invento para el cual se solicita la protección a través de la concesión de la patente en el expediente de la referencia, cumple cabalmente con los requisitos del artículo 14 y demás normas concordantes de la Decisión 486 de la Comisión de la Comunidad Andina.

Además, los argumentos que aquí se presentan demuestran que los fundamentos de la opositora carecen de fundamento válido y por tanto, no pueden ni deben ser tomados en cuenta para negar la patente solicitada.

En efecto, me referiré a cada una de las alegaciones que en contra de la patentabilidad del invento titulado **"COMPOSICIÓN FARMACÉUTICA QUE CONTIENE MELOXICAM PARA EL TRATAMIENTO DE ENFERMEDADES INFLAMATORIAS DOLOROSAS EN MEDICINA VETERINARIA"** presenta la opositora:

5.1. Ausencia de novedad:

Los documentos D1 a D3, revelan composiciones veterinarias que contienen meloxicam en el tratamiento de "mastitis aguda". Ninguno de estos documentos revela composiciones veterinarias que contengan meloxicam en el tratamiento de "mastitis moderada y suave" como se revela y reivindica explícitamente en la presente solicitud. Por lo tanto, la materia a proteger de las nuevas reivindicaciones 1 a 10 es novedosa frente a D1, D2 y D3.

Así entonces, la diferencia fundamental entre el indicio "mastitis aguda" y "mastitis moderada y suave" se describe en *"Antimicrobial Therapie in veterinary medicine; PRESCOTT J. F., BAGGOT J. D., WALKER R.D."* (Anexo 1). Este anexo, describe las diferentes progresiones de las formas de mastitis ("aguda", como "clínica" en la página 717 y 721; "suave y moderada", como "subclínica" en la página 724). La diferencia de las formas de mastitis se discute también en *"EMEA Guideline"* (Anexo 2) y *"Advances in the Therapy of Mastitis; ERSKINE R. J., DVM, PhD, KIRK J. H., DVM, MS, TYLER J. W., DVM, MPVM, PhD y DeGRAVES F.J., DVM, PhD"* (Anexo 3).

De acuerdo a lo mencionado anteriormente, los documentos citados no se consideran relevantes en el estado del arte de la presente solicitud por lo que no afectaría la novedad de la presente invención.

Por otro lado, el capítulo reivindicatorio ha sido modificado adicionalmente al hacer referencia a composiciones farmacéuticas y

no a usos de las mismas. Se suprimió la anterior reivindicación 1 y se reemplazo por:

"Composición farmacéutica que contiene meloxicam o una sal de meloxicam farmacológicamente aceptable de una base orgánica o inorgánica y uno o más vehículos con eficacia analgésica para el tratamiento de mastitis suave o moderada".

Con las anteriores modificaciones se puede ver de forma más clara la diferencia que existe entre los documentos citados por la opositora y la presente invención.

En vista de lo anterior, ninguno de los documentos citados por la opositora divulga todos y cada uno de los elementos que constituyen la presente solicitud, ya sea solos o combinados. Por lo tanto, no pueden afectar la novedad de la invención que reclama mi representada.

5.2. Aplicación Industrial: Menciona la apoderada de la sociedad opositora que la presente solicitud carece de aplicación industrial, debido a que algunas reivindicaciones reclaman el uso y el método terapéutico y éstos no son aplicables industrialmente porque primero no se obtiene un producto o proceso, y segundo, no es reproducible o repetible a escala industrial.

Al respecto, solicito tener en cuenta que, en primer lugar, el capítulo reivindicatorio ha sido modificado reivindicando la composición farmacéutica y no el uso de la misma. Por lo tanto esta objeción de patentabilidad ha sido subsanada.

Adicionalmente, solicito a ese Despacho tener en cuenta que demostrado el hecho de que la solicitud de patente de invención de la referencia cumple con los requisitos de novedad y nivel inventivo, se deduce que también cumple con el requisito de aplicación industrial, más aún si se tiene en cuenta que la invención de que trata esta solicitud tiene la facultad de ser utilizable y resulta materialmente realizable.

5.3. Los hechos anteriormente mencionados, permiten concluir que los documentos citados como fundamento de la oposición, no afectan los requisitos de novedad y aplicación industrial de la solicitud de patente que se tramita en el expediente de la referencia.

Adicionalmente, cualquier objeción en relación con estos requisitos de patentabilidad ha sido subsanada teniendo en cuenta la modificación al capítulo reivindicatorio aquí presentada.

En consecuencia, quedan desvirtuadas las afirmaciones de la opositora en contra de la ausencia de patentabilidad del invento para el cual solicita la protección mi representada.

6. MODIFICACIÓN DE LA SOLICITUD

Tal como lo indiqué en el capítulo anterior, junto con este escrito presento Formulario de Modificaciones y Correcciones, con nuevo capítulo reivindicatorio con diez (10) reivindicaciones, el cual solicito sea tenido en cuenta por ese Despacho.

De la misma manera el título de la invención ha sido adecuado a "COMPOSICIÓN FARMACÉUTICA QUE CONTIENE MELOXICAM PARA EL TRATAMIENTO DE ENFERMEDADES INFLAMATORIAS DOLOROSAS EN MEDICINA VETERINARIA".

7. PETICIONES

Con fundamento en lo anterior solicito a ese Despacho lo siguiente:

7.1. Declarar infundada la oposición presentada por PROCAPS S.A.

7.2. Declarar que el invento titulado "COMPOSICIÓN FARMACÉUTICA QUE CONTIENE MELOXICAM PARA EL TRATAMIENTO DE ENFERMEDADES INFLAMATORIAS DOLOROSAS EN MEDICINA VETERINARIA" para el cual mi representada solicita el otorgamiento de la patente de invención cumple con los requisitos del artículo 14 y concordantes de la Decisión 486 de la Comisión de la Comunidad Andina.

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7.3. Conceder la patente para el invento "COMPOSICIÓN FARMACÉUTICA QUE CONTIENE MELOXICAM PARA EL TRATAMIENTO DE ENFERMEDADES INFLAMATORIAS DOLOROSAS EN MEDICINA VETERINARIA" a nombre de mi representada BOEHRINGER INGELHEIM VETMEDICA GMBH.

8. ANEXOS

Anexo a este escrito los siguientes documentos:

8.1. Formulario de Modificaciones y Correcciones con el cual se presenta nuevo capítulo reivindicatorio y cambio de título.

8.2. Recibo de pago por concepto de la tasa correspondiente para modificaciones.

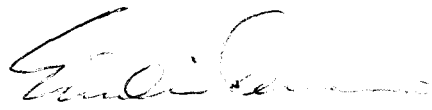
8.3. Copia de los documentos:

- Antimicrobial Therapie in veterinary medicine; PRESCOTT J. F., BAGGOT J. D., WALKER R.D.
- EMEA Guideline
- Advances in the Therapy of Mastitis; ERSKINE R. J., DVM, PhD, KIRK J. H., DVM, MS, TYLER J. W., DVM, MPVM, PhD y DeGRAVES F.J., DVM, PhD

9. NOTIFICACIONES

Mi representada y yo recibiremos notificaciones en la Secretaría de su Despacho o en mi oficina de abogado ubicada en la Carrera 4ª No. 72-35 de esta ciudad de Bogotá, D.C.

Bogotá,
Señora Jefe


EMILIO FERRERO
T.P.A No. 31.929

Espacio reservado para el adhesivo de radicación

FORMULARIO UNICO DE CORRECCIONES Y MODIFICACIONES

1	SOLICITUD DE: ☐ Corrección de Error Material ☒ Modificación a una solicitud	
2 SOLICITANTE	Nombre: BOEHRINGER INGELHEIM VETMEDICA GMBH Sociedad constituida y existente bajo las leyes de Alemania. Dirección: Binger Strasse 173, 55216 Ingelheim am Rhein, Alemania Domicilio: Ingelheim am Rhein, Alemania Teléfono: 546 09 70 Fax: 249 40 70 E-mail: clientes@camyp.com	IDENTIFICACION C.C. ☐ NIT ☐ C.E. ☐ Otro ☐ Número: _____
3 REPRESENTANTE O APODERADO	Nombre: Emilio FERRERO Dirección: Carrera 4ª No. 72-35 Teléfono: 347 36 11 Fax: 217 92 11 E-mail: cavelier@cavelier.com	IDENTIFICACION C.C. ☒ NIT ☐ C.E. ☐ Otro ☐ Número: <u>438.019</u> TP <u>31.929</u>
4	Número de expediente: <u>06.127.739</u>	
5	Descripción de la corrección o modificación solicitada: Presento nuevo capítulo reivindicatorio compuesto por 10 reivindicaciones para que sean tenidas en cuenta al momento de efectuar el examen de patentabilidad. Así mismo presento nuevo título para esta invención que es " COMPOSICION FARMACEUTICA QUE CONTIENE MELOXICAM PARA EL TRATAMIENTO DE ENFERMEDADES INFLAMATORIAS DOLOROSAS EN MEDICINA VETERINARIA ".	
6	Comprobante de pago No. <u>08-42,091</u> Fecha: <u>27 de mayo de 2008</u> <u>08-39,729</u> <u>19 de mayo de 2008</u> <u>08-39,728</u> <u>19 de mayo de 2008</u> <u>08-39,730</u> <u>19 de mayo de 2008</u>	
7	Anexos ☒ Comprobante de pago de la tasa por \$103.000, por concepto de modificaciones. ☐ Poderes, si fuere el caso ☐ Documento que acredita la existencia y representación legal de las partes, cuando sean personas jurídicas.	

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NOMBRE: EMILIO FERRERO

FIRMA: Emilio Ferrero

C.C. 438.019 de Usaquén T.P.A. 31.929

LMA

REIVINDICACIONES

1. Composición farmacéutica que contiene meloxicam o una sal de meloxicam farmacológicamente aceptable de una base orgánica o inorgánica y uno o más vehículos con eficacia analgésica para el tratamiento de mastitis suave o moderada.
2. Composición farmacéutica de acuerdo con la reivindicación 1, caracterizada porque el contenido de meloxicam o sal de meloxicam está en una concentración de 10-30 mg/ml.
3. Composición farmacéutica de acuerdo con las reivindicaciones 1 y 2, caracterizada porque una administración sencilla de la composición médica veterinaria es suficiente para el tratamiento de enfermedades inflamatorias dolorosas.
4. Composición farmacéutica de acuerdo con una de las reivindicaciones 1 a 3 precedentes, que corresponde a un intervalo de dosificación de 0,2 a 1,0 mg de sustancia activa/kg de peso corporal.
5. Composición farmacéutica de acuerdo con una de las reivindicaciones 1 a 4 precedentes, caracterizada porque el vehículo se selecciona de agua y/o aceite.
6. Composición farmacéutica de acuerdo con una de las reivindicaciones 1 a 5 precedentes, caracterizada porque están presentes uno o más aditivos adecuados.
7. Composición farmacéutica de acuerdo con una de las reivindicaciones 1 a 6 precedentes, caracterizada porque contiene o consiste esencialmente en sal de meloxicam, agua, opcionalmente uno o más aditivos seleccionados del grupo que consiste en tampones, solubilizantes, conservantes y, opcionalmente, espesantes.

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8. Composición farmacéutica de acuerdo con una de las reivindicaciones 1 a 7 precedentes, caracterizada porque contiene o consiste esencialmente en meloxicam, meglumina, agua, un polietilenglicol, un copolímero de polietilenopolioxipropileno, etanol, glicina y, opcionalmente, hidróxido de sodio y ácido clorhídrico y AEDT disódico.

9. Composición farmacéutica de acuerdo con una de las reivindicaciones 1 a 6 precedentes, caracterizada porque contiene o consiste esencialmente en meloxicam, uno o más aceites, opcionalmente uno o más antioxidantes y, opcionalmente, uno o más espesantes.

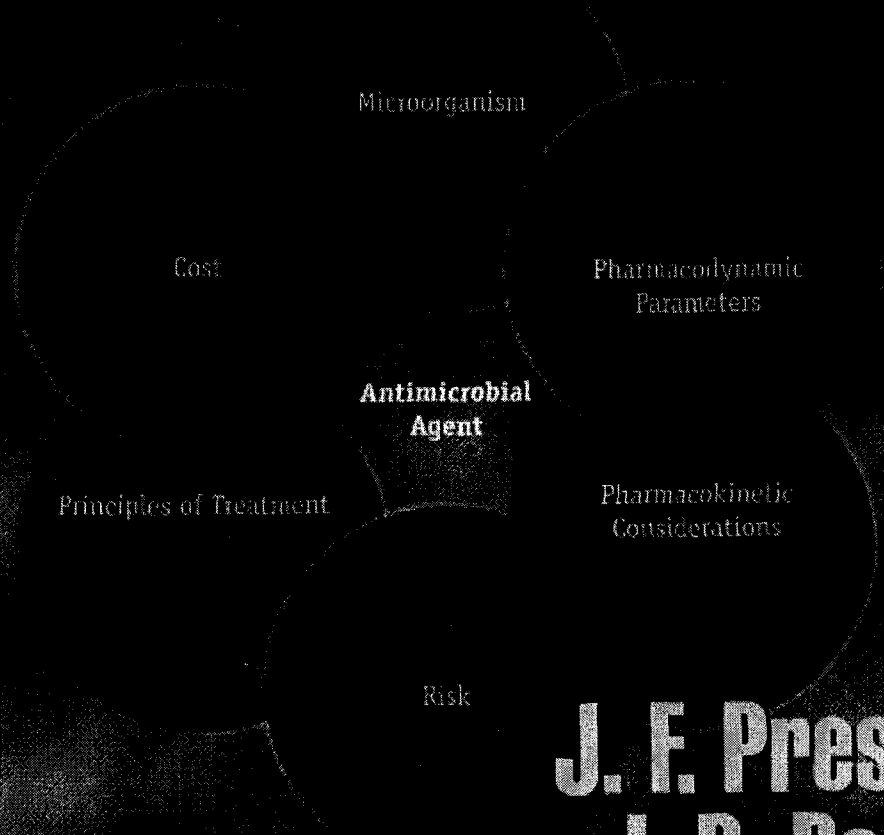
10. Composición farmacéutica de acuerdo con las reivindicaciones 1 a 9 precedentes, caracterizada porque la composición médica veterinaria se prepara para la administración por vía parenteral o intramamaria.

