GOBIERNO DE CHILE
FUNDACIÓN PARA LA
INNOVACIÓN AGRARIA

UNIDAD DE FORMACIÓN
DE RECURSOS HUMANOS PARA LA INNOVACIÓN

INSTRUCTIVO ELABORACIÓN
INFORME TÉCNICO Y DE DIFUSIÓN

Becas de Formación

AÑO 2006

(30)

INSTRUCTIVO PARA LA PREPARACION DEL INFORME TÉCNICO Y DE DIFUSIÓN

1. OBJETIVO

El objetivo de este informe es describir, analizar y evaluar la forma en que se desarrolló la propuesta, tanto desde el punto de vista técnico, como de su gestión administrativa y de las actividades de difusión realizadas. Específicamente, en este informe se deberán describir las visitas y tecnologías conocidas durante la iniciativa de formación, y junto con esto se deberá contemplar un análisis y reflexión respecto a los conocimientos adquiridos en la actividad y su aplicabilidad concreta en el país o en lugar de origen del participante, incluyendo los desafíos o limitantes que se presentan para su incorporación.

Adjunto al informe se deberá entregar una copia de todo el material o documentación recopilado durante la iniciativa de formación, incluyendo copia del material audiovisual.

El informe deberá adicionalmente describir las actividades de difusión realizadas, de acuerdo con el programa de difusión comprometido en su propuesta, adjuntando el material y documentación utilizada y entregada a los asistentes en dichas actividades.

2. PLAZOS Y ENTREGA DE INFORMES

Luego de terminada la iniciativa de formación y del regreso del participante, éste y/o la Entidad Patrocinante tienen un plazo máximo de 2 meses para realizar las actividades de difusión comprometidas en la propuesta. Después de realizada la última actividad de difusión comprometida, disponen de un plazo máximo de 15 días para la entrega a FIA del **Informe Técnico y de Difusión**.

Estos plazos están especificados en el contrato de ejecución respectivo y en la eventualidad de que exista un imprevisto que no le permita al participante y/o Entidad Patrocinante cumplir con dichos plazos, éstos deberán justificar y solicitar por escrito a la Dirección Ejecutiva de FIA la posibilidad de prorrogar los plazos estipulados, los cuales se autorizarán en la medida que exista una razón clara y justificada.

En la eventualidad de que los compromisos antes señalados no se cumplan, se procederá a ejecutar la garantía respectiva y el participante quedará imposibilitado de participar en nuevas iniciativas apoyadas por los diferentes Programas e instrumentos de financiamiento de FIA.

3. PROCEDIMIENTO

Los informes deben ser presentados, preferentemente en disquet o disco compacto y obligatoriamente en papel (dos copias) de acuerdo a los formatos establecidos por FIA, en la fecha indicada como plazo de entrega en el contrato firmado con el participante y/o Entidad Patrocinante. Los formatos de dichos informes (impresos y en disquet) son entregados por FIA al postulante o participante de la propuesta a través de este instructivo.

Los informes deberán ser dirigidos a las oficinas de FIA ubicadas en Avenida Loreley 1582, La Reina, Santiago, y podrán entregarse personalmente en dichas oficinas en horario hábil o enviarse por correo a domicilio en forma oportuna para que llegue en el plazo establecido.

FIA revisará los informes y dentro de los 45 días hábiles siguientes a la fecha de recepción (plazo máximo) enviará una carta al responsable de la propuesta o participante, informando su aceptación o no aprobación. En caso de no aprobarse el informe FIA comunicará en detalle las razones de dicha decisión. El responsable deberá corregir los reparos u observaciones que motivaron el rechazo, dentro del plazo determinado por FIA.

4. CONTENIDO Y FORMATO

La información presentada en el informe de avance técnico y de difusión debe ser presentada en un lenguaje claro y estar directamente vinculada a la información presentada en el informe financiero, siendo totalmente consistente con ella.

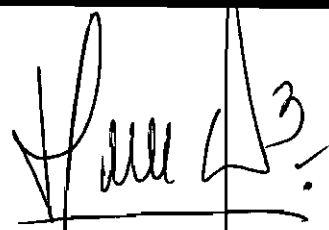
El informe debe incluir o adjuntar los cuadros, gráficos, fotografías y diapositivas, publicaciones, material de difusión, material audiovisual y otros materiales que apoyen o complementen la información y análisis presentados en el texto central.

El informe de avance técnico y de difusión debe incluir a lo menos información sobre todos y cada uno de los puntos mencionados a continuación, y siguiendo en lo posible el orden indicado. El envío de la información incompleta puede ser motivo de no aprobación de este informe.

En aquellos casos en que la estructura del informe que se entrega no permita incluir información, análisis desarrollados o actividades implementadas, es importante agregar las secciones que corresponda para informar adecuadamente sobre estos aspectos.

GOBIERNO DE CHILE
FUNDACIÓN PARA LA
INNOVACIÓN AGRARIA

CONTENIDO DEL INFORME TÉCNICO Y DE DIFUSIÓN

Fecha de entrega del Informe
Mayo 02 de 2007
Nombre del coordinador de la ejecución
Patricio Villalobos Biaggini
Firma del Coordinador de la Ejecución
 P. VILLALOBOS
Firma del representante legal de la Entidad Patrocinante

1. ANTECEDENTES GENERALES DE LA PROPUESTA
Nombre de la propuesta
Desarrollo de autovacunas hacia la industria Porcina en Chile
Código
FIA-FP-V-2006-1-P-073
Postulante o Postulantes
Patricio Villalobos Biaggini
Entidad Patrocinante o Responsable
LABORATORIO CENTROVET LTDA
Lugar de Formación (País, Región, Ciudad, Localidad)
Cambridge, Ontario, Canada.
Tipo o Modalidad de Formación (curso, pasantía, otros)
Pasantía
Fecha de realización (Inicio y término)
24 de Septiembre del 2006 al 07 de Octubre del 2007.

2. ALCANCES Y LOGROS DE LA PROPUESTA

Justificación y objetivos planteados inicialmente en la propuesta

Industria Porcina nacional

Chile exporta una gran variedad de productos, entre ellos carne de cerdo. Tanto éstos, como las políticas de acuerdos comerciales con los grandes mercados, sitúan a nuestro país entre los 20 países económicos más competitivos del mundo. Las exportaciones por rubro cerdos se estimaron para el 2006 en más de US$300 millones y su proyección anual es de un incremento del 32%. No obstante, esta pujante actividad, así como otras que involucra el manejo de especies en cautiverios y a gran escala, se ven amenazadas constantemente por diferentes patologías donde Chile no es la excepción.

Dada la complejidad del sistema animal hospedador (cerdos), muchas de estas patologías se hacen complejas, puesto que no siempre se identifican un patógeno único, sino que ocurren una variabilidad de otras bacterias asociadas, por los cuales el cuadro clínico es identificado como síndrome. Estas enfermedades están siendo combatidas por laboratorios de alto nivel de investigación, indicando complejidad en el tema. A pesar de que los mecanismos globales para combatir enfermedades son el uso de antibióticos y la vacunación. Frente a muchos de ellos, no existen vacunas comerciales registradas en nuestro país, o bien, éstas no son eficientes dado que muchas veces las vacunas son creadas con variantes inmunogénicas diferentes a las que afecta de un país a otro. Tal es el caso de enfermedades producidas por los patógenos *Staphylococcus hyicus*, *Haemophilus parasuis*, *Actinobacillus pleuropneumoniae* y *Streptococcus suis*,

Se pueden establecer dos factores comunes a todos ellos.

i) Los cuatro patógenos tiene requerimientos especiales diferentes del común de las bacterias.

ii) Estos patógenos corresponden a bacterias que pueden ser parte de la flora bacteriana normal de cerdos con variantes que se vuelven patogénicas.

Por ambas condiciones, es que estos patógenos, entre otros, son considerados bacterias fastidiosas o de difícil manipulación y requieren conocer al patógeno para aislar la especie asociada a la infección. De este éxito depende el desarrollo de una protección adecuada de cerdos que son vacunados.

En Chile, aunque se encuentran laboratorios de diagnóstico y de detección de patologías en cerdos, esto se limita prácticamente al diagnóstico clínico o molecular (PCR; serología, blots), no existiendo una gran experiencia en microbiólogos dedicados al aislamiento y estudios de patógenos de importancia en cerdos.

De acuerdo con este marco de discusión, es de extrema importancia la asesoría por personal con una experiencia adecuada, como lo es Industrias Gallant, Canadá.

Manejo de animales sanos no significa condiciones sanas

El uso de antibióticos ha sido la herramienta principal para mantener los animales sanos. Sin embargo, la transferencia y reordenamiento de genes de resistencia bacteriano, han hecho que cada vez que las dosis de uso de antibióticos sean mayores. En consecuencia, existen desventajas directas derivadas del uso constante de antibióticos para estos fines:

1. Aumento de los costos de producción, debido a que aumentan las tarifas crecientes de la enfermedad., y a la cantidad de tiempo para conseguir animales al peso comercial.