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Third Edition

Antimicrobial Therapy

in Veterinary Medicine



J. F. Prescott
J. D. Baggot
R. D. Walker

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Antimicrobial Drug Use in Bovine Mastitis

R. J. ERSKINE

Effective and economical mastitis control programs rely on prevention rather than treatment. Herds practicing mastitis prevention produce higher-quality milk at less cost than herds that do not. Nonetheless, therapeutic intervention is an important part of a control program for bovine mastitis. This chapter will describe the strategies of antimicrobial therapy for bovine mastitis, with a practical emphasis.

The concept of administering intramammary infusions of antiseptic solutions as a treatment for mastitis caused by infectious agents has been present for at least a century. Widespread availability and use of antimicrobials in animal agriculture in the 1950s cultivated the development of a wide variety of commercial products for intramammary infusion in the 1960s and 1970s. Initial successes suggested 75% efficacy (cures) in both lactating and dry-cow formulations. However, there has been growing realization that in many cases therapeutic attainments fall short of expectations. Chronic intramammary infections (IMI) with extensive fibrotic change caused by pathogens such as *Staphylococcus aureus* pose difficult therapeutic problems, and it is unlikely that typical labeled-dose regimens, which may provide effective antibacterial concentrations above MIC for 24–48 hours, will eliminate the pathogen from affected mammary glands. Additionally, the major developmental thrust for antimicrobials as a treatment for mastitis has been directed against Gram-positive organisms, particularly staphylococci and streptococci. However, in many herds the emergence of pathogens with greater resistance to antimicrobial therapy, such as Gram-negative rods and *Mycoplasma bovis*, has been a substantial cause of loss.

Nonetheless, mastitis remains the most frequent reason for antimicrobial use on dairy farms and thus contributes to a substantial portion of

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total drug and veterinary costs incurred by the dairy industry. Although these costs are minimal, relative to the economic losses attributable to lost production resulting from inflammation, application of management practices that decrease the prevalence of contagious pathogens, such as *Streptococcus agalactiae* and *Staphylococcus aureus*, has shifted the focus of mastitis control and economic losses to IMI caused by environmental pathogens that are associated with more frequent episodes of clinical mastitis. The cost of clinical mastitis is greater than U.S. \$100/case and \$40 to \$50 per cow in a herd per year. Discarded milk following treatment may account for as much as 70% of lost marketable milk, and in herds that do not have a judicious treatment program, losses from discarded milk alone can exceed \$100 per cow per year. Thus, there is increased awareness among producers of treatment-related costs, and the economic cost of extensive antimicrobial therapy for mastitis.

Michigan Department of Agriculture records, collected from Michigan dairies that had at least one occurrence of inhibitory residues in marketed milk in 1995-1997, revealed that antimicrobial therapy of mastitis accounts for 90% of the occurrences. Furthermore, both the consuming public and the regulatory sector have increased their awareness of the possible health hazards posed by antimicrobials and other drugs in milk used for human consumption (see Chapter 33). Whether these concerns are real or perceived, frequency of milk testing and regulatory control of drug use on dairy farms are increasing and are likely to increase in complexity. Thus, because of accountability to dairy producers and the consuming public, we must address two key issues other than efficacy. Therapy must be economically viable and must not increase risk of drug residues in marketed milk.

Considerations before Treatment

Assessing Efficacy

Therapy of infectious disease should assist host defenses in eliminating invading pathogens and/or reduce pathophysiological consequences of infection without degrading host defenses. Logically, emphasis in research and clinical application of antimicrobial drugs for mastitis treatment has focused on the elimination of infectious agents. However, therapeutic success for some IMIs may be better measured by evaluating reduction of clinical symptoms, rather than total elimination of the pathogen from the gland. Ultimately, the best outcome of mastitis therapy is a positive effect on the amount of marketed milk received from the treated cow, along with long-term survival of the cow as a milk producer in the herd.

Determination of IMI status and definition of cures are dependent on bacteriologic culture of milk samples and on the sensitivity and specificity

of this technology to assess the outcome correctly. The conventional definition of an IMI is either the presence of the same microorganism in 2 of 3 consecutive cultures (different sampling dates) or the presence of the pathogen in both samples of duplicate samples (collected at the same time). If these guidelines are followed, the chance of determining an IMI based on false positive isolations from contamination is relatively low. One isolation from a single sample of the contagious pathogens *Streptococcus agalactiae* and *Staphylococcus aureus* is probably indicative of an IMI, because of the high prevalence of these organisms as intramammary pathogens and the low rate of environmental contamination of milk samples with these pathogens. Mammary gland quarters that have an IMI caused by a particular pathogen before treatment but do not have an IMI caused by the same pathogen after treatment would be defined as cured. Conversely, quarters that remain bacteriologically positive after treatment are not cured. Although this is a rather simple premise of efficacy, a survey of mastitis therapy trials can result in numerous, and perhaps misleading, definitions of bacteriologic cures. The number of times a quarter is sampled before and after therapy, the volume of milk sample inoculated, the sample used to inoculate the culture medium, the time when sampling occurs, and elapsed time between collection of consecutive samples are dissimilar among many reports. In both research and in clinical practice, the definition of cures is often insufficiently conservative. Misleading belief in bacteriologic cures that are really false-negative culture results can readily develop if care is not taken in data analysis, particularly when assessing therapeutic outcomes for IMI caused by such invasive pathogens as *S. aureus* and *Streptococcus uberis* and by Gram-negative rods such as *Pseudomonas* and *Klebsiella*. Bacteria exposed to antimicrobials may be inhibited in growth and can remain so for some time after the termination of therapy. Intracellular survival, abscess formation, and L forms, an antimicrobial-induced variant of *Staphylococcus aureus* and some other Gram-positive bacteria that lacks a cell wall, can reduce the probability of successful isolation of bacteria following routine aerobic culture of milk samples. A 30-day refractory period of decreased probability to isolate bacteria in milk has been demonstrated for *S. aureus* IMI, and *Pseudomonas aeruginosa* can be isolated from affected quarters subsequent to a case of clinical mastitis more than 12 months after initial therapy, despite repeated negative cultures. Additionally, many chronic IMIs result in intermittent shedding of bacteria in milk so that one, or even two samples, collected after treatment may be inadequate to ensure the absence of bacteria in the affected quarter. The conclusion is that bacteriologic cures should be reviewed critically before success of therapy can be affirmed.

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The other potential goal of therapy is to attain clinical cures, with or without bacteriologic cures. This may be desirable to allow an affected cow's milk to be sold or to ameliorate a potential severe or life-threatening IMI. Clinical cures attained by antimicrobial therapy can be more obvious than bacteriologic cures, but assessment can be tainted by subjective outcomes. Clinical mastitis is defined as abnormal milk, with or without quarter enlargement and systemic signs. Return to normal appearance is accepted as a clinical cure; however, relapses (ie, clinical mastitis cases that become apparent again before milk withholding periods following therapy are complete) and recurrences should be noted as part of the therapeutic evaluation. Particularly for systemic (acute) clinical mastitis cases, clinical chemistries and/or objective measures such as heart rate and rectal temperature can be measured. Best indicators for efficacy are dry matter intake, milk production, the amount of produced milk actually marketed, and culling/death rates after treatment.

Pharmacokinetic Considerations

Intramammary administration by infusion through the teat canal offers a direct method of obtaining relatively high concentrations of drug in the affected quarter of the gland. For this reason, this method of administration continues to be the most frequently employed and can be particularly successful with Gram-positive cocci that are susceptible to beta-lactam drugs as a means to reduce the signs of clinical mastitis and, in some instances, to eliminate IMI. Additionally, intramammary infusions can help eliminate existing IMI or prevent new IMI caused by Gram-positive cocci during the dry period. Nonetheless, there are drawbacks to this route of administration. Most of the products labeled for use in dairy cattle have been directed for use against Gram-positive cocci and thus have little or no activity against Gram-negative pathogens. Various extralabel products have been employed clinically and in research to broaden the potential spectrum of activities over that available in commercial products. With such products, it is difficult to ascertain correct milk and meat withdrawal periods following extralabel drug use. Intramammary infusion with preparations not prepared under sterile conditions can result in superinfection caused by environmental pathogens. Often the secondary IMI caused by such pathogens as *Pseudomonas aeruginosa*, *Candida* spp., and *Nocardia* spp. result in worse clinical implications than if no intramammary infusion was administered at all. Additionally, it is difficult to ascertain correct milk and meat withdrawal periods following extralabel drug use. Fibrin casts and microabscess formation typical of more chronic IMI interfere with distribution of infused drugs to the site of infection in the terminal alveoli, thus not allowing effective concentrations

at the site of IMI. Finally, the relatively short duration of dosing typical of intramammary therapy limits the period of effective concentration in the gland needed to eliminate more chronic or invasive IMI. For these reasons, the systemic administration of antimicrobials has received attention as an adjunct or alternative therapy to intramammary therapy.

As a basis for practical pharmacokinetics of mastitis therapy, Ziv (1980) suggested that the ideal antibiotic for parenteral mastitis therapy would (1) have low MIC against the majority of udder pathogens, (2) have high bioavailability from IM injection sites, (3) be weakly basic or otherwise non-ionized in serum, (4) be sufficiently lipid soluble, (5) have a low degree of protein binding, (6) have a long $t_{1/2}$ in the body, (7) retain activity in inflammatory secretions, and (8) have clearance from body organs and tissues similar to the clearance of the drug from the blood—ie, not result in drug accumulation in specific organs. Systemically administered sulfonamides, penicillins, aminoglycosides, and early-generation cephalosporins do not readily penetrate the mammary gland. Macrolides, trimethoprim, tetracyclines, and fluoroquinolones distribute well to the mammary gland; however, only the fluoroquinolones have broad-spectrum activity against many Gram-negative pathogens. This class of drugs is undergoing intense scrutiny for use in food-producing animals, because of bacterial resistance concerns for humans, and is therefore banned by the Food and Drug Administration for any dairy-bred bovine in the United States.

Systemic use of antimicrobials has been moderately successful for improving cure rates as compared with intramammary infusions for chronic *S. aureus* IMI in dry cows and lactating cows (Owens et al., 1988; Soback et al., 1990; Sol et al., 1990). Antimicrobials such as fluoroquinolones and tetracyclines would be good pharmacokinetic candidates because of their large volume of distribution (lipophilic) and relatively long half-life. Because of a high degree of resistance to antimicrobials in commercial intramammary products, systemic antimicrobial therapy with alternative antimicrobial drugs has been attempted for the treatment of acute mastitis caused by Gram-negative bacteria. In general, poorer results are attained than with Gram-positive pathogens, although drug selection for some of the studies did not follow sound pharmacologic principles. For example, ceftiofur (IV) has high protein binding and therefore low bioavailability, erythromycin (IM) has poor efficacy against coliforms, and gentamicin is not lipid soluble and therefore does not diffuse into tissues. Treatment of experimental *E. coli* infections with cefquinome, a third-generation cephalosporin that has good tissue distribution and low MIC for Gram-negative bacteria, was determined to be beneficial in reducing deleterious clinical outcomes of experimentally induced infections (Shpigel et al., 1997). Thus, from a pharmacologic perspective, mastitis treatment should utilize bacterial culture and apply sound pharma-

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cokinetic and pharmacodynamic principles if systemic therapy is indicated as an adjunct for intramammary dosing. Most parenteral dosing involves extralabel drug use. This frequently increases the risk of antimicrobial residues in milk and meat of treated cows, as well as the need to withhold products from market for longer periods of time.