2. Mayor contaminación ambiental, puesto que los animales no alcanzan a retener y asimilar las dosis ingeridas.

3. Ocurrencia de ingestión de altas dosis de antibióticos presentes en las carnes comestibles.

4. La resistencia por microorganismos a los antibióticos está sobrepasando rápidamente nuestra habilidad de sintetizar nuevas drogas (tratamientos ineficientes).

De acuerdo con ello, el comité de dirección científico de la Unión Europea manifiesta que el uso de antibióticos para prevenir enfermedad, constituye uso erróneo y que se debe prohibir en conjunto. Si bien, está autorizado el uso de 8 tipos de antibióticos en este tipo de alimento, la tendencia es a evitar el uso global en la industria alimenticia animal (EU meeting, 1999).

Alternativas limpias

La prohibición de administrar antibióticos con la alimentación en la industria agroalimentaria puede tener consecuencias importantes, y no todas positivas. La falta de antibióticos probablemente cause un aumento de las infecciones (ejemplo: las entéricas en las poblaciones de lechones destetados) creando un riesgo probablemente grave para la salud de los consumidores. Hallar formas alternativas de proteger la salud de los cerdos es una demanda urgente y actual. Las vacunas comerciales incluyen los serotipos bacterianos que eran más frecuentes en el momento en que se registro. Sin embargo, muchas veces estos serotipos no coinciden con los que ahora actúan en la explotación, por eso las autovacunas son más efectivas.

Las autovacunas son actualmente la manera más adecuada de proteger cada granja sobre una base modificada para requisitos particulares. Son la mejor alternativa cuando no hay vacunas comerciales disponibles, y responden adecuadamente a las nuevas enfermedades y a las patologías de menor importancia. Asimismo, ayudan a reducir el uso de antibióticos y a combatir bacterias resistentes. Se ha demostrado su eficacia, y se utilizan extensamente en muchos países. El uso de vacunas inactivadas ha demostrado su eficacia en combinación con otras medidas para la profilaxis. Experimentalmente se usan diferentes antígenos como bacterias sonicadas, proteínas flagelares, y bacterias modificadas genéticamente. Se ha demostrado que todos ellos inducen algún grado de protección.

No obstante la potencialidad de las autovacunas, en Chile no se registran actualmente autovacunas contra patógenos importantes que afectan la industria porcina.

Centrovet Ltda. con experiencia a su haber, ha desarrollado tres tipos de autovacunas para la industria salmonera. Esta positiva experiencia, así como su gran aporte en el mercado nacional en productos veterinarios y su excelencia en el servicio, han situado a nuestra empresa como líder en el mercado veterinario. La confianza depositada en nosotros nos impulsa explorar en investigación y desarrollo de vacunas y autovacunas para la industria porcina.

A través de la siguiente propuesta Centrovet Ltda. pretende obtener financiamiento para apoyar la inversión que esta haciendo en el tema, de manera de dar un fuerte impulso en el desarrollo de productos de origen biológico para combatir enfermedades que afectan a los cerdos. La demanda por soluciones alternativas a los antibióticos es actual, y la experiencia en el tema de empresas con amplia trayectoria en la investigación, desarrollo y producción de autovacunas, como lo es Laboratorios Gallant en Canadá, es una excelente oportunidad de captación de experiencia y favorecer los tiempos y conocimientos de la aplicación de autovacunas en Chile. Esta experiencia ganada favorecerá de forma indirecta el mercado nacional, y por ende mantener la competitividad de Chile, frente a otras economías mundiales en el tema.

Las tendencias en el rubro veterinario se encaminan hacia fusiones entre empresas de diferentes continentes, con fuerzas competitivas particulares que permitan crear sinergias entre ellas. Con esta lógica es que la empresa ha decidido enfrentar la competencia fortaleciendo su cartera de productos innovadores, diferenciándose de sus competidores. Es por esto que el presente proyecto esta alineado con los objetivos estratégicos de Centrovet.

OBJETIVO GENERAL

Captar conocimientos adecuados para el aislamiento, manejo y preparación de vacunas autógenas contra patógenos complejos, para posteriormente aplicar los conocimientos adquiridos en el desarrollo de autovacunas de aplicación en Chile.

OBJETIVOS ESPECÍFICOS

1 Recuperar bacterias patógenas desde cerdos post-mortem recuperados en terreno.

2. Aprender técnicas de reconocimiento de los patógenos *Staphylococcus hyicus*, *Haemophilus parasuis*, *Actinobacillus pleuropneumoniae* y *Streptococcus suis*, en su asociación a órganos enfermos.

3. Determinar los serotipos patógenos dentro de un inverso bacteriano.

4 Aislar, purificar e identificar las bacterias patógenas para su cultivo masivo.

5. Aprehender políticas de manejo y cuidado de animales de nivel internacional para favorecer el mejor esquema de desarrollo de autovacunas en Chile y obtener la confianza de este manejo por las autoridades reguladoras competentes nacionales (Servicio Agrícola y Ganadero).

Objetivos alcanzados tras la realización de la propuesta

Los objetivos planteados fueron alcanzados íntegramente. El último objetivo, relativo a Chile, se planteó como una consecuencia de la estancia en Canadá.

Esto significa que se logró capturar conocimientos adecuados para el aislamiento, manejo y preparación de vacunas autógenas contra patógenos complejos, para posteriormente aplicar los conocimientos adquiridos en el desarrollo de autovacunas de aplicación en Chile.

De acuerdo con los objetivos específicos:

1. Se logró recuperar bacterias patógenas desde cerdos post-mortem recuperados en terreno. Se trabajaron muestras de pulmón, corazón y partes intestinales. Algunas fotografías pueden ser vistas en el archivo adjunto, correspondiente a la presentación de la actividad de difusión.

2. Se ensayaron técnicas de reconocimiento de los patógenos *Staphylococcus hyicus, Haemophilus parasuis, Actinobacillus pleuropneumoniae y Streptococcus suis*, en su asociación a órganos enfermos. Además se prepararon y ensayaron medios cultivo usados para e crecimiento de ellos.

3. Cuando correspondió, se realizaron pruebas de serotipificación y pruebas estándares para la identificación de estos patógenos.

4. Por lo tanto, se aislaron, purificaron e identificaron bacterias patógenas para su cultivo masivo.

El objetivo final, aún está en ejecución, puesto que se discutieron las políticas de manejo y cuidado de animales de uso veterinario en Canadá. En este sentido se intenta el desarrollo de autovacunas para proponer a la autoridad competente en Chile algunos de estos principios para ser aplicados en Chile.

Resultados e impactos esperados inicialmente en la propuesta

La presente propuesta pretendió lo siguiente:

1. Aumentar y potenciar nuestros conocimientos para impulsar el desarrollo del concepto de autovacunas en la Industria porcina en Chile.

2. Aprehender políticas de manejo y cuidado de animales de nivel internacional para favorecer el mejor esquema de desarrollo de autovacunas en Chile y obtener la confianza de este manejo por las autoridades reguladoras competentes nacionales (Servicio Agrícola y Ganadero-Gobierno de Chile).

3. Establecer una cooperación comercial y de conocimientos con laboratorios internacionales, con el fin de potenciar proyectos en conjunto en relación al desarrollo de autovacunas.

4. Difundir el uso de autovacunas como alternativa limpia al uso de antibióticos para el control de enfermedades en la industria porcina

5. Mejorar la competitividad como empresa y posicionarla a un alto nivel en el desarrollo de autovacunas.

6. Favorecer el bienestar de los animales en cautiverio, lo cual directamente favorecerá los costos de producción a través tiempos adecuados de cultivo y tasas de conversión adecuadas.

7. Mantener y mejorar la confianza de productores porcinos chilenos en Centrovet, a través de buenas políticas de manejo y cuidado contra enfermedades.

Resultados alcanzados

Describir si se lograron adquirir los conocimientos, experiencias, alianzas u otros resultados que se esperaban alcanzar a través de la participación en la actividad de formación y del desarrollo de las actividades de difusión. Si hay resultados que no se alcanzaron total o parcialmente, indicar las razones que a juicio del participante explican dicha situación.

La estancia en Gallant Laboratories, incluyó el procesamiento de muestras provenientes de granjas canadienses con problemas reales. Se siguieron protocolos para el procesamiento de una orden de atención veterinaria. Es decir, se participó desde la recepción de una muestra, hasta el envió de una partida de vacunas desarrolladas. Entre ambos extremos se realizaron aislamientos, reconocimientos de las patologías a través de signos clínicos de las muestras, se aislaron e identificaron bacterias, se realizaron ensayos de serotipificación, se prepararon medios de cultivo complejos, y se discutieron las normas que rigen el desarrollo de estos productos.
Con estos conocimientos es posible afirmar que las autovacunas son una alternativa concreta para combatir enfermedades en Canadá.

De acuerdo a los resultados esperados se obtuvo lo siguiente:

1. Se obtuvieron conocimientos frescos acerca de como manipular los patógenos en cuestión y acerca de las autovacunas en el mercado canadiense. Se adquirieron conocimientos de como manejar estos patógenos y en la actualidad se están estudiando en nuestro laboratorio.

2. En base a las materia políticas. Se entendió que las regulaciones en Canadá son inspeccionadas por la Canadian Food Inspection Agency (CFIA) y que esta entidad regula el uso de autovacunas, a través de estrictos controles de calidad de los laboratorios fabricantes, independiente de la existencia de vacunas comerciales. Comparativamente en Chile, la entidad reguladora es el Servicio Agrícola Ganadero (SAG), y la aceptación del uso de una autovacuna depende de que no haya una vacuna registrada, o bien de que la vacuna no sirva como método de control de la enfermedad, lo cual debe ser fuertemente fundamentado.

Mediante experimentación, se logró visualizar que los ratones son un buen modelo de ensayo para ensayar las pruebas de seguridad como alterativa a los cerdos.

3. Se obtuvo cooperación entre una empresa canadiense y una chilena. Actualmente, la empresa canadiense está disponible para eventuales consultas, y nos sirve de referencia para efectos comparativos con Chile.

4. Laboratorios Gallant cuenta con diversos datos estadísticos que indican que las autovacunas son una alternativa concreta para solucionar problemas puntuales en granjas porcinas. Asimismo, durante la pasantía se pudo constatar que el laboratorio recibió variadas órdenes que indicaron los mismos resultados. La experiencia de los 9 años de funcionamiento de empresas Gallant demostraron ser elocuentes al momento de procesar las órdenes. Denotaban un sistema eficiente y dinámico en cada proceso de desarrollo de las autovacunas.

5. En el caso concreto de Centrovet, la idea es mejorar la competitividad como empresa y posicionarla a un alto nivel en el desarrollo de autovacunas. Este tema no sólo se está

desarrollando en Centrovet, sino que además existe inquietud por el tema de parte de los productores de cerdo. Aspectos que fueron percibidos durante la actividad de difusión.

6. Favorecer el bienestar de los animales en cautiverio, lo cual directamente favorecerá los costos de producción a través tiempos adecuados de cultivo y tasas de conversión adecuadas.

Aunque este resultado esperado es abstracto en este etapa del proyecto. La idea final es el manejo adecuado de animales para investigación. En este sentido, la pasantía en Gallant laboratories fue clave. Se obtuvieron datos del número de animales necesarios para ensayos de vacunas. Más importante aún, fue constatar el uso de modelo ratón en vez de cerdos para los ensayos de seguridad de vacunas preparadas. Esto es importante directamente para reducir el tamaño de las instalaciones y los costos en el desarrollo de vacunas.

7. Mantener y mejorar la confianza de productores porcinos chilenos en Centrovet, a través de buenas políticas de manejo y cuidado contra enfermedades.

Resultados adicionales

Describir los resultados obtenidos que no estaban contemplados inicialmente como por ejemplo: formación de una organización, incorporación de alguna tecnología, desarrollo de un proyecto, firma de un convenio, entre otros posibles.

Como toda actividad comercial que incluye animales en cautiverio, la Industria porcina no es la excepción. Adicionalmente a los patógenos nombrados en la propuesta, existen otras diversas patologías altamente complejas por su forma de cultivo, como a la novedad que les conlleva.

De modo que otros resultados prácticos obtenidos fueron los siguientes:

1. Se recibió entrenamiento práctico de otros patógenos como micoplasmas, *Escherichia coli* ETEC, *Clostridium perfringens e Erysipela*.

2. Se recibió información práctica acerca del diagnóstico y estado de actualidad de circovirus porcino en Canadá.

3. Se recibió entrenamiento práctico acerca de la línea de proceso completa en la creación de autovacunas en Canadá.

4. Se visitaron grandes centros de apoyo en la investigación al Laboratorio Gallant, como lo es la Universidad de Guelp y otro laboratorio animal donde se realizaban los ensayos de seguridad animal.

5. Se mantiene una cooperación dinámica entre la Empresa Gallant y el Coordinador de la propuesta, con motivo de mantener un flujo de información acerca de las variantes que afectan a un país u otro (cooperación científica).

Aplicabilidad

Explicar la situación actual del rubro y/o temática en Chile (región), compararla con las tendencias y perspectivas presentadas en la iniciativa de formación y explicar la posible incorporación de los conocimientos adquiridos por parte del postulante, en el corto y mediano plazo; los procesos de adaptación necesarios, y los apoyos tanto técnicos como financieros necesarios para hacer posible su incorporación en nuestro país (región).

A diferencia de Canadá, donde cada empresa diside si demanda la fabricación de una autovacuna en particular. Es decir el mercado es el que regula la presencia de autovacunas y la entidad gubernamental (CFIA) sólo fiscaliza que las prácticas sean seguras a través de controles de calidad estrictos hechos a los laboratorios. En chile, es la autoridad competente (SAG) la que evalúa la posibilidad de desarrollo de autovacunas, la cual dependerá de si existen vacunas similares registradas o si estas son ineficientes en el mercado chileno.

Otra diferencia, es que el Laboratorio Gallant está autorizado para realizar diagnóstico y desarrollo de vacunas en Canadá. En Chile, las entidades que desarrollan vacunas, no pueden realizar diagnóstico, y por ende aislamientos. Las cepas deben ser aportadas por laboratorios de diagnóstico diferentes. Sin embargo, en la búsqueda de esos laboratorios, y tal como lo manifiestan los diferentes productores, no existen laboratorios competentes que diagnostiquen enfermedades de cerdos. En este sentido, el primer resultado obtenido a través de esta pasantía, es aportar con conocimientos a aquellos laboratorios que lo hacen. De tal manera, que Centrovet ha concretado la apertura de un laboratorio de diagnóstico independiente de la casa matriz para la recepción de muestras.

La factibilidad de llevar a la práctica los conocimientos adquiridos, es real y creciente. Como es sabido, existe un aumento de demanda en la carne de cerdo. Por lo tanto se prevee un aumento en el registro de las patologías y la necesidad de personal especializado que sea capaz de llevar registros y proyectar soluciones.

El postulante, proyecta una propuesta a fondos semilla, para aumentar la velocidad de adaptación de estos conocimientos al servicio del sector productivo. Como se ha percibido durante el evento de difusión, los productores demandan por soluciones prácticas y rápidas que le permitan tomar decisiones prontas para evitar o aminorar posible pérdidas económicas por mal manejo de las patologías. Con esto se quiere decir que existe un vacío en el tema en Chile, y que sólo algunas empresas nacionales están dispuestas a esta orientación.

Detección de nuevas oportunidades y aspectos que quedan por abordar.

Señalar aquellas iniciativas que surgen para realizar un aporte futuro para el rubro y/o temática en el marco de los objetivos iniciales de la propuesta, como por ejemplo la posibilidad de realizar nuevas actividades.

Indicar además, en función de los resultados obtenidos, los aspectos y vacíos tecnológicos que aún quedan por abordar para ampliar el desarrollo del rubro y/o temática.

Tal como se menciona en el párrafo anterior, desde el punto de vista del coordinador, existe un vacío en el tema en Chile, y que no está claro que haya una rápida reacción de las empresas nacionales hacia el tema. De acuerdo a la información en la propia página de Agrosuper, el mayor productor de cerdo en Chile, se proyecta duplicar la producción de carne de cerdo nacional en el 2007. Esto prevee la necesidad de un mayor apoyo a este sector desde el punto de vista de salud animal. Centrovet, está ejecutando temáticas para implementar en un corto plazo un laboratorio de diagnóstico que fortalezca las deficiencias detectadas.

Para ampliar el desarrollo de la temática, creemos que es necesario el aporte de todos los sectores participantes. Por parte de los laboratorios farmacéuticos, es necesario que inviertan en la preparación de personal de manera de favorecer la interacción entre el sector productivo y la investigación. Por parte de los productores, se espera una apertura y acercamiento a centros de investigación para abordar las temáticas complejas como lo algunas de las enfermedades porcinas. Por último, se espera que entidades regulatorias apoyen a ambos tipo de entidades de manera que las normas puedan acercarse lo más posible solucionar los problemas de manera rápida, pero segura. Pensamos que esto favorecerá una dinámica de competencia a nivel mundial.