The Cow's Point of View

Cows that have concurrent metabolic disease or inadequate nutrition or are subjugated to stress, including calving, are more likely to be affected by infectious agents. Although neutrophil efficiency for phagocytosis and killing in the mammary gland is poorer than in other tissues, it is the most critical defense mechanism of the mammary gland, once the teat end barrier is breached by an invading pathogen. In vitro, neutrophil phagocytosis and killing are positively correlated with beneficial clinical outcomes of experimental IMI, and thus preventive measures such as core-antigen vaccines and antioxidant supplementation of dietary rations, which improve antibacterial function of neutrophils, reduce the incidence and severity of clinical mastitis. Conversely, increased incidence and severity of clinical mastitis cases in cows in the periparturient period are believed in part to be caused by impeded ability for neutrophil migration into the gland. Additionally, the understanding of mammary resistance to infection has increased, so that the neutrophil is now recognized to be part of a larger, complex symphony of immune effectors that includes macrophages, lymphocytes, immune modulators such as cytokines, inflammatory mediators, and acute phase reactants. Potential differences among cows in immune response, coupled with the realization of the diverse nature of pathogens that invade the bovine mammary gland, result in highly variable responses to even the best of preventive programs. Based on the premises that antibiotics do not eliminate an infection without a functional immune system, and that ability to manipulate immune function is limited at the time of treatment, mastitis therapy should be initiated on an assessment, even on a crude scale, of immune competence of the treated cow. Some crude predictions of therapeutic failure include intramammary infections in older cows, infections of long duration, consistent shedding of pathogens over time, and multiple infected quarters. Genetic markers in dairy cattle may in the future allow identification of potentially immune-impaired breeding lines, as well as target therapeutic efforts and expectations of treated animals.

***Antimicrobial Therapy of the Lactating Cow**

Treatment of Acute Clinical Mastitis—Coliforms

Acute, or systemic, mastitis is most often caused by coliform (lactose-fermenting *Enterobacteriaceae*) and other Gram-negative organisms.

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However, numerous other pathogens, including *Mycoplasma bovis*, Gram-positive cocci, Gram-negative rods, and mycotic organisms can result in severe mastitis. Cases can be life threatening to the cow and are often accompanied by marked production loss. If the cow survives, affected cows often perform poorly and may undergo premature culling. From a cowside appraisal, treatment of these cases is a forced decision—ie, treatment is indicated, if only to relieve the cow of systemic signs. Supportive care is usually indicated and, in the case of coliform mastitis, may be the most beneficial component of the therapeutic regimen. The obvious basis for antimicrobial therapy is knowledge of the causative pathogen. However, this is not attainable for some hours after initial case recognition, and thus the practical problem remains of basing treatment on best clinical guess. In addition, most antimicrobial therapeutic regimens currently used as a treatment of coliform mastitis in the United States are not labeled for use in dairy cows. As a result, in the United States, the veterinarian or herd manager often formulates therapeutic management of coliform cases using extralabel drugs.

Experimental challenge models have clarified much of the pathophysiology of mastitis resulting from infection with Gram-negative organisms. Following infection, bacterial numbers in milk increase rapidly. Depending on the size of the challenge, peak bacterial concentrations in milk often occur within a few hours. Typically, a subsequent rapid decline in bacterial concentration follows neutrophil migration into the gland. Though often severe, experimental coliform infections usually clear spontaneously and rarely are more than 10 days in duration. The resulting inflammation and leukocytosis in the affected quarter may persist for weeks, or the quarter may become agalactic, despite inability to isolate bacteria on culture.

Much of the inflammatory and systemic changes observed during the course of acute coliform mastitis result from the release of lipopolysaccharide (LPS) endotoxin from the bacteria. Although there is considerable release of LPS during the rapid proliferation of the Gram-negative bacteria, the majority of release occurs following bacterial phagocytosis and killing by neutrophils. The release of LPS results in subsequent activation of the cyclooxygenase and lipoxygenase pathways, releasing prostaglandins, leukotrienes, and thromboxanes, compounds that are potent mediators of local inflammatory and systemic circulatory events. LPS induces the release of macrophage-derived cytokines, initiating a wide range of systemic responses to inflammation, including fever, leukocytosis, protein synthesis and release by hepatocytes, and serum iron sequestration, collectively termed the acute phase response. Consequently, in order to reduce the severity of acute coliform mastitis, either bacterial growth must be inhibited to reduce exposure of the quarter and the cow

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to LPS, or the effects of the LPS release must be neutralized. From a practical standpoint, therapy of acute coliform infections cannot begin until clinical signs appear. Clinical recognition of coliform mastitis usually occurs after peak bacterial numbers have been attained. Thus, by the time therapy is initiated, maximal release of LPS has likely occurred. This raises concerns regarding the advantages of antimicrobial therapy in alleviating the effects of acute coliform mastitis. Thus, the primary therapeutic concern is the treatment of endotoxin-induced shock with fluids, together with other supportive care. The reader is referred to other sources for in-depth reviews of these concepts (Anderson, 1989; Erskine et al., 1993).

Antimicrobial therapy may be of secondary importance relative to supportive treatment of endotoxic shock, but it remains a practical part of a therapeutic regimen for acute mastitis. Although useful for determining the pathogenesis of coliform IMI, experimental challenge models cannot be considered as universally applicable for all cases. Occasionally, coliform infections result in chronic mastitis. Septicemia can occur, although it is believed to be an infrequent result of infection. In addition, numerous other pathogens cause acute clinical mastitis, which can be difficult to distinguish from cases caused by coliforms at initial presentation. Chronic IMI caused by pathogens such as *S. aureus* can become acute or even gangrenous at times of neutropenic dysfunction, such as parturition. Severe overwhelming IMIs that lead to sepsis have been recorded for pathogens such as *Pseudomonas aeruginosa* and *Bacillus cereus*. The need for antimicrobial therapy in cows with grossly abnormal milk but with improved appetite, attitude, and milk production should be evaluated critically. Unnecessary extension of therapy in these instances results in increased discarded milk expense for the dairy producer and risk of antimicrobials in marketed milk.

Selection of a potentially efficacious antimicrobial agent for the therapy of acute coliform mastitis is primarily dependent on in vitro culture and sensitivity. A review of antimicrobial susceptibility data for Gram-negative pathogens is presented in Table 31.1. Antimicrobials such as aminoglycosides and cephalosporins are often selected for use, because a higher proportion of isolates display sensitivity or lower MIC to drugs of this class than to available penicillins or macrolides. Selection of aminoglycosides, however, appears to be erroneous, for reasons discussed above and confirmed by experimental data. Gentamicin IM was not more efficacious in preventing agalactia or death resulting from acute clinical mastitis, or in improving other clinical outcomes, when compared with administration of erythromycin IM or no systemic antimicrobials (Jones and Ward, 1990). In addition, cows experimentally challenged with *E. coli* and dosed with 500 mg of intramammary gentamicin as frequently as

TABLE 31.1. Antimicrobial susceptibility (%) of Gram-negative pathogens

Pathogen	Gram-negative	Coliform	Coliform	Gram-negative	Coliform	Coliform	Coliform	<i>E. coli</i>
Number of Isolates	214 ^a	1,695 ^b	172 ^c	46 ^d	53 ^e	70 ^f	ND ^g	27 ^h
	%							MIC ₉₀ (µg/ml)
Amoxicillin	ND	ND	ND	ND	ND	ND	59	>200
Ampicillin	ND	48	44	61	ND	73	20	200
Cephalothin	53	65	79	83	87	79	ND	128
Erythromycin	ND	ND	ND	ND	0	ND	ND	ND
Gentamicin	96	ND	91	98	100	96	ND	2
Neomycin	48	87	63	48	55	83	68	ND
Norfloxacin	ND	ND	ND	ND	ND	ND	ND	<0.25
Novobiocin	ND	ND	ND	ND	0	ND	ND	ND
Penicillin	0	4	ND	0	0	1	ND	ND
Spectinomycin	ND	ND	ND	ND	ND	ND	45	128
Streptomycin	69	60	34	30	42	59	55	ND
Sulfadiazine-trimethoprim	ND	ND	ND	72	ND	ND	82	128
Tetracycline	30	66	24	43	60 ⁱ	61	68	>200

Note: Data expressed as percentage of isolates susceptible from disk assay, except for *E. coli* (Muckle et al., 1986). ND, not done.

^aMcDonald et al. (1977).

^bDavidson (1980).

^cLarson et al. (1981).

^dAnderson et al. (1982).

^eSchultze (1983).

^fAnderson (1989).

^gMackie et al. (1988); 4-year study.

^hMuckle et al. (1986).

ⁱOxytetracycline.

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every 12 hours did not have lower peak bacterial concentrations in milk, duration of infection, convalescent somatic cell or serum albumin concentrations in milk, or rectal temperatures than those of untreated challenged cows (Erskine et al., 1992). Additionally, gentamicin readily diffused through the milk: blood barrier, as indicated by detectable concentrations in serum throughout the treatment period and the first 12 hours after the last dose. Consequently, urine gentamicin concentrations were detectable 14 days after the last infusion, and concentrations of 1 µg of gentamicin/g in renal tissue were detected in cows 6 months after the trial. The American Veterinary Medical Association has approved a voluntary ban on the extralabel use of aminoglycosides in cattle.

Ceftiofur sodium, a third-generation cephalosporin, has been reported to have excellent activity against Gram-negative pathogens, with a suggested MIC for *E. coli* of 0.25–1.0 µg/ml. However, mammary tissue and milk concentrations of ceftiofur following systemic treatment remain below these concentrations (Owens et al., 1990; Erskine et al., 1993). This is probably a consequence of low bioavailability because of a high degree of protein binding, as previously discussed. Three doses of parenteral sulfadiazine-trimethoprim have also failed to achieve clinical improvement in experimental *E. coli* infections (Pyorala et al., 1994). However, three doses IM of cefquinome, a third-generation cephalosporin with good tissue distribution, was found to improve clinical outcome of experimentally infected cows when compared with use of cloxacillin or ampicillin (Shpigel et al., 1997). This is one of the few studies that demonstrated efficacy for parenteral use of antimicrobials in cows with experimental coliform mastitis, but it also demonstrates the importance of selecting antimicrobials with low MIC for targeted organisms in addition to beneficial pharmacokinetic properties.

Treatment of Acute Clinical Mastitis—Noncoliforms

Therapy for causative pathogens of acute mastitis other than coliforms has not been well studied. In general, results can be expected to be poor for Gram-negative and Gram-positive rods, mycotic or algal organisms, and for *Mycoplasma bovis*. Some benefit may be gained in reducing the clinical severity, and perhaps in outright bacteriologic cure, of IMI caused by Gram-positive cocci. For these pathogens, parenteral administration of antimicrobials that exhibit relatively good activity against these organisms and distribute well to the gland, such as oxytetracycline, fluoroquinolones, and macrolides, may be the best choices for therapy.

PSEUDOMONAS SPECIES

Aminoglycoside susceptibility is often reported, and outside the United States, chloramphenicol, polymixin B or C, and furazolidone have

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been reported to have success. However, these conclusions are based on clinical observations and not on controlled studies, and the reality is that antimicrobial therapy is not likely to achieve bacteriologic cures or affect clinical outcome. *Pseudomonas* IMI can become dormant, and virtually undetectable from bacteriologic culture of milk, only to recur as severe mastitis months after therapy. In other words, false-negative cultures from affected cows following a case of severe mastitis are common. Additionally, fatal septic involvement of these IMIs has been reported. Thus, treatment should be focused on supportive care and on antimicrobial use to prevent sepsis. Effective control, by reducing the exposure of udders to the reservoir of infection, is critical when approaching mastitis outbreaks caused by this organism.

SERRATIA SPECIES

Serratia spp. are not readily invasive, and for IMI to occur, large numbers of bacteria must be placed on or near the gland. About half of the infections are reported to be clinical, although subclinical herd outbreaks are possible, and new IMI can occur during the dry period. The clinical nature of IMI caused by these organisms may depend on the initial exposure, and chronic infections that last much longer than typical Gram-negative infections may occur. Spontaneous recovery has been reported. Thus, as with *Pseudomonas* spp., treatment for the purpose of bacteriologic cure is not likely to be beneficial.

ARCANOBACTERIUM PYOGENES

Arcanobacterium (Actinomyces) pyogenes is one of several organisms involved in the etiology of European "summer mastitis." It is frequently associated with teat injury and clinical mastitis observed in dry cows. Cases are predominantly malodorous and clinical; however, because the clinical signs are not specific, diagnosis and therapy should be based on milk culture and antimicrobial susceptibility. Long-acting antibiotic treatment has been reported to be successful at dry off, and tylosin has been reported to be effective in Denmark (Katholm, 1988). However, because of decreased production and low rate of cure, culling is frequent. For more typical cases of clinical mastitis in lactating cows, treatment of this pathogen should be attempted with intramammary infusions of beta-lactams. Because of the intense propensity of this organism to form purulent abscesses, it is recommended to include systemic administration of procaine penicillin G or oxytetracycline. More-chronic IMIs are not likely to be cured bacteriologically, and therapy of recurring cases should be approached judiciously, as bacteriologic cures become unlikely and high concentrations of the pathogen are often shed, raising concern over the potential reservoir of infection that affected cows become for herdmates.