3. ASPECTOS RELACIONADOS CON LA ORGANIZACIÓN Y EJECUCIÓN DE LA PROPUESTA

Programa de actividades realizado

Fecha	Actividad	Objetivo	Lugar
23/09/2006	Viaje a Canadá	Investigación en autovacunas	Laboratorios Gallant
25/09/2006	Reunión acuerdos de investigación	Difundir el uso de autovacunas	Laboratorios Gallant
25/09/2006	Inicio de entrenamiento en autovacunas	Entrenamiento en el aislamiento, identificación y...	Laboratorios Gallant
06/10/2006	Finalización estancia de Investigación	Preparación de vacunas de microorganismos fastidiosos	
07/10/2006	Regreso a Chile		Chile
29/11/2006	Seminario	Difundir el uso de autovacunas	Productores de cerdos
02/05/2007	Entrega de informe	Rendición de cuentas	FIA

Al comparar las actividades programadas en la propuesta aprobada con las actividades que realmente se realizaron, cuando corresponda, señalar las razones por las cuales algunas de las actividades programadas no se realizaron como estaba previsto o se modificaron.

Incorporar en este punto fotografías relevantes que contribuyan a describir las actividades realizadas.

Todas las actividades fueron realizadas pero se incluyeron, sin embargo, postergación en dos de las actividades que se mencionan.

1. Se postergó y dio aviso de postergación de la actividad de difusión, pues esta coincidió con un encuentro mundial, realizado en Brasil, y al que asiste un gran porcentaje de personas trabajando en el rubro. Se prefirió postergar para dar una ventana mayor a dos semanas y así ser más efectivos en el llamado.

2. Se postergó la entrega del informe final, puesto que el coordinador de la propuesta dejó de ejercer funciones en la empresa, en el momento que la propuesta debía ser entregada.

Programación y resumen de la estancia

RESUMEN ESTANCIA EN GALLANT LABS-CANADÁ

Informe Técnico - Centrovet, Chile
Patricio Villalobos Biaggini

La visita a Gallant Customer Laboratorios, Cambridge, Notario, Canadá se realizó en un marco de cooperación entre esta empresa y Laboratorio Centrovet-Chile, y con aportes del Fondo de Innovación Agraria, mediante la adjudicación de un proyecto de Formación.

Dado que Gallant Labs posee una gran trayectoria en el manejo de patógenos para la creación de autovacunas para cerdos en Canadá, el proyecto fue postulado con los siguientes objetivos:

Objetivos

1. Recuperar bacterias patógenas desde cerdos post-mortem recuperados en terreno.
2. Aprender técnicas de reconocimiento de los patógenos *Staphylococcus hyicus, Haemophilus parasuis, Actinobacillus pleuropneumoniae y Streptococcus suis*, en su asociación a órganos enfermos.
3. Determinar los serotipos patógenos dentro de un inverso bacteriano.
4. Aislar, purificar e identificar las bacterias patógenas para su cultivo masivo

El programa de actividades realizado en Laboratorios Gallart se describe más adelante, y en cada tópico se resume el trabajo realizado, con las observaciones derivadas de cada actividad.

Day 1- September 25, 2006

Breakfast Meeting- get to know each other and discuss plans for training
Tour of Gallant Custom Laboratories Inc.-meet the staff

Sign Confidentiality agreement
Review Regulatory requirements of Chile
List how it affects:
-diagnostic testing
-quality control
-documentation
-production
-sales

Day 2- September 26, 2006

Review documentation procedures for:
Diagnostic testing and results- how to organize the University lab and reports
Vaccine orders
Production
Quality Control
Product Release and regulatory requirements
Shipping product to client
Discuss and review forms, as needed for lab work and Standard Operating Procedures

Day 3- September 27, 2006

Diagnostic Lab- Sample log in, labelling specimens, verifying submission information, and processing sample.
-identify request
-determine what materials are needed

Preparing lab media and specimen
Post mortem sample processing
Swab sample processing
Other types of samples

Day 4 – September 28, 2006

Diagnostic – Primary isolation of Day 3 specimens
-identification and purification of isolates
-planning further testing

Note: We have saved several representative cases for you to practice your diagnostic techniques. You will be able to take them through the whole process and we will guide you on how to isolate and identify the organisms of interest.

Day 5-September 29, 2006

Diagnostics- identification of isolates from Day 3 Specimens and plan any further testing.
Primary isolation of Day 4 specimens –purify isolates and plan further testing
Travel to lab to observe swine handling.
Tour of University of Guelph

Day 8- October 2, 2006

Diagnostic –complete reports of Day 3 specimens
-evaluate results and discuss recommendations to clients
-identification of isolates from Day 4 and plan further testing

Day 5-September 29, 2006

Diagnostics- identification of isolates from Day 3 Specimens and plan any further testing.
Primary isolation of Day 4 specimens –purify isolates and plan further testing
Travel to lab to observe swine handling.
Tour of University of Guelph

Day 8- October 2, 2006

Diagnostic –complete reports of Day 3 specimens
-evaluate results and discuss recommendations to clients
-identification of isolates from Day 4 and plan further testing

Bacterin Production- process to decision

Production- order processing
-recovery of isolates
-planning –media, isolates, regulatory, documentation

Day 9 – October 3, 2006

Diagnostic – report for Day 4 specimens
-evaluate results and discuss recommendations to clients

Vaccine Production
-Media preparation and planning
-Equipment planning
-Timelines-using Chilean regulation requirements
-Review isolates and seeding procedures
-Review production parameters
-Documentation
Technical- set up several seeds of production volumes

Day 10 – October 4, 2006

Vaccine Production
Technical – seeds to production volumes
Observe bottling and packaging
Observe blending procedure

Animal test
Travel to contract lab for mouse test observation

Day 11- October 5, 2006

Production –complete production of cultures by inactivation
- quality control testing
- documentation
- planning for blending and bottling

Day 12 – October 6, 2006 –Last day

Production- Observe quality control tests.

Descripción de La empresa

Gallant Labs es un laboratorio de I&D en Vacunas y de Diagnóstico. Realizan diagnóstico de enfermedades relacionadas a cerdos, aves, pavos, pollos, vacas y caballos, siendo las muestras de cerdo, las de mayor frecuencia. Estructuralmente, el laboratorio de diagnóstico esta junto al de I&D, los cuales entregan el patógeno puro hacia el área de producción.

El Laboratorio cuenta con un Staff de 7 personas de planta y 3 técnicos rotantes de apoyo, cuando las dosis son altas. El personal de Planta corresponde a 2 técnicos microbiólogos, 4 profesionales microbiólogos y Jackie Gallart, que también es Microbióloga. La empresa desarrolla diagnóstico e identificación de algunos patógenos de los géneros *Staphylococcus, Haemophilus, Actinobacillus, Escherichia coli, Clostridium, Salmonella, Streptococcus.* Se realiza sólo diagnóstico de PRRS mediante Real Time RT-PCR. La empresa desarrolla exclusivamente vacunas autógenas, dado que el mercado porcino en Canadá es amplio.

De las regulaciones

Este tipo de empresa, tiene un acceso más expedito para el desarrollo y venta de vacunas autógenas. Gallant Labs puede recibir órdenes de pedido directas desde los veterinarios a cargo de las granjas. Si bien el Gobierno canadiense realiza un estricto control de las vacunas en Canadá, este control es mediante auditoría a la empresa fabricante, a través de un control estricto a las contra-muestras en los controles de calidad y áreas de proceso de la empresa.

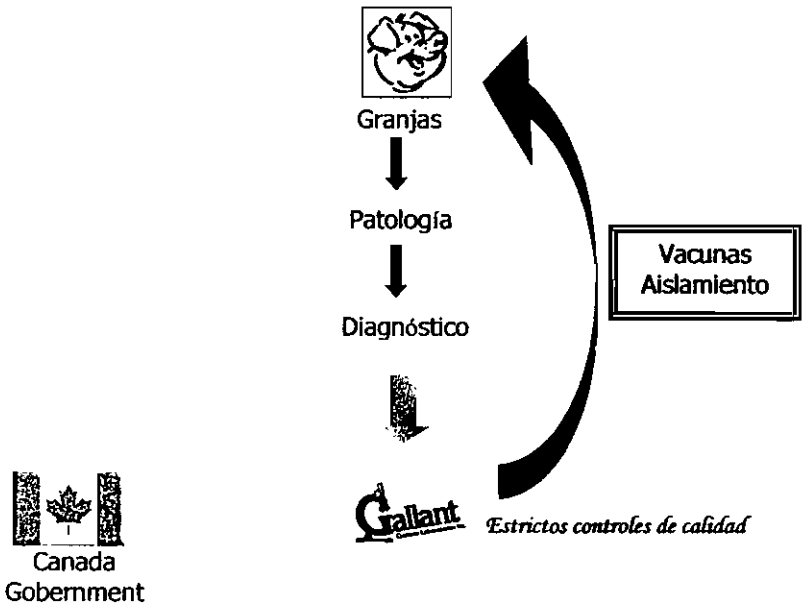
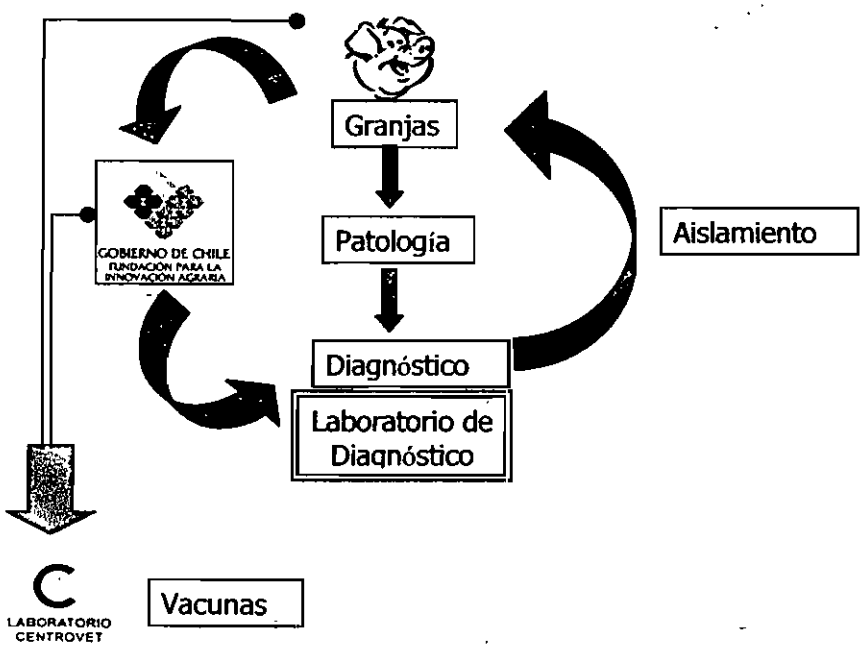