NOCARDIA SPECIES, YEASTS, AND FUNGI

Infection with *Nocardia* spp. and *Candida* spp. are characterized by sudden onset of udder swelling and lack of positive clinical response to conventional antimicrobial therapy. Some cases, particularly caused by *Candida*, are subclinical. Clinical cases are often associated with extended antimicrobial treatment and contaminated udder infusions or devices. Because the clinical signs are similar to bacterial infections, diagnosis should be based on milk culture. Treatment should include discontinuation of antimicrobial therapy and culling of chronically infected cows. Clinically recovered cows may shed organisms for up to 8 months. Clotrimazole, ketoconazole, miconazole, nystatin, and sulfamethoxypyridazine have all been used, with reported clinical success, in the treatment of *Candida*. However, these studies were based on clinical impressions, and none of these drugs is approved for use in food-producing animals in the United States.

OTHER PATHOGENS

Prototheca zopfii, a colorless alga, is a common inhabitant of the dairy environment, being associated with wet areas contaminated with manure. Chronic subclinical and clinical infections occur, and successful treatments have not been reported. Infected cows should be isolated and removed from the herd. Mycoplasmal infections occur primarily during milking, and carrier cows are the major reservoir of infection. Routine microbiological identification procedures will not detect the presence of these organisms in milk, as special media and growth conditions are required. Successful treatment methods have not been discovered for *Mycoplasma* mastitis, and because of the potential contagious nature of infection, infected cows should be segregated or culled from noninfected cows in the herd.

Occasionally, premature agalactia in chronically infected quarters, particularly quarters infected with pathogens refractile to therapy, is promoted as an alternative to culling the cow. This may have some benefit in genetically superior animals within a herd or in cows that are to be maintained until calving. The goal is to eliminate the infection by causing fibrosis of the affected quarter, thus reducing the risk of further pathogenic change or systemic effects on the cow, as well as reduced risk of infection for other cows. Infusion of 60 ml of 2.0% chlorhexidine-diacetate (Nolvasan, solution—Fort Dodge) into affected quarters is administered twice at 24-hour intervals. The quarter should be stripped out before the second infusion. Care should be taken that milk from noninfected quarters is not sent to market before prior testing for inhibitors in milk. This regimen is not recommended for a majority of chronically infected animals and should be used judiciously by practitioners.

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*Treatment of Mild Clinical Mastitis

Of bacteriologic cultures of milk samples collected from cows with clinical mastitis, 30–35% will not yield any microorganism. Unlike treatment of severe clinical mastitis cases, treatment of mild cases (abnormal milk with or without local inflammation of the affected quarter, no systemic signs) is a more voluntary therapeutic decision. Many mild mastitis cases are coliform IMIs that are resolved before treatment is necessary. Additionally, numerous mild clinical mastitis cases are temporary setbacks in the balance between pathogen and host defenses that occur in more chronic IMIs. A high proportion of clinical cases, particularly those caused by Gram-positive cocci, observed in cows during the first 60 days after calving are manifestations of infections that started during the dry period. Additionally, chronic IMIs often display recurring episodes of clinical mastitis at regular intervals, and a small proportion of cows often account for a disproportionate number of cases. This poses the major question about treatment of mild clinical cases. Does antimicrobial therapy enhance cure rate over that attained by no treatment (spontaneous cure)? Therapeutic and spontaneous cure rates for clinical mastitis cases caused by coliforms and staphylococci are very similar. However, by contrast, it may be advantageous to treat streptococcal IMIs if they are not chronic. Information on therapeutic efficacy of mild clinical mastitis cases is not extensive. In one study, clinical symptoms were resolved in 74% of clinical cases caused by *Streptococcus uberis* and 84% of cases caused by *S. dysgalactiae* after a single intramammary infusion of cefoperazone (Wilson et al., 1986). Bacteriologic cure was achieved in only 60–65% of the cases. A comparison of cure rate in treated and untreated cows was not reported. In a study of three Californian dairies, bacteriologic cure assessed at 4 and 20 days after treatment with amoxicillin, cephalixin, or oxytocin (no antimicrobial) did not differ for mild clinical mastitis cases caused by any pathogen, although antimicrobial treatment resulted in better clinical cure rates for cases caused by pathogens other than streptococci and coliforms (Guterbock et al., 1993). Thus, renewed interest in a "no antibiotic" approach to mild clinical mastitis cases promotes a convenient regimen to avoid costs of discarded milk and residue risks that are inherent in antimicrobial therapy. However, an economic analysis of the California data determined that although milk production and survival in the herd did not differ between antimicrobial- and non-antimicrobial-treated cows, the rates of both relapses and recurring cases were higher in non-antimicrobial-treated cows, especially among streptococcal cases. A case report from a Colorado dairy also reported a marked increase in the incidence of clinical mastitis, in the prevalence of IMI, and in the subsequent increase in herd somatic cell count associated with streptococcal IMI following adoption of a no-antibiotic approach to clini-

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cal mastitis (Cattell, 1996). Administration of supportive care, IV oxytetracycline, and intramammary cephalirin to cows with varying degrees of clinical mastitis severity resulted in a higher frequency of normal udder appearance at 10 days (clinical cure) and bacteriologic cure in 14 days for streptococcal IMIs and clinical cure for coliform IMI than in cows administered supportive care only (Morin et al., 1998).

The conclusion is that common sense and individual herd history should determine the course of therapeutic planning for mild clinical mastitis cases in dairy herds. For initial occurrences of clinical episodes for any affected quarter, especially those caused by streptococci, labeled use of commercial intramammary infusions is the best approach. However, if infected quarters recur with regularity, and in the absence of systemic signs, repeated treatment of what now has become chronic IMI is not warranted. Any moderate increase in cure rates gained by extensive parenteral therapy for chronic IMI in lactating cows will not overcome the expense of discarded milk and other related treatment costs.

Treatment of Subclinical Intramammary Infections

As with mild clinical mastitis cases, subclinical mastitis does not present an urgent potential loss of gland function or life to the cow. No significant economic losses will occur as a result of prolonging initiation of therapy until bacterial culture can be completed. Antimicrobial susceptibilities of isolated pathogens often reveal potentially beneficial therapy with beta-lactam and macrolide drugs (Tables 31.2 and 31.3).

TABLE 31.2. Antimicrobial susceptibility (%) of *Staphylococcus aureus*

Number of Isolates	214 ^{a,e}	— ^{b,e}	28 ^f	106 ^g
	%			MIC ₉₀ (µg/ml)
Ampicillin	47	33	ND	ND
Cephalothin	100 ^c	100 ^c	100	1
Erythromycin	94	98	96	0.5
Gentamicin	ND	ND	100	ND
Neomycin	89	94	86	1
Novobiocin	97	98	93	0.25
Penicillin	0	30	4	ND
Streptomycin	ND	ND	39	4
Tetracycline	88	88	93	0.5 ^d

Note: Data expressed as percentage of isolates susceptible from disk assay, except Craven et al. (1986). ND, not done.

^aClinical isolates.
^bSubclinical isolates.
^cCephaloridine.
^dOxytetracycline.
^eMackie et al. (1988).
^fSchultze (1983).
^gCraven et al. (1986).

TABLE 31.3. Antimicrobial susceptibility (%) of streptococci

Number of Isolates	7 ^a	41 ^a	5 ^b	257 ^a	68 ^b	52 ^b	96 ^c
Pathogen	<i>Streptococcus agalactiae</i>	<i>S. dysgalactiae</i>	<i>S. dysgalactiae</i>	<i>S. uberis</i>	<i>S. uberis</i>	Enterococci	Gram-positive cocci
Ampicillin	ND	ND	ND	ND	ND	ND	59
Cephalothin	100 ^d	100 ^d	80	100 ^d	96	63	96
Cloxacillin	ND	ND	60	95	81	10	ND
Erythromycin	100	100	100	97	72	48	ND
Gentamicin	ND	63	100	11	72	67	82
Neomycin	ND	ND	80	ND	29	44	89
Novobiocin	ND	ND	80	ND	88	48	ND
Penicillin	100	100	100	100	93	60	49
Streptomycin	ND	ND	10	ND	10	35	39
Tetracycline	100	27	100	98	66	48	63

Note: Data expressed as percentage of isolates susceptible from disk assay. ND, not done.

^aMcDonald et al. (1976).

^bSchultze (1983).

^cAnderson et al. (1982).

^dCephoxazole.

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However, many subclinical IMIs selected as potential therapy candidates are chronic, and particularly in the case of *Staphylococcus aureus*, prediction of therapeutic outcome by in vitro testing is poor in chronically infected quarters when compared with predictions regarding newly acquired IMIs (Owens et al., 1997). Thus, drug distribution following intramammary administration may not be efficacious, because of extensive fibrosis and microabscess formation in the gland. In addition, it is critical to assess the cow's immune status from a perspective of duration of infection, number of quarters infected, and other variables, as previously discussed.

Therapy is administered on the premise that treatment costs will be outweighed by compensatory production gains following elimination of infection. In the case of contagious pathogens, elimination of an IMI may also result in a decrease of the reservoir of infection for previously noninfected cows. Most often, the predominant pathogens causing subclinical mastitis are streptococci and staphylococci. In particular, the contagious pathogens *Streptococcus agalactiae* and *Staphylococcus aureus* offer a study in opposites with respect to therapeutic strategies.

Prevalence of *S. agalactiae* IMI can be rapidly reduced by "blitz" treatment. With this method an entire herd, or more economically, all the infected cows in a herd, are treated with antimicrobials. The most efficacious and cost-effective regimen is to employ intramammary beta-lactam therapy using approved commercial intramammary products, although use of erythromycin has been successful. All four quarters of infected cows should be treated to ensure elimination of the pathogen from the cow and prevent possible within-cow cross-infection of a noninfected quarter by an infected quarter. Cure rates can often be from 70% to 90%. Much of this success relies on the high degree of antimicrobial susceptibility of *S. agalactiae*, as well as its being essentially an obligate udder pathogen. It is necessary to monitor treated herds by somatic cell count and bacteriology to further identify and treat cows that were not initially identified as being infected. Usually, 30-day monitoring intervals after each treatment will be successful. A small percentage of cows will not respond to therapy and are best segregated or culled. Additionally, failure to use postmilking teat dipping and total dry-cow treatment to prevent new IMIs while eliminating existing IMIs by blitz treatment will ultimately result in considerable expense and frustration on the part of the producer, as well as possible reinfection of the herd. Parenteral therapy is not likely to offer any benefit over intramammary therapy, and in one study, 90% cure rates were achieved with intramammary penicillin-novobiocin therapy as opposed to 9% with IM ceftiofur, an elegant demonstration as to the importance of using pharmacokinetic considerations for parenteral drug selection (Erskine et al., 1996).

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Other common streptococci causing IMI are *S. dysgalactiae*, *S. bovis*, and *S. uberis*. As with *S. agalactiae*, most of these streptococci are very susceptible in vitro to penicillin. Despite this apparent susceptibility, these streptococcal infections are not as easily cured as those caused by *S. agalactiae*. Controlled clinical data on the therapy of streptococcal infections is not extensive. Intramammary administration of beta-lactams or, if parenteral treatment is desired, procaine penicillin G administered at 22,000 IU/kg of body weight twice daily is considered a good drug regimen. However, efficacy and cost-effectiveness of this therapeutic regimen are at present difficult to assess. Furthermore, penicillins are poorly distributed to the mammary gland following parenteral administration. In strains with in vitro susceptibility to a combination of penicillin and novobiocin, intramammary therapy has obtained cure rates of 90% for subclinical IMIs caused by *S. agalactiae*, *S. uberis*, and *S. dysgalactiae* and 77% for other streptococci (Owens et al., 1997). Thus, in vitro susceptibility correlates well with clinical outcome for IMIs caused by streptococci.

Staphylococcus aureus IMIs often result in deep-seated abscesses. Therapy is difficult, especially since resistance to antimicrobial drugs (particularly beta-lactams) is more common than in streptococci, and *S. aureus* may survive within phagocytic cells following phagocytosis, where antimicrobial concentrations are reduced. Additionally, *S. aureus* has numerous extracellular substances that impart an ability to survive in the presence of a hostile host immune system. Consequently, elimination of infections by antimicrobial treatment is often not successful.

Experimentally induced *S. aureus* IMIs have cure rates of 25–55% of infected quarters when evaluated for 21–60 days after infection. However, natural infections are usually of longer duration before detection and subsequent therapy and thus are more refractory to therapy. The use of intramammary cefaperazone for the treatment of naturally occurring clinical *S. aureus* mastitis resulted in bacteriologic cures for only 39% of the cases, as measured 14 days after treatment (Wilson et al., 1986). However, as with experimental infections, these results may be optimistic, because assessment of bacteriologic cures depends on the time period during which the infection is monitored after treatment; relapses of 28 days after treatment have been reported. Treatment of IMIs caused by *S. aureus* identified as susceptible to penicillin-novobiocin resulted in cures of 70% in quarters with IMI of 14 days duration and 35% in chronically infected quarters (Owens et al., 1997). This not only reaffirms the difficulty in attaining successful therapy but also suggests a poor correlation between in vitro culture and susceptibility and therapeutic outcome in chronic IMIs caused by this organism. Consequently, novel combinations of drugs, including parenteral treatment regimens, may be needed to eliminate a higher proportion of infections. In experimental infections in lac-

tating cows, the combined use of IM procaine penicillin G and intramammary amoxicillin achieved a better cure rate (18 of 35 infected quarters) than intramammary amoxicillin alone did (10 of 40 infected quarters (Owens et al., 1988). Parenteral administration as an adjunct to intramammary therapy has been further supported by an additional study that determined that administration of 11 mg/kg of oxytetracycline in addition to intramammary antimicrobials improved cure rates for lactating cows with *S. aureus* IMI over cure rates in cows that received intramammary infusions only (Sol et al., 1990). Additionally, therapy that is administered for longer periods than those typical for labeled intramammary regimens (4 days rather than 2 days) maintains drug concentrations above MIC of *S. aureus* in the gland for longer times and results in better efficacy (Ziv and Storper, 1985).