Diagrama de flujo que ocurre en Canadá desde que el productor acusa una patología hasta que una posible vacuna es desarrollada. Tiempo transcurrido es menor a dos meses.



Potencial diagrama de flujo que ocurre en Chile desde que el productor acusa una patología hasta que una posible vacuna puede ser desarrollada.

Gallant Labs mantiene un estricto control de las vacunas, personal y en sus áreas de proceso, en esto hay un manejo muy profesional de todo el desarrollo de una vacuna. Las vacunas preparadas son almacenadas almenos por 18 meses luego de su preparación.

Algunos de los controles de calidad existentes son por ejemplo: el trabajo que realiza una persona es chequeado constantemente por un encargado de la documentación, que ante cualquier duda, repite o hace repetir la prueba, o bien se detiene el proceso, y se chequean los procedimientos. Existen ensayos de control biológico periódicos hacia el autoclavado de material, lo flujos laminares, chequeo de la pureza de los cultivos durante los crecimientos. Finalmente, el control de mayor cuidado es el relacionado a los ensayos de seguridad y envasado de las vacunas.

Actividades

Del proceso de las muestras

- Se procesaron órdenes de pedido completas, incluyendo desde la toma de muestras hasta, pasando por diagnóstico y controles de calidad hasta el envasado de una vacuna.
- Se realizaron y siguieron los protocolos de recepción y procesos de muestras. En general, se puede decir que el sistema es muy fluido, las órdenes son procesadas en prácticamente 45 días desde recibida la muestra.
- Se procesaron muestras de swabbing rectal e intestinal, de pulmón, hígado y corazón, provenientes de casos reales.
- Se manipularon tejidos de animales postmortem, y luego se realizaron los procesamientos microbiológicos de ellas. Se llevaron a cabo los protocolos necesarios para identificar los microorganismos recuperados en cada muestra.
- Con esta estadía, se tiene la lista de materiales y las especificaciones técnicas para el manejo particular de cada patógeno en laboratorios chilenos. Se tiene fotografías de todos los procesos realizados.
- Se copia una tabla resumen de los medios y tratamientos para cada cepa.

De los medios de cultivo

- Se copiaron las recetas de los medios de cultivo para cada patógeno.
- Se diferenciaron y realizaron procedimientos particulares en la preparación de medios para tratar exitosamente el crecimiento de patógenos con fines de producción.

De los ensayos de seguridad

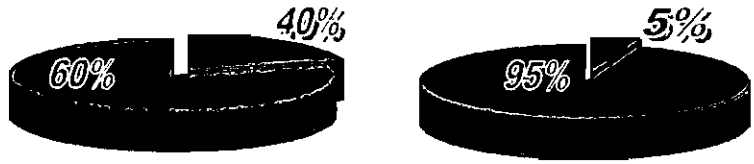
- Gallant Labs requiere de los servicios de un centro veterinario privado para realizar las pruebas de seguridad de las vacunas preparadas. La empresa se denomina Animal Fitness, la cual es controlada por el Gobierno Canadiense.
- Para ensayar cada vacuna diseñada contra los patógenos preparados, ocho ratones blancos son inyectadas con 1 ml de la vacuna. Se analiza la conducta y el estado sanitario diario de los ratones durante 7 días. Si uno de los ocho ratones muere o se enferma, la vacuna es desechada y se chequean las inactivaciones y los controles de calidad microbiológicamente.
- Este protocolo es realizado para todos los patógenos, excepto para *Actinobacillus pleuropneumoniae*, el cual se ensaya usando 2 a 5 cerdos por ensayo. A pesar de haber visitado el Dr. Friendship de La Universidad de Guelp, quien realiza los ensayos de seguridad en cerdos para Gallant Labs, lamentablemente, no se coincidió con un

ensayo de evaluación. Cabe mencionar que el Dr. Friendship trabaja en el diagnóstico de circovirus. Sus datos indicarían que Circovirus tiene una incidencia del 50% en granjas porcinas canadienses.

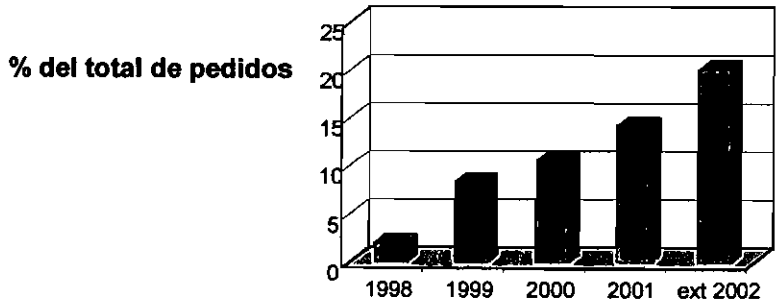
Detalles importantes a tener presente

1. Gallant Labs realiza la identificación específica con materiales estándares que pueden ser rápidamente adoptadas en nuestros laboratorios. Sin embargo, para determinar el serotipos de las especies, es necesario comprar los sueros respectivos. Gallant Labs los obtiene comercialmente desde una empresa Canadiense y en la Universidad de Montreal

En la grafica se puede observar, los que la actividad de ambos departamentos en Gallant Labs. Sin embargo, según Jackie Gallant, el diagnóstico es necesario para mantener y captar clientela.



- Diagnostico
- Autovacunas
- Diagnostico Order de pedidos para Gallant Labs



Contribución de vacunas combinadas en un número total de órdenes.

GOBIERNO DE CHILE
FUNDACIÓN PARA LA INNOVACIÓN AGRARIA

Contactos Establecidos

Presentar los antecedentes de los contactos establecidos durante el desarrollo de la propuesta (profesionales, investigadores, empresas, etc.), de acuerdo al siguiente cuadro:

Institución/ Empresa/Organi-zación	Persona de Contacto	Cargo	Fono/Fax	Dirección	E-mail
Gallant Laboratories	Jackie Gallant	Gerente	1 519 620-2488	1425 Bishop St N Cambridge, Ontario N1R 6J9, Canadá	gallantcustomlabs@on.aibn.com
Gallant Laboratories	Lori Mossier	Científico	1 519 620-2488	1425 Bishop St. N Cambridge, Ontario N1R 6J9, Canadá	gallantcustomlabs@on aibn com

Material Recopilado

Junto con el informe técnico se debe entregar un set de todo el material recopilado durante la actividad de formación (escrito y audiovisual: artículos, fotos, libros, diapositivas, cd) ordenado de acuerdo al cuadro que se presenta a continuación (deben señalarse aquí las fotografías incorporadas en el punto 4):

Tipo de Material	Nº Correlativo (si es necesario)	Caracterización (título)
Artículo	1 2 3 4 5	1. Circular FARMA-SAG para autocavunas 2. Manual para diagnóstico de enfermedades de cerdos 3. PCV2. Artículo del Internacional Pigletter 4. Artículo pérdidas económicas PWMS. Connors 2006 5. Diagnosis Circovirus. Gordon & McNeilly. IPVS. 2006.
Foto		
Libro		
Diapositiva		
CD		

4. PROGRAMA DE DIFUSIÓN EJECUTADO

Programa de difusión ejecutado

En esta sección se deberán describir detalladamente las actividades de difusión realizadas, tales como publicaciones, charlas, seminarios u otras actividades similares, comparando con el programa establecido inicialmente en la propuesta. Se deberá también describir y adjuntar el material de difusión preparado y/o distribuido en dichas actividades.

La información a entregar sobre cada actividad de difusión es la siguiente:

- Tipo de actividad realizada y objetivo principal (incluye elaboración de publicaciones)
- Fecha y lugar de realización
- Temas tratados o exposiciones realizadas
- Destinatarios de la actividad: especificar el tipo y número de personas que asistieron a la actividad (productores, académicos, investigadores, profesionales, técnicos, etc.). Se deberá adjuntar el listado de asistentes según formato indicado más adelante.
- Nombre y tipo de las organizaciones u otras instituciones relevantes en el tema o sector que tuvieron representación en la asistencia al evento.
- Identificación de los expositores que estuvieron a cargo de las presentaciones, indicando su vinculación con la iniciativa y lugar de trabajo
- Indicar si se trató de una actividad abierta a todos los interesados, abierta a quienes se inscribieron previamente, o limitada a quienes fueron especificamente invitados.
- En el caso de los seminarios, deberá adjuntarse el Programa de la actividad que se realizó.

- **Tipo de actividad realizada y objetivo principal (incluye elaboración de publicaciones)**

Charla de difusión de la actividad, cuyo objetivo es transmitir la experiencia, conocimientos y plantear el tema de autovacunas, en términos de sondear su necesidad de uso y mostrar la efectividad en un gran mercado como lo es Canadá.

- **Fecha y lugar de realización** Miércoles 29 de Noviembre de 2006 a las 19:00 hrs, en la sala de conferencias del Restaurante Babaria, (Paine Ltda), ubicado en Panamericana Sur Km. 40. Paine Fono: 02-8242607)

- **Temas tratados o exposiciones realizadas**

"Perspectivas para el Desarrollo de Autovacunas para la Industria Porcina en Chile"

- **Destinatarios de la actividad: especificar el tipo y número de personas que asistieron a la actividad (productores, académicos, investigadores, profesionales, técnicos, etc.). Se deberá adjuntar el listado de asistentes según formato indicado más adelante.**

Dirigido a productores porcinos

- **Nombre y tipo de las organizaciones u otras instituciones relevantes en el tema o sector que tuvieron representación en la asistencia al evento.**

Departamento de Veterinaria de la Universidad Santo Tomás.

- **Identificación de los expositores que estuvieron a cargo de las presentaciones, indicando su vinculación con la iniciativa y lugar de trabajo**

La presentación fue iniciada por el gerente de Centrovet, Don Alberto Farcas, y la propuesta fue narrada por el señor Patricio Villalobos, coordinador de esta propuesta.

- **Indicar si se trató de una actividad abierta a todos los interesados, abierta a quienes se inscribieron previamente, o limitada a quienes fueron específicamente invitados.**

La actividad fue abierta a todos los interesados.

- **En el caso de los seminarios, deberá adjuntarse el Programa de la actividad que se realizó.**

Se adjunta copia de la presentación realizada

Material entregado en las actividades de difusión

Entregar un listado del material elaborado y distribuido con motivo de la actividad o material audiovisual exhibido como video, datashow, entre otros.

Además, se debe entregar adjunto al informe un set de todo el material entregado en las actividades de difusión (escrito y audiovisual) ordenado de acuerdo al cuadro que se presenta a continuación.

También se deben adjuntar fotografías correspondientes a la actividad desarrollada. El material se debe adjuntar en forma impresa y en un medio magnético (disquet o disco compacto).

Tipo de material	Nombre o identificación	Preparado por	Cantidad
Apuntes	Farma / MP2	SAG	30
Apuntes	Carta tipo de solicitud	El coordinador	30
Apuntes	Presentación	El coordinador	30
Apuntes	Guía toma de muestras	El coordinador	30
Carpeta	Carpeta FIA	FIA	30

Participantes en actividades de difusión Es necesario registrar los antecedentes de todos los asistentes que participaron en las actividades de difusión. El listado de asistentes a cualquier actividad deberá al menos contener la siguiente información:	
Nombre	
Apellido Paterno	
Apellido Materno	
RUT Personal	
Dirección, Comuna y Región	
Fono y Fax	
E-mail	
Nombre de la organización, empresa o institución donde trabaja / Nombre del predio o de la sociedad en caso de ser productor	
RUT de la organización, empresa o institución donde trabaja / RUT de la sociedad agrícola o predio en caso de ser agricultor	
Cargo o actividad que desarrolla	
Rubro, área o sector a la cual se vincula o en la que trabaja	

29 de Noviembre de 2006

Asistencia Charla: Datos solo para estadística de FIA

"Perspectivas para el Desarrollo de Autovacunas para la Industria Porcina en Chile" el

	Nombre	Rut	Empresa	Firma
1	Antonio Avilés		U Católica	
2	Juan Villegas		Agrícola Los Tilos	
3	Daniel Adi		Daniel Adi	
4	José Vera		Agrícola ASA	
5	Juan Sepúlveda		AASA	
6	José López		AASA	
7	David Atherton		Comercio y Servicio	
8	Gloria Pereira		Comercio y Servicio	
9	Marcela Pereira		Comercio y Servicio	
10	Joaquín Reyes		Comercio y Servicio	
11	Juan Hernández		Ag. Los Tilos	

12 Fernand Sae		Pdte. de ch.le	
13 Danilo Gonzales A.		Pdtes de chile	
14 Cristian Gutierrez.		//	
15 Luis Liberon		//	
16 Dagoberto Carvajal		Sta Inés de Melipilla	
17 Romualdo Cordocedo		//	
18 Donatia Sanchez		Sta. Inés de Melipilla	
19 Pedro Pablo Riquelme		//	Pedro P.
20			
21			
22			
23			
24			
25			
26			

Evaluación de las actividades de difusión

Especificar el grado de éxito de las actividades propuestas, señalando las razones de los problemas presentados y sugerencias para mejorarlos en el futuro. Señalar también las razones por las cuales se hicieron modificaciones al programa propuesto inicialmente, en los casos que corresponda.

Como puede ser extraído de los puntos expuestos en esta propuesta, la evaluación de las actividades de formación y de transferencia es muy positiva. La experiencia y los conocimientos adquiridos en el lugar visitado, fueron de alto enriquecimiento cultural y científico.

La transferencia se recibió muy abiertamente y con buena disponibilidad.
No existen sugerencias.

Hubo retrazo en la entrega del informe final, debido a que durante los meses de Diciembre y Enero, La empresa trabajó con menos personal, y con transformaciones de infraestructura, lo cual evito obtener documentación necesaria para el proyecto. Posteriormente el proponente tomó licencia y las respectivas vacaciones.

5. EVALUACIÓN DE LA PROPUESTA

Organización durante la actividad (indicar con cruces)[1]

Ítem	Bueno	Regular	Malo
Recepción en país o región de destino según lo programado	x		
Cumplimiento de reserva en hoteles	x		
Cumplimiento del programa y horarios según lo establecido por la entidad organizadora	x		
Facilidad en el acceso al transporte	x		
Estimación de los costos programados para toda la actividad	x		

Evaluación de la iniciativa de formación

En esta sección se debe evaluar la actividad en relación a los siguientes aspectos:

a) Efectividad de la convocatoria

La convocatoria tuvo una efectividad del 60-70%.

b) Grado de participación de los asistentes (interés, nivel de consultas, dudas, etc)

A pesar de la convocatoria, el nivel de participación fue elevado. Se logró "sentir" la necesidad de abordar el tema, no sólo desde el punto de vista de patología, sino que además de asesorar el rubro desde un punto de vista de registro de patologías.

c) Nivel de conocimientos adquiridos en función de lo esperado (se debe indicar si la actividad contaba con algún mecanismo para medir este punto)

La actividad no contaba con mecanismos para medir este punto. No obstante, la Dra. Gallant interaccionaba frecuentemente con el participante, y el personal de laboratorio siempre realizó una interacción dinámica de manera de asegurase la buena transmisión de los conocimientos. Por lo demás se realizó una presentación final al terminar el curso, en la que se corrigieron algunos errores y se reforzaron algunas dudas.

[1] En caso de existir un ítem Malo o Regular, señalar los problemas enfrentados durante el desarrollo de la actividad de formación, la forma como fueron abordados y las sugerencias que puedan aportar a mejorar.

La transferencia de datos en Chile, fue clara. Aunque el sector productivo no está interiorizado en los conceptos biológicos que evocan las enfermedades, ellos saben de la experiencia en terreno. De acuerdo a esto, y a la timidez característica de los productores a preguntar situaciones propias en público, las preguntas fueron post-presentación. Es decir, durante la cena, se pudo constatar el grado de interés, aclarar dudas y proyectar visitas. En resumen, se detectó interés y recepción clara de los conceptos planteados.

d) Calidad de material recibido durante la actividad de formación

Material de primera calidad. En todo momento se observó una preocupación del estado y la forma de entrega del material. Se observó una buena disposición en este como en otros aspectos. La Dra Gallant y su equipo no escatimaron en tiempo ni recursos.

e) Nivel de adecuación y facilidad de acceso a infraestructura/equipamiento necesario para el logro de los objetivos de la actividad de formación.