These studies suggest that therapy of chronic subclinical IMI may be more successful when it includes parenteral as well as intramammary therapy and is preferentially administered for periods long enough to ensure drug levels above MIC, to allow effective killing of the pathogen. However, cure rates attained may not be much better than those attained from spontaneous cure. Additionally, definitions of cure must be made critically, and affected quarters monitored bacteriologically for at least 30 days to encompass the refractory period when IMI are present but bacteria may not be isolated.

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The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines and Inspections

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EMEA/CVMP/344/99-FINAL-Rev.1

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

**GUIDELINE FOR THE CONDUCT OF EFFICACY STUDIES
FOR INTRAMAMMARY PRODUCTS FOR USE IN CATTLE**

APPROVAL BY CVMP FOR RELEASE FOR CONSULTATION	15 - 17 JUNE 1999
TRANSMISSION TO INTERESTED PARTIES	18 JUNE 1999
DEADLINE FOR COMMENTS	17 DECEMBER 1999
DISCUSSION IN EFFICACY WORKING PARTY	7 FEBRUARY 2000
ADOPTION BY CVMP	8 MARCH 2000
DATE OF COMING INTO EFFECT	8 SEPTEMBER 2000
REVISION 1 (includes further clarifications to paragraph 5.4)	12 FEBRUARY 2003

Public

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GUIDELINES FOR THE CONDUCT OF EFFICACY STUDIES FOR INTRAMAMMARY PRODUCTS FOR USE IN CATTLE

This note for guidance replaces the three previous guidance notes (III/9041/90, III/3576/91, III/3173/92) in Volume 7A (1998).

1. OBJECTIVE

The objective of the trial is to assess the efficacy of a medicinal product administered via the teat duct for the treatment of clinical and/or subclinical mastitis during lactation, or for the elimination of udder infections at drying-off and the prevention of new infections during the dry period. Clinical mastitis is defined as mastitis with clinical signs in the quarter (swelling, heat, pain) and/or changes in the appearance of milk (clots or flakes, watery appearance, discoloration), with or without general signs (fever, loss of appetite). Subclinical mastitis is defined as mastitis without clinical signs but a high milk somatic cell count in the quarter and a positive bacteriological isolation from the milk.

2. METHODS AND TREATMENTS

2.1 Comparative trials

Comparative trials should be carried out in two groups of cows:

- cows/quarters treated with the test product,
- control cows/quarters treated with an authorized product, placebo or no treatment. The choice of the type of control group should be justified by the applicant.

The trial must be carried out on a sufficient number of herds and cows. The described statistical methods should include definition of study population, sample size calculation, randomization and blocking procedures, confounding factors, statistical models, and level of significance. Cows selected in each herd should not exceed 20% of the total number of cases treated in the complete study, unless justified.

Treatment should be allocated within each herd in a random manner using blinding as far as is practically possible. The balancing of the groups to include control and treated cows must be carried out at the individual herd level and on the total population.

The milk samplings and microbiological investigation must be carried out in accordance with the methods recommended by the International Dairy Federation (IDF) (see Bulletin No 132, 1981), or by other adequate reference.

2.2 Reference product

Where possible, it is advisable to use a reference product with the same claims, approved in accordance with Directive 2001/82/EC.

2.3 Test product

The conditions of administration, dosage and frequency of administration should be justified, described and should be those proposed.

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2.4 Milk withdrawal period

In a blinded study, the milk withdrawal period should be the same for each product (test and reference products) and correspond to the product with the longest withdrawal period. If blinding is not used, then the withdrawal period of the individual products should be observed.

In dry cow treatment, the products must be given to cows with dry periods of a sufficient length to ensure that antibiotic residues will not persist in the milk intended for human consumption following calving.

2.5 Treatment unit

In clinical mastitis the treatment and statistical unit is the individual udder quarter, and only one quarter is to be treated in any one cow.

In the dry cow treatment and subclinical mastitis, the treatment unit is the cow but the statistical unit is the individual quarter. In the dry cow all four quarters receive the same treatment.

3. SELECTION OF ELIGIBLE HERDS AND COWS

3.1 Identification

Study cows should be selected from herds with proper cow identification and recording of health data.

3.2 Defining inclusion/exclusion criteria

The population of eligible cows must be described and the inclusion/exclusion criteria defined for:

- cows which are to be included in the trial;
- cows which are to be excluded from the trial;
- cows which are to be excluded post inclusion from the trial.

In subclinical mastitis trials, all lactating cows with presence of pathogens in conjunction with quarter somatic cell count (SCC) > 300 000 cells/ml in both pre-treatment milk samples, may be eligible for the trial.

In clinical mastitis trials, all lactating cows with clinical mastitis which can be treated with intramammary treatment only are eligible for the trial. Cows with severe systemic signs or toxic mastitis should not be included.

In dry cow treatment, all lactating cows approaching the end of lactation are eligible for the trial.

3.3 Exclusion criteria

The following cows are to be excluded from the trial:

- cows with intercurrent diseases;
- cows which were given systemic or intramammary anti-infectious or anti-inflammatory treatments within a 30-day period before the trial;
- cows with visible teat damage;
- in clinical mastitis: cows with mastitis in two or more udder quarters;
- in clinical and subclinical mastitis: cows with a daily milk yield less than 5 litres of milk prior to onset of clinical signs.

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3.4 Herd and cows information

The history of the herd and cows must be recorded after the inclusion of a cow in the trial and before the commencement of the treatment.

Farm:

- name and address of herd owner;
- number of dairy cows;
- methods of herd management, milking, and dry cow management;
- teat disinfection procedures if practised;
- bulk milk SCC in the herd over preceding months (if available).

Cows:

- name or identification number;
- breed;
- number of lactations;
- date of calving;
- estimated or measured milk yield at time of treatment;
- cow milk SCC if available during preceding months;
- history of previous mastitis treatments if available;
- in clinical mastitis: carefully recorded clinical signs at the time of treatment;
- in the dry cow treatment: the milk yields of cows at drying-off and the method of drying-off (e.g. once-a-day milking, abrupt cessation of milking, etc.).

4. REPORTING

4.1 Data required

Data required from each treated cow:

Clinical mastitis:

The cow is examined clinically (general condition, milk quality, udder consistency) just before treatment, and in connection with the first bacteriological post-treatment sampling. In addition to these examinations, clinical examination should be made in cases where clinical developments make it necessary. If no clinical cure has occurred at the first post-treatment sampling, the case is excluded from further sampling.

Milk samples for bacteriological analysis are taken before treatment and two times after treatment. The post-treatment samples should be taken at least 7 days apart between day 14 and day 28 from the cessation of the treatment. Quarter milk SCC is determined from the second post-treatment sample.

Subclinical mastitis:

Milk samples for bacteriological analysis are taken twice before treatment and twice after treatment. Two positive isolations of the same pathogen before treatment are required (two consecutive samples taken with one day apart; if only one sample out of these two is positive, diagnosis must be confirmed with a third sample). The post-treatment samples should be taken at least 7 days apart between day 14 and day 28 from the cessation of the treatment. Quarter milk SCC is determined from the pre-treatment samples, and from the second post-treatment sample.

Dry cow treatment:

Milk samples for bacteriological analysis must be taken before drying-off and after calving from all the quarters. Two samplings before treatment are required (two consecutive samples taken with one day apart within one week before drying-off; if one sample out of these two is positive, diagnosis must be confirmed with a third sample). The first post-treatment sample is taken before the first regular milking after calving, and the second post-treatment sample 4 to 7 days later. Cow milk SCC should

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be determined from the second post-treatment sample. The cow must be clinically examined after calving for any pathological changes of the udder or of the appearance of the milk. Cases of clinical mastitis occurring during the dry period and during the post-calving investigational period shall be recorded.

The *in vitro* antimicrobial susceptibility of the bacteria isolated from the pre-treatment sample to the antimicrobials used in treatment should be determined using accepted procedures.

4.2 Admission criteria for cows or quarters in the final analysis of data

- a) Cases which are uninterpretable, due to a lack or loss of information, shall be listed in the final report and their distribution in each group shall be analysed.
- b) Data collected from any udder/quarter of a cow, which had to be treated with additional antibiotic or other supportive treatment associated with the mastitis within the individual cow experimental period, should be included in the final data analysis. These cases should be classified as treatment failures. Cows treated with antibiotics or anti-inflammatories due to intercurrent diseases during the experimental period should be excluded from the trial. Data from cows in which additional quarters had to be treated within the individual cow study period shall be excluded from the final analysis and listed separately for each treatment group.

5. INTERPRETATION OF RESULTS

5.1 Bacteriological status

Bacteriological status is the key parameter in evaluating success of treatment. Bacteriological cure must be evaluated for each treated infected udder quarter and must be based on the total elimination (eliminated from both post-treatment samples) of the pathogens which were present at the time of treatment. In clinical and subclinical mastitis claims, and in treatment claim of dry cow treatment, quarters with new infections in the originally infected, treated quarters (growth of bacterial species different from the original ones) in one or both post-treatment samples can be classified as a bacteriological cure. The number and type of these occurrences in each treatment group should be included in the final study report. A high frequency of these occurrences is not acceptable and needs further clarification.

5.2. Clinical mastitis

In clinical mastitis studies, the clinical cure must be evaluated for each infected quarter and must be based on the return to normal of the parameters concerning the cow's general condition, the quality of the milk and the consistency of the udder. A case is regarded as a clinical cure if the milk has a normal appearance and the condition of the udder and the cow's general condition are normal in the clinical examination at time of the first post-treatment sampling. If there is no clinical cure, the case is to be excluded from the second sampling and classified as treatment failure. Only cases of mastitis in which bacteria are isolated in the pre-treatment sample will be used in calculating cure rates. Results from quarters with no growth in the pre-treatment sample can be reported separately.

5.3. Clinical and subclinical mastitis

In clinical and subclinical mastitis trials, individual quarter milk SCC is determined from the second post-treatment samples. Mean SCCs are calculated from the results for each treatment group and separately for bacteriologically cured and not cured quarters. The mean SCC results for each treatment group will be used to give numerical trends, but these data are generally not included in the final judgement criteria.

The data should be expressed as numbers of quarters and number of cows cured clinically, bacteriologically, and based on individual quarter milk SCC (subclinical mastitis only), see table 1. For clinical mastitis studies, cows with no clinical cure in the clinical examination at the first post-

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treatment sampling are included in the final calculations of the bacteriological cure rates as failures. For subclinical mastitis studies, it is preferable to present combined cure rates based on individual quarter data (bacteriological cure + quarter milk SCC < 300 000 cells/ml).

Table 1. An example for data presentation for each treatment group (further details are given in the text).

		Post-treatment Cure			
		Clinical cure		Bacteriological cure	Bacteriological + SCC cure ¹
	No of quarters/cows	n	%	n	%
Treatment A					
Treatment B					

¹ For subclinical mastitis only

5.4 Dry cow treatment

An application for a dry cow treatment may support either a claim for cure or a prevention claim or both cure and prevention claims together. However, efficacy of treatment (claim for cure) and the prevention of new infections (prevention claim) must be evaluated separately.

Treatment effect is evaluated for each pathogen over all the treated udder quarters. A new infection corresponds to the appearance of a bacterial species different from that isolated at drying-off or the appearance of bacterial growth in a quarter not infected at drying-off. Mean cow milk SCCs for each treatment group determined from the second milk samples after calving are calculated for each treatment group. The mean SCC results will be used to give numerical trends, but these data are generally not included in the final judgement criteria. In the treatment claim, if the bacterial species growing in one or both post-calving sample(s) is the same as the original one at drying-off, it is a “treatment failure”; if the post-calving sample(s) has growth of other bacterial species, it is considered as a new infection and “treatment success”.

For the prevention claim, only healthy quarters with no bacterial growth at drying-off are eligible. The same quarter cannot be taken into account both for prevention and treatment claim. No growth in both post-calving samples is “prevention success” for prevention claim and “cured i.e. treatment success” for treatment claim. For prevention claim, bacterial growth in one or both post-calving samples is always “prevention failure”.

6. PRESENTATION OF DATA

The data to be presented are described in the annex to Directive 81/852/EEC as modified by directive 92/18/EEC.

A record from each individual case should be presented in the final report.

In particular, the data on the bacteriological response for each organism for each treated quarter must be summarized and tabulated separately for each bacterial species and treatment group. Results from the *in vitro* susceptibility are enclosed in the final report.