No hubo restricciones respecto a aprender de la temática. Tanto la Dra Gallant como su personal dieron amplias facilidades de acceso a sus equipos e infraestructura. Realmente resulto una sombro el grado de disposición de los investigadores canadienses.

f) Indique las materias que fueron más interesantes, más desarrolladas a lo largo de la actividad de formación y las que generan mayor interés desde el punto de vista de la realidad en la cual se desenvuelve el participante.

Para el participante, todas las materias tomadas fueron de interés y las más desarrolladas fueron las concernientes a los requerimientos de los patógenos vistos. Aunque el participante posee una maestría en Microbiología, no poseía experiencia en el tema de patógenos fastidiosos. El interés y robustez de lo adquirido se reflejaron en la adaptación de lo aprendido en el laboratorio en Chile, el cual está realizando cultivos propios de los patógenos.

g) Problemas presentados y sugerencias para mejorarlos en el futuro

No se registraron problemas y por lo tanto no tenemos sugerencias.

GOBIERNO DE CHILE
FUNDACIÓN PARA LA INNOVACIÓN AGRARIA

Aspectos relacionados con la postulación al programa de formación o promoción
a) Apoyo de la Entidad Patrocinante (cuando corresponda) _X_ bueno ___ regular ____ malo Justificar: La entidad patrocinante apoyo en todo momento la iniciativa. Si embargo, no hubo apoyo en la continuidad de la planificación estratégica en el que estaba inserta la presente propuesta.
b) Información recibida por parte de FIA para realizar la postulación _X__ amplia y detallada ____ aceptable ____ deficiente Justificar: Excelente apoyo por parte del personal de FIA
c) Sistema de postulación al Programa de Formación o Promoción (según corresponda) _X__ adecuado ____ aceptable ____ deficiente Justificar: No hay comentarios
d) Apoyo de FIA en la realización de los trámites de viaje (pasajes, seguros, otros) (sólo cuando corresponda) _X__ bueno ____ regular ____ malo Justificar: Muy oportuno y eficiente
e) Recomendaciones (señalar aquellas recomendaciones que puedan aportar a mejorar los aspectos administrativos antes indicados) No da pie a comentarios.

GOBIERNO DE CHILE MINISTERIO DE AGRICULTURA SAG	**Registro y Control de Productos Farmacéuticos de Uso Veterinario FARMA/IT5**	
	Autovacunas	

EXIGENCIAS TÉCNICAS.

I.- El médico veterinario tratante debe adjuntar la siguiente información:

a) Nombre y ubicación del lugar en que se encuentran o han encontrado los animales enfermos. En el caso de animales acuáticos debe indicarse además, el Código Sernapesca asignado para el o los centros de cultivo involucrados

b) Individualización del propietario de los animales o identificación de la empresa

c) Número de animales y/o biomasa existentes en el lugar o centros afectados.

d) Manejo sanitario general del lugar (predio, plantel o centro de cultivo) señalando los productos farmacológicos y vacunas utilizados y los resultados obtenidos. Para el caso de los animales acuáticos, se deberá complementar la información enviada según la resolución Sernapesca N° 1720 de 2004 , con los respectivos resultados.

e) Diagnóstico clínico de la enfermedad.

f) Informes de los diagnósticos y aislamientos realizados, señalando claramente el origen de las muestras y describiendo las técnicas con el suficiente detalle como para ser repetidas por el Servicio, con especificación expresa del agente que se utilizará para la fabricación de la autovacuna. Los informes deben identificar al laboratorio de diagnóstico, deben estar firmados por el profesional responsable y deben señalar la fecha de aislamiento, la cual no debe ser anterior a 15 meses.

g) Porcentaje de mortalidad y/o morbilidad asociado al diagnóstico de la enfermedad.

h) Número de animales y/o biomasa a inmunizar adjuntando el programa de vacunación.

j) Individualización del médico veterinario solicitante, señalando nombre completo, RUT, número de teléfono, fax y correo electrónico.

II.- Envío de la cepa del microorganismo actuante:

El envío de la cepa del microorganismo actuante al Laboratorio de Control Biológico deberá realizarse en condiciones de mantención adecuada (liofilizada, refrigerada o congelada) en triplicado, en envase hermético sellado y rotulada de acuerdo a una codificación que permita su trazabilidad (género o familia del microorganismo, especie de la cual proviene el aislado, plantel, sector, región, mes, año, etc.), adjuntando los antecedentes señalados en el punto b) del numeral I.

, a fecha

Dr.....
Director Servicio Agrícola y Ganadero
Región .....
Presente

Estimado Dr.....

Por intermedio de la presente, y cumpliendo la normativa vigente en el Reglamento de Productos Farmacéuticos de Uso Exclusivamente Veterinarios, específicamente en lo que dice relación a la fabricación y uso de Autovacunas y Productos Experimentales (art. 46 al 53), vengo a solicitar a usted la autorización para utilizar una Autovacuna .....aquí colocar contra qué patógeno.

Dicha solicitud obedece a que nos hemos visto afectados de brotes de la enfermedad mencionada en .....aquí colocar los lugares geográficos afectados, los cuales se han caracterizado por .....aquí colocar una descripción del historial de la enfermedad, de como a evolucionado clínicamente, como se a comportado epidemiológicamente, y especialmente explicar lo que se ha hecho para tratarla, haciendo énfasis en la necesidad de vacunar preventivamente con una autovacuna eficiente para detener su propagación.

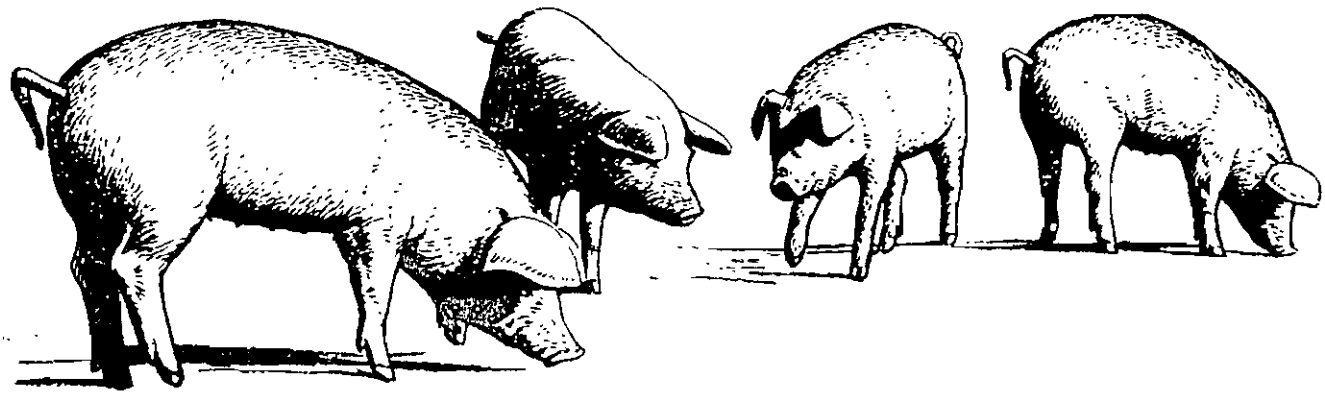
El producto solicitado será utilizado en .....aquí colocar el nombre y ubicación geográfica del centro donde será utilizada la vacuna, bajo la supervisión del Dr. .....individualizar al médico veterinario que será el responsable de la vacunación, definir la especie de destino, tamaño (edad o peso), número de animales que serán vacunados y el esquema de vacunación que se usará.

Por todo lo anteriormente descrito, solicito a usted que nos autorice la elaboración y uso de .....aquí colocar el número de dosis, autovacuna que será elaborada por el Laboratorio Centrovet limitada, ubicado en Av. Los Cerrillos N° 602, Santiago.