ADVANCES IN THE THERAPY FOR MASTITIS

Ronald J. Erskine, DVM, PhD, John H. Kirk, DVM, MS,
Jeff W. Tyler, DVM, MPVM, PhD,
and Fred J. DeGraves, DVM, PhD

Effective and economic mastitis control programs rely on prevention rather than treatment. Herds practicing mastitis prevention produce higher quality milk more cost effectively than herds that do not. Nonetheless, practitioners are requested by clients to manage therapy for mastitis cases. This article describes therapeutic alternatives for bovine mastitis.

Therapy of infectious disease should either assist host defenses in eliminating invading pathogens or reduce pathophysiologic consequences of infection without degrading host defenses. Logically, emphasis in mastitis therapeutics has focused on the elimination of infectious agents by use of antimicrobial agents. Therapeutic success for some infections may be better measured by evaluating reduction of clinical symptoms, however, rather than total elimination of the pathogen from the gland. Ultimately, the best endpoints of efficacy are milk production and long-term survival.

The greatest advance in antibiotic therapy of mastitis has been philosophic rather than scientific: The single most noteworthy development is the growing consensus among veterinarians and dairy managers as to the limited role antibiotics play in treatment and control programs. Factors that have fostered this altered mindset have included economic assessment of treatment programs, changes in the Pasteurized Milk Or-

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dinance, and critical evaluation of clinical efficacy of therapy. Antibiotic therapy for mastitis must be restricted to instances in which the practitioner is reasonably assured of (1) therapeutic efficacy, (2) economic benefit (either improved production or reductions in the reservoir of infectious bacteria that offset direct and indirect treatment costs), and (3) production and sale of residue-free milk and meat. Clear evidence that antimicrobial therapy for individual cows can improve health and productivity may be restricted to dry cow intramammary therapy and lactating cow therapy for *Streptococcus agalactiae* mastitis. Clinical trials and experimental studies have demonstrated no benefits to antibiotic therapy in cattle with either clinical or subclinical gram-negative mastitis.^{28, 38, 44, 55} Treatment of lactating cows with *Staphylococcus aureus* mastitis often is unsuccessful.⁷⁹ The question asked by practitioners and mastitis researchers has shifted from "Which antibiotic should I use?" to "Are antibiotics indicated?"

PHARMACOLOGIC CONSIDERATIONS

The ideal antibiotic for systemic treatment of mastitis would be weakly basic, poorly bound to plasma proteins, and lipid soluble. It would retain activity in inflammatory secretions and have antimicrobial activity against mastitis pathogens. Systemically administered sulfonamides, penicillins, aminoglycosides, and cephalosporins do not readily penetrate the mammary gland. Macrolides (erythromycin, tilmicosin), trimethoprim, tetracyclines, and fluoroquinolones distribute well to the mammary gland, but none of these compounds is approved for systemic use in the lactating dairy cow. New antimicrobial drugs are released on the veterinary market continually; some of these compounds likely will prove valuable in the therapy for mastitis. Recent areas of active investigation include systemic therapy for subclinical mastitis, distribution patterns of various antimicrobial agents, development of resistant L-forms following antimicrobial therapy, and resistance patterns of common mastitis pathogens. The reader is referred to articles on these topics published in a recent issue of this publication.^{79, 89}

SUBCLINICAL MASTITIS THERAPY

Subclinical mastitis does not present an urgent potential loss of gland function or loss of the cow's life. Therefore, treatment is provided on the premise that costs will be outweighed by compensatory production gains following elimination of infection. In the case of contagious pathogens, elimination of infection also may result in a decreased risk of new infections in previously noninfected cows. Although host immune defenses play a critical role, the degree of therapeutic success in treating subclinical mastitis relies predominantly on the type of causative pathogen, and often not on the drug regimen selected. The predominant

pathogens causing subclinical mastitis most often are streptococci and staphylococci. In particular, the contagious pathogens *Streptococcus agalactiae* and *Staphylococcus aureus* offer a study in opposites with respect to therapeutic success.

"Blitz" treatment is one method that rapidly reduces the herd prevalence of *Streptococcus agalactiae*. With this method, an entire herd or substantial part of it is treated with antimicrobial agents. It is efficacious, cost-effective, and, because it uses Food and Drug Administration (FDA) approved antimicrobial products, should not result in drug residue problems in milk.^{25, 26} Much of this success relies on the high degree of antimicrobial susceptibility of *Streptococcus agalactiae*, as well as it being essentially an obligate udder pathogen.^{25, 25, 25} Whole herd culture and treatment of infected cows, followed by repeat culture and treatment at routine intervals, is a frequently successful approach to blitz treatment. Alternatively, Dairy Herd Improvement somatic cell count (SCC) data from herds with a high prevalence of *Streptococcus agalactiae* ($\geq 25\%$ of the herd infected) can offer a cost- and labor-efficient means of identifying cows for treatment, provided that preliminary milk cultures of selected cows have identified *Streptococcus agalactiae* as a major herd problem.²⁵ Finally, failure to use post-milking teat dipping and total dry cow treatment to complement blitz treatment will ultimately result in considerable expense and frustration on the part of the producer, as well as possible reinfection of the herd.

Other streptococci causing intramammary infections (IMI) are *Streptococcus dysgalactiae*, *Streptococcus bovis*, *Streptococcus uberis*, and the enterococci.²⁵ As with *Streptococcus agalactiae*, most of these streptococci are very sensitive in vitro to antibiotic agents, especially penicillin. In one study, virtually 100% of *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Streptococcus bovis*, and group G isolates were sensitive to penicillin.⁵⁶ Only 63% of the enterococci were sensitive to penicillin, however.⁵⁶ Despite this apparent sensitivity, some streptococcal infections remain refractory to therapy. Information on the therapy of streptococcal infections is not extensive. Wilson et al.⁸⁴ reported 74% of clinical cases caused by *Streptococcus uberis* and 84% of cases caused by *Streptococcus dysgalactiae* resolved after a single intramammary infusion of cefoperazone.⁸⁴ Bacteriologic cure was achieved in only 60% to 65% of the cases.⁸⁴ A comparison of cure rate in treated versus untreated cows was not reported, however. Procaine penicillin G, administered at 22,000 IU per kilogram body weight twice a day would appear to be the drug regimen of choice. Efficacy and cost-effectiveness of this therapeutic regimen is difficult to assess, however, and withholding periods for milk and meat would be extended considerably compared with the use of labeled doses. Furthermore, penicillins are poorly distributed to the mammary gland following parenteral administration.

IMIs caused by *S. aureus* often result in deep-seated abscesses.⁵⁵ Treatment is difficult, especially because *Staphylococcus aureus* resistance to antimicrobial drugs (particularly β -lactams) is more common than among streptococci⁵⁵, and *S. aureus* may survive intracellularly following phagocytosis where antimicrobial concentrations are reduced. In addi-

tion, *S. aureus* has numerous extracellular substances that impart an ability to survive in the presence of a hostile host immune system.^{37, 40} As a result, elimination of infections by antimicrobial treatment often is not successful.

Studies of efficacy of treatment of *Staphylococcus aureus* mastitis indicate cure rates of 25% to 55% of infected quarters in experimental infections that were evaluated for 21 to 60 days after infection.^{60, 63} Natural infections are usually of longer duration, however, and therefore more refractile to therapy. Wilson et al⁶⁴ reported that intramammary cefaperazone for the treatment of clinical *Staphylococcus aureus* mastitis resulted in bacteriologic cures for only 39% of the cases, measured 14 days after treatment. These results may be optimistic given that assessment of bacteriologic cures may depend on the time period the infection is monitored after treatment; a greater number of infections relapse as time after treatment increases.^{26, 60}

Alternatively, treatment of dry cows rather than lactating cows may be more effective in eliminating existing streptococcal and staphylococcal IMI.^{22, 57, 65, 73} Dry cow treatment may decrease the incidence of new infections in quarters that are uninfected at the start of the dry period.⁵⁷ Efficacy of conventional dry cow treatments in eliminating and preventing new *Staphylococcus aureus* IMI may be low, however. In a retrospective study, Ziv et al⁹⁰ determined that new infection rates of *Staphylococcus aureus* in quarters were 11% when treated with one of three commercially available intramammary infusion products containing (1) cloxacillin, (2) neomycin and cloxacillin, or (3) a combination of nafcillin, procaine penicillin, and dihydrostreptomycin, versus 4.9% in nontreated control quarters. Soback et al⁷⁸ determined that dry cows administered intramammary cephalixin benzathine had a cure rate of 30.8% and a new IMI rate of 51.7%, versus 33.3% and 29.3% in untreated quarters.

Consequently, novel combinations of drugs—including treatment regimens not approved by the FDA for use in dairy cattle—may be needed to eliminate a higher proportion of infections. In experimental infections in lactating cows, Owens et al⁶³ reported the combined use of intramuscular procaine penicillin G and intramammary amoxicillin achieved a better cure rate (18 of 35 infected quarters) than intramammary amoxicillin did alone (10 of 40 infected quarters). Similarly, Soback et al⁷⁴ determined that subcutaneous norfloxacin nicotinate administered at the start of the dry period achieved a better cure rate (66.6%) and lower new infection rate over the dry period (17.1%) for *Staphylococcus aureus* infections than those of untreated cows (33.3% and 29.3%, respectively) or cows administered intramammary cephalixin benzathine preparations (30.8% and 29.3%, respectively).

These studies provide evidence for three important considerations in the treatment of *Staphylococcus aureus* mastitis: (1) FDA approved and labeled drug use is not likely to result in a high frequency of cures. (2) Because of increased discarded milk costs and risk of residues in milk following extra-label drug use, dry cows should be treated preferentially over lactating cows. And (3) treatment should include systemic drug regimens, preferably antimicrobial agents that distribute well in mam-

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mary tissue and abscesses—i.e., tetracyclines, macrolides, and fluoroquinolones. Alternatively, the most effective treatment of *Staphylococcus aureus* may be to cull the infected cow from the herd.

Numerous cytokines, which regulate cell function during immune and inflammatory processes, recently have been identified and purified from cattle. With subsequent identification of the genes responsible for cytokine synthesis, recombinant production has been made possible.¹⁷ Cytokines that have demonstrated promise for use in the prevention or treatment of bovine mastitis are interleukin-1 (IL-1) and interleukin-2 (IL-2), interferons, and colony stimulating factor.^{17, 61, 62, 76} Intramammary IL-1 β and IL-2 increased mammary gland polymorphonuclear leukocyte diapedesis.¹⁸ In addition, IL-2 activated inducible superoxide production and phagocytosis in neutrophils.¹⁸ Preliminary trials with cows experimentally infected with *Staphylococcus aureus* determined that 38% of infected glands were cured when treated intracistemally with recombinant bovine IL-1 β and 42% with IL-2.¹⁷ This was similar to cure rates achieved with different intramammary formulations of sodium cephalixin. Thus, interleukins may modulate and even enhance nonspecific cellular defenses, although clinical availability is not yet a reality.

Novel uses of antimicrobials and other potential treatments such as cytokines have not been studied extensively under field conditions, and potential gains in efficacy derived from these treatments therefore are speculative at best. Whether or not an increased rate of therapeutic success would be justified by the increased costs in drugs and labor in treating dry cows also needs to be determined, but reduced potential for discarded milk compared with antimicrobials may make cytokine use attractive.

THERAPY OF CLINICAL MASTITIS

Although bacteriologic culture of milk is indicated to determine the most appropriate treatment, therapy usually is initiated before results can be obtained. Selecting an initial therapeutic regimen for clinical mastitis cases therefore usually is based on severity of infection, past bacterial culture history, and the experience of the practitioner. Mild cases that display little more than abnormal milk may respond well to "stripping out" the affected quarter regularly. Oxytocin may be administered to assist in milk letdown. Commercial intramammary antimicrobial preparations may be indicated to reduce clinical signs if abnormal signs persist for several days, or in moderate cases with marked swelling of the gland. It is questionable whether antimicrobial therapy has any benefit for recurring or persistent infections, and repeated attempts to treat these infections likely will result in no therapeutic benefit, with increased discarded milk costs. In a large Florida dairy in which streptococci were the predominant clinical mastitis pathogen, only 6.1% of lactations had more than 28 days of discarded milk, but these lactations were responsible for 52.7% of the milk discarded because of mastitis.⁵⁹ Bartlett et al⁹ reported that 70% of lost marketable milk resulting from clinical mastitis was due to withholding, as opposed to 30% from decreased production.

Antibiotics may be indicated in more severe cases. Labeled products with known milk withdrawal periods are preferred for initial treatment, whereas "extra-label use" drugs must be used judiciously.

ANTIMICROBIAL THERAPY OF ACUTE CLINICAL MASTITIS

Veterinary and drug costs are higher for the treatment of severe (systemic involvement) clinical mastitis cases than for less severe cases.³⁴ In addition, veterinary practitioners probably intervene more frequently in therapeutic management of acute mastitis caused by coliform bacteria (lactose fermenting, gram-negative rods) than in infections caused by any other pathogen. This, in part, is because coliforms are the single most common causative agent isolated from clinically severe mastitis cases.^{7, 35} In addition, most antimicrobial therapeutic regimens used as treatment of coliform mastitis in the United States are not labeled by the FDA for use in dairy cows. Cows managed with extra-label drugs are a potential residue liability for both dairy farmers and practitioners.

Experimental challenge models have clarified much of the pathophysiology of mastitis resulting from infection with gram-negative organisms.^{13, 27, 30, 70} Following infection, bacterial numbers in milk increase rapidly. Depending on the size of the challenge, peak bacterial concentrations in milk often occur within a few hours.^{27, 70} Typically, a rapid decline in bacterial concentration follows neutrophil migration into the gland. Although often severe, experimental coliform infections usually clear spontaneously and rarely last more than 4 to 9 days.^{27, 70} The resulting inflammation and leukocytosis in the affected quarter may persist for several weeks, or the quarter may become agalactic, despite the inability to isolate bacteria on culture.^{23, 27}

Many of the inflammatory and systemic changes observed during the course of acute coliform mastitis result from the release of lipopolysaccharide endotoxin (LPS) from the bacteria.^{13, 23} Most LPS release occurs following bacterial phagocytosis and killing by neutrophils.^{13, 23} This results in subsequent activation of the cyclooxygenase and lipoxygenase pathways, releasing prostaglandins, leukotrienes, and thromboxanes, compounds that are potent mediators of local inflammatory and systemic circulatory events.^{6, 36} Also, endotoxin induces the release of macrophage-derived cytokines, initiating a wide range of systemic responses to inflammation, often termed the *acute phase response*. As a result, to reduce the severity of acute coliform mastitis, either bacterial growth must be inhibited to reduce exposure of the quarter and the cow to LPS, or the effects of the LPS release must be neutralized. From a practical standpoint, therapy of acute coliform infections cannot begin until clinical signs appear. Clinical recognition of coliform mastitis usually occurs after peak bacterial numbers have been attained.^{13, 27, 30} Thus, by the time therapy is initiated, maximal release of LPS likely has occurred. This raises concerns regarding any advantage of antimicrobial therapy in alleviating the effects of acute coliform mastitis.

Selection of a potentially efficacious antimicrobial agent for the therapy of acute coliform mastitis primarily depends on in vitro culture and sensitivity.⁴ Antimicrobials such as aminoglycosides and cephalosporins, therefore, often are selected for use, based on favorable in vitro antimicrobial susceptibility patterns.⁴

Jones and Ward determined that 2 g of intramuscular gentamicin every 12 hours were not more efficacious in preventing agalactia or death resulting from acute clinical mastitis than intramuscular erythromycin or no systemic antimicrobial agents.³⁸ Systemic gentamicin also was no more effective in restoring appetite in affected cows than seen in the other groups.³⁹ Cows experimentally challenged with *Escherichia coli* and dosed with 500 mg intramammary gentamicin every 12 hours did not have lower peak bacterial concentrations in milk, shorter duration of infection, lower convalescent somatic cell or serum albumin concentrations in milk, or lower rectal temperatures than untreated challenged cows.²⁹ Antimicrobial inhibition was detected in milk from infected quarters of two cows at least 7 days following the last infusion of gentamicin.²⁹ In addition, gentamicin readily diffused through the milk-blood barrier, as indicated by detectable concentrations in serum throughout the treatment period and the first 12 hours after the last dose. Urine gentamicin concentrations were detectable in two cows 14 days after the last infusion. Two gentamicin-treated cows that were culled 6 months after the trial were found with kidney tissue concentrations of 1 µg gentamicin/g.²⁹ With increased interest and sophistication in drug residue testing among regulatory agencies, practitioners should carefully consider the 30- to 45-day half-life of aminoglycosides in the bovine kidney.

Ceftiofur sodium, a third-generation cephalosporin, has been reported to have excellent activity against gram-negative pathogens,^{75, 87} with a suggested MIC for *Escherichia coli* of 0.25 µg/mL. Although not approved as a treatment for mastitis, ceftiofur is approved for use in lactating dairy cattle, and if the labeled dose of 1 to 2.2 mg/kg every 24 hours is administered intramuscularly, no milk withholding time is required. The efficacy of ceftiofur as a treatment for clinical mastitis remains unproved, however. Owens et al⁶⁴ determined that systemic ceftiofur was not more efficacious in curing IMIs caused by *Staphylococcus aureus* than intramammary infusions of ceftiofur or cephalixin. Mammary tissue and milk concentrations of ceftiofur following systemic treatment remained less than 0.04 and 0.4 µg/g, respectively.⁶⁴ Similarly, experimentally *Escherichia coli*-challenged cows dosed intravenously with 3 mg of ceftiofur/kg every 12 hours had concentrations less than 0.2 µg/mL of milk throughout the trial (Erskine RJ, unpublished data). Thus, despite extra-label dosing and acute mammary inflammation, systemic ceftiofur is not likely to attain concentrations in milk that are effective in reducing bacterial numbers. If systemic ceftiofur has a benefit in the treatment of acute coliform mastitis, it likely would be for sepsis.

It may be time we shift the emphasis of our treatment strategy from "What antimicrobial will result in a cure?" to one of "What treatment alternatives exist?" Unlike most clinical mastitis episodes caused by

other pathogens, in which presence of the pathogen in the gland is the primary problem, the pathogenesis of acute coliform mastitis often confronts us with endotoxin-induced shock.

Although antimicrobial therapy may be of secondary importance relative to treatment of endotoxic shock, it may be indicated for acute mastitis. Coliform infections occasionally result in chronic mastitis. Septicemia may occur, although a Cornell University study suggested this is an infrequent result of infection.⁶⁰ In addition, although coliform organisms are the predominant clinical mastitis agent in many herds, numerous other pathogens cause clinical mastitis that is not distinguishable from that caused by coliforms. Consequently, until culture results can confirm the causative agent, antimicrobial agents may be indicated. Caution should be exercised in continuing therapy in cows that have already eliminated the infection, but continue to display clinical signs of mammary inflammation. Thus, antimicrobial therapy in cows with grossly abnormal milk, but with improved appetite, attitude, and milk production should be evaluated critically. Milk culture may assist in making the decision.

FLUID AND ELECTROLYTE THERAPY

Oral fluid therapy has documented efficacy in the treatment of hypovolemic shock syndromes (vibrio cholera in humans and diarrhea in calves). Recently completed studies, however, have observed static body weight and expanded plasma volume in cows with endotoxin-induced mastitis.^{60, 61} These results directly question dehydration as a primary shock mechanism in cows with acute mastitis. The mechanisms involved in endotoxic shock and, hence, coliform mastitis are more complex than simple dehydration. Cardiogenic, peripheral vascular capacitance, neurogenic, and other mechanisms contribute directly to decreased tissue perfusion and indirectly to the clinical manifestations of shock. Furthermore, effective oral fluid therapy hinges on normal gastrointestinal absorption of administered fluid. At least gastrointestinal motility and probably other gut functions are impaired in the endotoxemic state.⁵¹ A general rule of thumb is that oral fluid administration is most effective in subjects with a primary dehydration, normal gastrointestinal absorption, and less than 10% dehydration. Many cows with acute mastitis are effectively excluded using these criteria.

Although cumbersome, difficult, and potentially time consuming, intravenous fluid therapy deserves strong consideration. Effective fluid therapy is recognized as one of the most beneficial treatment options in cases of endotoxin-induced shock. Further, the absence of any potential for violative residues in milk or meat makes this method of treatment particularly attractive.²⁸ Intravenous fluids may be administered rapidly in the first hour of treatment (40 mL/kg or 20 L) and at a reduced rate (10–20 mL/kg) thereafter. More rapid fluid administration rates may increase central venous pressures and create risks for pulmonary edema.

Total doses will vary from 20 to 60 L depending on cow size, hydration status, and presence of ongoing fluid losses.^{4, 16} Fluids generally are administered using a 12- to 14-gauge catheter, but 3- to 4-inch 12-gauge needles may be sufficient for short-term use in placid, well restrained cows.

Rational fluid therapy is logically premised on the correction of deficits and replacement of ongoing losses. Unfortunately, mastitis case summaries describing perturbations in serum chemistry are noticeably lacking in the veterinary literature. Textbook descriptions of serum chemistry results in cases of clinical coliform mastitis are cursory.^{11, 16} Common abnormalities identified in these texts and anecdotal observations include hypocalcemia, hypoglycemia, acidosis, and hypovolemia. Serum chemistry data from eight cows treated at the Auburn University Large Animal Clinic for clinical coliform mastitis are presented in Table 1. These data are drawn from cows with clinical signs consistent with acute mastitis and a bacteriologically confirmed diagnosis of gram-negative mastitis. Given the varying stage of the disease process at the time of presentation and the referral nature of this practice, these data probably are not entirely representative of the clinicopathologic status of cattle treated in ambulatory practice. The data presented in Table 2 are drawn from four cows administered an intramammary infusion of 1 mg endotoxin. Each cow was treated with a small volume (approximately 3 L) of isotonic saline 4 hours after intramammary challenge.⁵² We will use these tables and referenced material to illustrate anticipated serum chemistry changes in cattle with coliform mastitis. Hypoglycemia probably is a minor consideration in the development of fluid therapy protocols in cattle with acute mastitis. Although low serum glucose concentrations are commonly seen in the late phases of septic shock,⁵² this observation is less consistent in ruminant models of gram-negative sepsis.⁶² Data in

Table 1. SELECTED SERUM CHEMISTRY RESULTS IN EIGHT CATTLE WITH CLINICAL COLIFORM MASTITIS

	Reference Range	Case Range	Case Mean	Comments
Glucose (mg/dL)	48-70	55-92	70.5	4 of 8 cows > 70 mg/dL
Calcium (mg/dL)	8.2-10.0	6.3-10.5	7.88	6 of 8 cows < 8.2 mg/dL 1 of 8 cows > 10.0 mg/dL
Creatinine (mg/dL)	0.2-1.4	0.6-5.6	1.68	3 of 8 cows > 1.4 mg/dL
Urea nitrogen (mg/dL)	7-17	9-64	29.0	5 of 8 cows > 17 mg/dL
Total protein (g/dL)	5.8-8.5	6.3-8.0	7.05	All cows within reference range
Sodium (mmol/L)	134-144	136-144	140.9	All cows within reference range
Potassium (mmol/L)	3.77-4.89	2.0-4.9	3.58	2 of 8 cows < 3.77 mmol/L
Total CO ₂	20.9-26.1	15-28	22.0	2 of 8 cows < 20.9 mmol/L 1 of 8 increased
Sorbitol dehydrogenase (IU/L)	13-18	5.0-27.3	12.4	5 of 8 cows < 13 IU/L 2 of 8 cows > 18 IU/L

Table 2. SELECTED SERUM CHEMISTRY RESULTS IN 4 COWS WITH ENDOTOXIN-INDUCED MASTITIS AT VARIOUS TIMES AFTER ENDOTOXIN INFUSION

	0 hrs	3 hrs	6 hrs	12 hrs	24 hrs	48 hrs
Glucose (mg/dL)	50.8	62.0	64.3	66.0	68.3	61.0
Calcium (mg/dL)	9.3	8.8	7.7	8.4	8.4	8.4
Creatinine (mg/dL)	0.78	0.80	0.88	0.75	0.63	0.85
Urea nitrogen (mg/dL)	23.5	23.8	23.8	24.8	24.0	19.3
Total protein (g/dL)	8.20	7.45	7.20	7.03	7.43	7.85
Sodium (mmol/L)	135.0	132.5	133.3	131.5	130.0	130.3
Potassium (mmol/L)	3.98	4.10	3.68	3.75	4.13	4.25
Total CO ₂ (mmol/L)	25.0	25.5	23.0	22.0	24.3	24.5
Sorbitol dehydrogenase (IU/L)	22.1	18.7	18.5	17.6	17.0	13.9

the tables substantiate the relative rarity of true hypoglycemia in cases of coliform mastitis. The authors (Erskine, Tyler) have seen serum glucose concentrations greater than 200 mg/dL in a cow with peracute *Staphylococcus aureus* mastitis. Although recommended by some sources,¹¹ large volume replacement with isotonic (5%) dextrose or frequent administration of 50% dextrose in 500-mL increments probably is of minimal clinical benefit unless novel therapies that promote cellular uptake and utilization of glucose are employed. Excessive glucose supplementation will raise serum glucose concentrations in excess of the renal threshold and promote free water losses in animals with already suspect hydration status. Some cows likely will benefit from daily administration of 500 mL 50% dextrose, administered directly or added to large volumes of intravenous fluids, particularly in the later stages of acute coliform mastitis.

Hypocalcemia frequently has been documented in experimental gram-negative sepsis.^{21,22} Clinical observations in cows with gram-negative mastitis substantiate these results. Not all down cows are hypocalcemic, however. In the absence of serum chemistry results, physical examination findings should be used to support the diagnosis of hypocalcemia. These findings include ataxia, muscle fasciculation, delayed pupillary light responses, dilated abdominal viscera on auscultation and percussion, cool extremities, and bloat. Cows with these abnormalities may require calcium therapy. Cows with fulminant sepsis, recognized by marked scleral injection, fever, heart rates greater than 100 beats/minute, and diarrhea, are at greater risk for calcium-induced arrhythmias. The subcutaneous route of calcium administration has a wide margin of safety in cases of acute mastitis and, consequently, is favored by some practitioners, although it may not be adequate for advanced cases and may result in abscess formation. Ideally, 500 mL of a calcium borogluconate solution (10 g calcium), is administered in 10 to 20 L of an intravenous electrolyte solution. The carrier solution should not contain bicarbonate or bicarbonate equivalent salts because these alkalinizing agents complex with calcium and form precipitates. When economic or practical considerations preclude large-volume intravenous fluid ther-

apy, calcium should be administered slowly, with concurrent cardiac auscultation.

Hypokalemia is expected in any disease condition causing prolonged anorexia. Balanced electrolyte solutions typically contain 5 to 7 mmol/L, but higher concentrations of potassium can be administered safely as long as the rate of administration remains below 0.5 mmol/kg/hour. No toxicity would be anticipated when administering solutions with a potassium concentration less than 12 mmol/L, as long as fluid administration is limited to 20 L/hour.

Although some cows with acute mastitis develop metabolic acidosis,³² this is not a consistent finding (see Table 1). Consequently, bicarbonate administration should be limited to cows with documented, substantial decreases in serum bicarbonate or total carbon dioxide (CO_2 < 18 mmol/L). Total deficit may be calculated as $0.5 \times \text{body weight in kg} \times (25 - \text{total serum } \text{CO}_2)$. Many clinicians dilute the 5% solution 3 to 1 using distilled water, yielding a 1.3% (isotonic) solution. Serum acid-base status may be re-evaluated at 3- to 4-hour intervals, with incremental bicarbonate administration performed as dictated by test results. Total CO_2 measurement devices premised on liberation of serum CO_2 and bicarbonate are readily adapted to on-farm testing using heparinized whole blood (Ruffin DC, Tyler JW, Spano JS: Comparison of plasma and whole blood total CO_2 determinations with traditional acid-base determinations in cattle, J Am Vet Med Assoc, submitted).

Hypertonic fluids have been advocated for use in cattle with mastitis, dehydration, and neonatal diarrhea.³² Few controlled studies examining this form of therapy have been performed. Although positive changes have been observed in hemodynamic parameters following rapid intravenous administration of 5 mL/kg 7.5% sodium chloride solution, no clear benefit has been established when this form of therapy has been compared with equimolar amounts of sodium salts administered as isotonic solutions.^{16, 14, 15} Intravenous hypertonic fluid therapy was used in an endotoxin-induced mastitis model.³¹ Therapy caused significant expansion of circulating plasma volume and modest decreases in the amount of lost milk production when compared with cows receiving equal volumes of isotonic sodium chloride.^{30, 32} Neither clinical nor biochemical evidence of salt poisoning was observed in these studies.³² Practitioners are cautioned that this form of therapy is experimental. Dehydrated cows with impaired renal function maintained at high ambient temperatures may be at an increased risk for sodium ion toxicosis. Interest in this mode of therapy has arisen because of economic rather than medical reasons. Practical considerations preclude large volume isotonic fluid therapy in many farm settings. Small volumes of hypertonic solutions may be administered rapidly and inexpensively during ambulatory visits.

When economically justifiable and feasible, intravenous fluid therapy is an important adjunct in the treatment of acute mastitis. In the absence of serum chemistry results, near-isotonic sodium chloride-based solutions supplemented judiciously with calcium, potassium, and glu-

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cases likely are the most suitable choices for intravenous fluid in cattle with acute mastitis.

STEROIDAL ANTI-INFLAMMATORY THERAPY

There is experimental evidence that corticosteroids stimulate the production of lipocortin. This may prevent phospholipase A₂ from contacting its substrate and, as a result, prevent arachidonic acid release.³³ This corticosteroid mechanism would prevent the formation of arachidonic acid metabolites, which would disrupt the formation of cyclooxygenase and lipoxygenase products. The time lag for lipocortin production makes early administration of corticosteroids necessary for maximal effectiveness. Because corticosteroids affect the initial phase of arachidonic acid release, experimental models of endotoxemia often require pretreatment with corticosteroids to significantly reduce mortality rates. The efficacy of corticosteroids is greatly reduced when administered after signs of disease are present because large amounts of arachidonic acid metabolites have already been produced. Corticosteroid impairment of immune function also may play a role in clinical outcome of disease. However, bacterial clearance was not adversely influenced by dexamethasone administration in a coliform challenge model of mastitis in lactating doe goats.⁵ This supports the observation of dairy practitioners that corticosteroids may be effective (or at least not detrimental) in the therapy of coliform mastitis.

The use of steroids in the treatment of acute coliform mastitis has been widely advocated. Dexamethasone, nine α -fluoroprednisolone, betamethasone, and flumethasone have been used in the treatment of experimental coliform mastitis.^{44, 49} Dexamethasone and isoflupredone have been approved for use in lactating dairy cows in the United States. Corticosteroids can be expected to reduce clinical signs of systemic disease, udder edema, and swelling. The influence of steroid therapy on survival and production has not been studied adequately.

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the synthesis of prostaglandins and thromboxanes by cyclooxygenase inhibition. All NSAIDs inhibit the formation of prostaglandin E₂ in the brain to reduce fever.⁴ This response is not necessarily always beneficial because the inflammatory response may be an important host defense mechanism. All NSAIDs do not necessarily act in the same manner. This suggests that there may be other mechanisms of action, some of which may be of more therapeutic benefit than cyclooxygenase inhibition.³¹ It has been demonstrated that several NSAIDs chelate iron, potentially making it unavailable to catalyze the production of extremely toxic hydroxyl radicals from less toxic superoxide anions and hydrogen peroxide.^{8, 31}

Other mechanisms, such as lipooxygenase inhibition and lysosomal membrane stabilization, also may be important.^{19,56} Differences in efficacy and toxicity also may be explained by the physicochemical characteristics of NSAIDs.¹² Characteristics such as polarity, acidity, and lipophilicity cause different NSAIDs to achieve different concentrations in various tissues within species and in similar tissues across species.

NSAIDs have been used for symptomatic relief of the clinical signs of endotoxic shock in several species of domestic animals and are widely used by dairy practitioners.^{39,47} NSAIDs that have been used in experimental coliform mastitis or in experimental endotoxin-induced mastitis include aspirin, flunixin meglumine, phenylbutazone, flurbiprofen, carprofen, and ibuprofen.^{20,50,53} NSAIDs used to treat acute coliform mastitis can be expected to reduce the severity of systemic clinical signs—e.g., rectal temperature, heart rate, respiratory rate, and udder pain. The effect of NSAIDs on survival and milk production has not been studied adequately, and NSAIDs have not been approved for food animals in the United States.

THERAPY OF UNUSUAL PATHOGENS

With the exception of *Mycoplasma*, the pathogens discussed in this section are common inhabitants of the dairy cow's environment. Strict attention therefore is required in the collection of milk samples for microbiologic analysis to avoid contaminants and maintain the reliability of the diagnosis. Selective media and extended incubation periods often are necessary. Bacterial cures from treatments are not common and many animals eventually are culled with chronic, refractory mastitis. In many cases, the organisms are susceptible only to medications not approved by the FDA for treatment of lactating cows. Caution should be taken under these conditions to prevent antibiotic contamination of meat and milk going to market. Some therapeutic agents thought to be effective by mastitis researchers in other countries are banned in the United States.

Pseudomonas

Treatment of individual cows should be based on antimicrobial culture and susceptibility test results. Although some clinical improvement is possible, bacteriologic cures seldom are attained. Aminoglycoside susceptibility often is reported. Outside the United States, chloramphenicol, polymyxin B or C, and furazolidin have been reported to have success.^{21,43} β -lactams and tetracyclines are the least effective. Effective control, by reducing the exposure of udders to the reservoir of infection, is critical when approaching mastitis outbreaks caused by this organism. Water, especially that used to prepare udders for milking, often is the source of the organism and methods to eliminate this reservoir of infection have been described.^{24,44}

Serratia

Serratia are not readily invasive, and for IMIs to occur, large numbers of bacteria must be placed on or near the gland.⁴³ About half the infections are reported to be clinical,⁷⁸ although subclinical herd outbreaks are possible.⁶⁹ The clinical nature of the organism may depend on the initial exposure, and chronic infections that last much longer than typical gram-negative infections may result.^{69, 79, 85} Spontaneous recovery has been reported. Many infections occur during the first half of the dry period. Thus, as with *Pseudomonas*, treatment for the purpose of bacteriologic cure is not likely to be beneficial.

Actinomyces pyogenes

Actinomyces (Corynebacterium) pyogenes is one of several organisms involved in the etiology of European "summer mastitis." It frequently is associated with teat injury and clinical mastitis in dry cows.⁷² Cases predominantly are malodorous and clinical. Because the clinical signs are not specific, diagnosis and therapy should be based on milk culture and antimicrobial susceptibility testing.⁶⁸ Long-acting antibiotic treatment has been reported to be successful at dry off,² and tylosin has been reported to be effective in Denmark.⁴¹ Because of decreased production and low rate of cure, however, affected animals often are culled.

Yeasts/Fungi

Infection with *Nocardia* and *Candida* is characterized by sudden onset of udder swelling and lack of positive clinical response to conventional antimicrobial therapy.^{1, 45, 46, 64, 71} Some cases, particularly those caused by *Candida*, are subclinical. Clinical cases often are associated with extended antimicrobial treatment and contaminated udder infusions or devices.^{46, 54, 66, 77} Given that clinical signs are similar to those of bacterial infections, diagnosis should be based on milk culture. Treatment should include discontinuation of antimicrobial therapy and culling of chronically infected cows.⁴⁵ Clinically recovered cows may shed organisms for up to 8 months.⁴⁶ Clotrimazole, ketoconazole, miconazole, nystatin, and sulfamethoxypyridazine all have been used, with reported clinical success, in treating *Candida* infections.⁴⁵ These studies were based on clinical impressions, however, and none of the drugs just mentioned are approved for use in the United States.

Other Pathogens

Prototheca zopfii, a colorless algae, is a common inhabitant of the dairy environment associated with wet areas contaminated with ma-

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nure. Chronic subclinical and clinical infections occur, and successful treatments have not been reported.⁴³ Infected cows should be isolated and removed from the herd. Mycoplasmal infections occur primarily during milking, and carrier cows are the major reservoir of infection.⁴⁶ Routine microbiologic identification procedures will not detect the presence of these organisms in milk; special media and growth conditions are required. Successful treatments have not been discovered for mycoplasmal mastitis and, because of the potentially contagious nature of the infection, affected cows should be segregated from non infected cows in the herd or culled.

Premature agalactia in chronically infected quarters, particularly quarters infected with pathogens refractile to therapy, occasionally is promoted as an alternative to culling the cow. This may have some benefit in genetically superior animals within a herd, or for cows to be maintained until calving. The goal is to eliminate the infection by fibrosing the affected quarter, thus reducing the risk of further pathogenic change or systemic effects on the cow, as well as reducing risk of infection for other cows. One method frequently used by practitioners is the infusion of 60 mL of 2% chlorhexidine-diacetate solution, into the affected quarter twice, 24 hours apart. The quarter should be stripped out before the second infusion. Care should be taken that milk from noninfected quarters is not sent to market before prior testing for inhibitors in milk. This regimen is not recommended for a majority of chronically infected animals, and should be used judiciously.

SUMMARY

Methods to enhance mammary resistance to bacterial infection and to reduce the effects of existing infections without the use of antimicrobial agents are becoming more attractive, primarily because of increasing pressure from consumers and regulatory agencies to decrease the risk of drug residues in milk. Because of the difficulty in obtaining satisfactory results with existing drug formulations, new approaches in the treatment of mastitis should emphasize better understanding of mammary gland pharmacokinetics, ameliorating the pathologic effects of infection, and enhancing natural defenses. Efficacy studies should emphasize milk production and long-term survival of cows to allow economic evaluations.

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FECHA : MAYO 27 DE 2008

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DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
CAVELIER ABOGADOS	CONSIGNACION	BANCO DE BOGOTA	062754387	766113	27/05/2008	47,547,000.00

***** CONCEPTO *****

CANT.	RENTIS/ICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-08	PRORROGA DE TERMINOS	75,000.00
31		PRORROGA DE TERMINOS	75,000.00
TOTAL :			\$ 75,000.00

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***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
CAVELIER ABOGADOS	CONSIGNACION	BANCO DE BOGOTA	062754387	1750688	19/05/2008	75,145,000.00

***** C O N C E P T O *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO	
1	50005-01-09	OTROS SERVICIOS DE PROPIEDAD I	21 CERTIFICACIONES	11,000.00
TOTAL :				\$ 11,000.00

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NIT : 900176089-2

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FECHA : MAYO 19 DE 2008

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
CAVELIER ABOGADOS	CONSIGNACION	BANCO DE BOGOTA	062754387	1750686	19/05/2008	75,145,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-09	OTROS SERVICIOS DE PROPIEDAD I	21 CERTIFICACIONES
			11,000.00
		TOTAL :	\$ 11,000.00

SON: ONCE MIL PESOS

RESPONSABLE : _____

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