Esperando una buena acogida a la presente, le saluda atte,

Dr. .....
Médico Veterinario
Jefe de Salud

SWINE DISEASES DIAGNOSTIC MANUAL



2005

Allen D. Leman Conference

St. Paul, MN

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Newport Laboratories Swine Diagnostic Submission Guide

Clinical Signs	Stage of Production	Common Causes	Tissues To Submit	Fresh	Fixed
Diarrhea	Farrowing	E.coli, Clostridium Rotavirus, TGE virus, Coccidiosis	Small & large intestine Fecal swab	X X	X
Diarrhea	Nursery	E.coli, Clostridium	Small & large intestine Fecal swab	X X	X
Diarrhea	Grow-Finish	Ileitis (Lawsonia)	Small intestine (ileum)	X	X
Respiratory: Cough, rapid breathing (thumping)	Farrowing Nursery Grow-Finish	Swine influenza virus Mycoplasma, PRRS Pasteurella	Lung Nasal swab Serum	X X X	X
CNS/Brain disease: Down-paddling, dizzy, head tilt, convulsions	Nursery	Strep suis, H. parasuis, Edema disease	Brain Cerebrospinal Fluid, (CSF) Brain/CSF swabs	X X X X X	X
Sudden death	Nursery	Strep suis, H. parasuis	All organs (except stomach & intestine)	X	X
	Grow-Finish	Hemorrhagic Bowel Syndrome Ulcers	Small intestine (Ileum) Stomach	X X	
	Gestation Farrowing	Clostridium	Liver, spleen kidney	X X	
Reproductive Disease: Abortions mummies stillborns weakborns	Gestation Farrowing	Parvovirus, PRRS Swine influenza virus	Aborted fetuses mummies stillborns weakborns sow & pre-suckle pig serum	X X X X X	

NEWPORT LABORATORIES
"Guiding the Way"

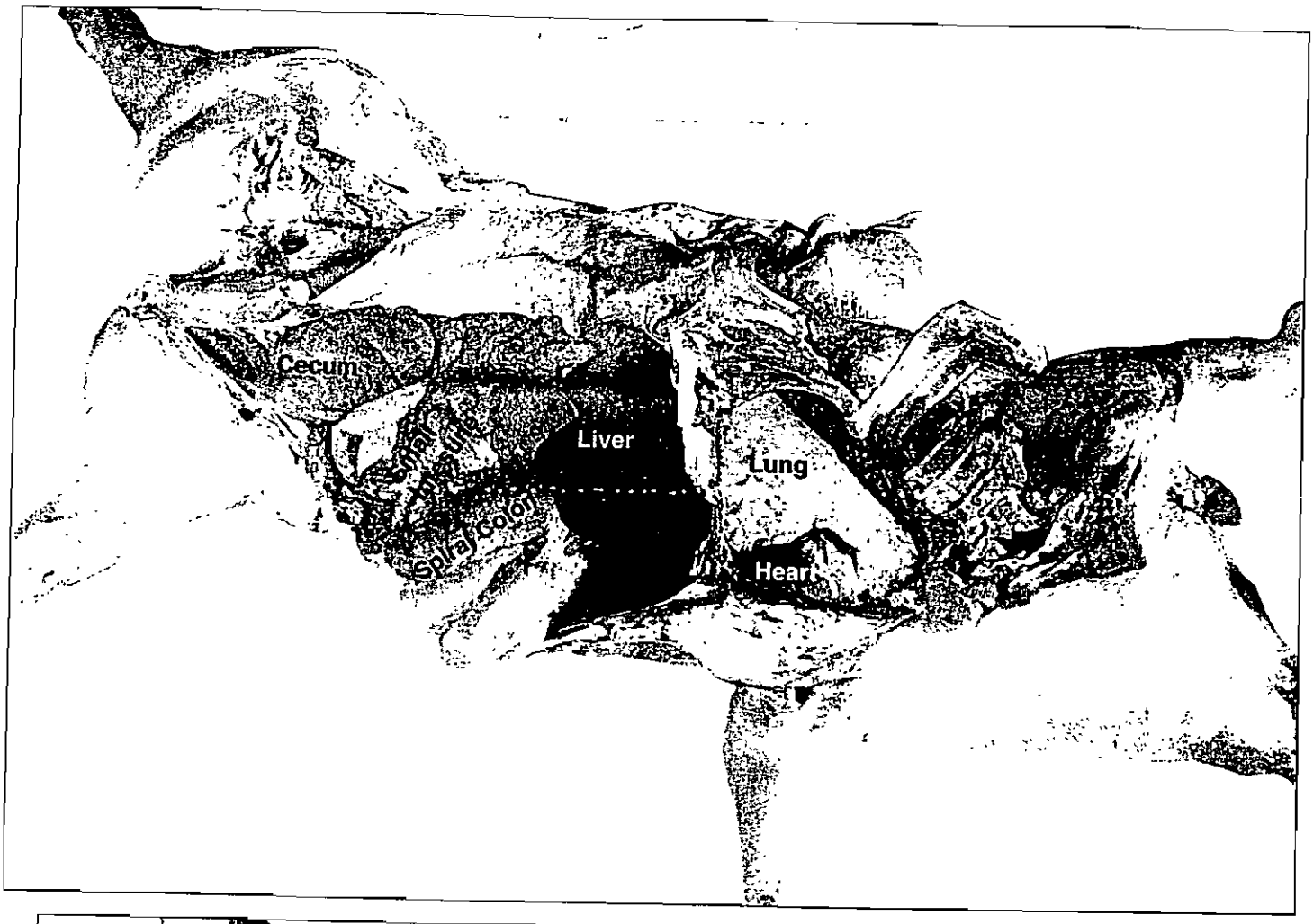
To insure good diagnostic results when collecting and submitting samples, do the following:

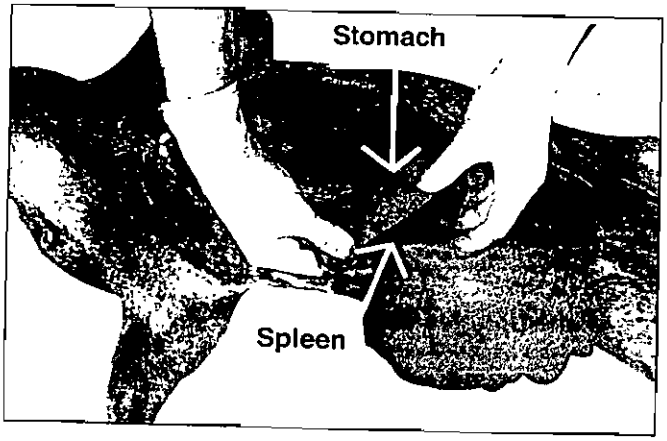
- Collect and submit fresh samples from recently dead or euthanized pigs.
- For formalin-fixed (preserved) samples, place samples in formalin immediately. Handle formalin very carefully! Avoid skin contact and breathing in fumes.
- Submit "fist size" fresh samples and formalin-fixed samples that are 1/4 inch thick.
- Put samples in plastic bags that can be closed or tied securely. Leaking sample bags cause major problems for everybody!
- Always bag GI (stomach or intestine) samples separately from other tissues.
- If samples from different sites or ages are submitted together, identify them accordingly.
- Submit all tissues, swabs and serum tubes in leak proof diagnostic shippers.
- Pack adequate number of frozen cold packs to insure that the samples arrive at the lab chilled.
- Use insulated diagnostic shipper with plastic liner to prevent leakage. Fold in plastic liner before taping box shut.
- Do not freeze samples.
- Send a completed diagnostic submission form with the samples. Place the form in a sealed plastic bag or on the outside of the styrofoam box.
- Ship overnight using a reliable shipper. Avoid weekend shipping.

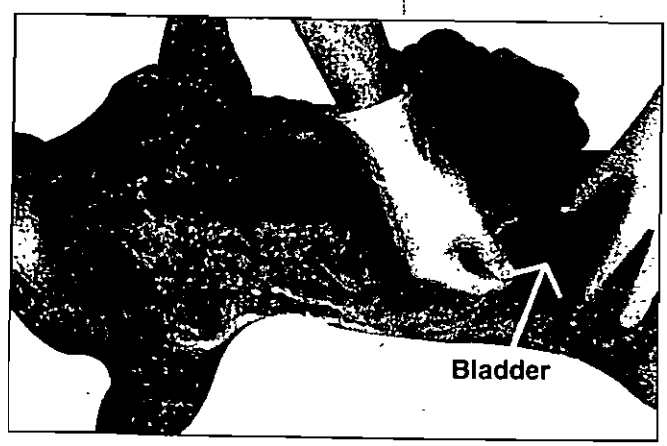
Ship To:

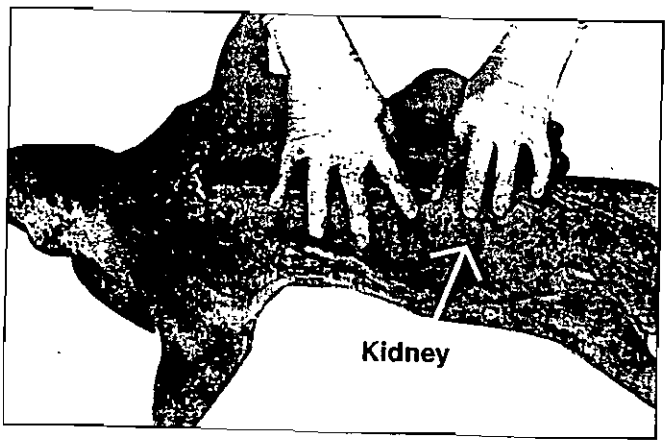
Newport Diagnostic Lab
600 Oxford Street
Worthington, MN 56187
Phone: 800-220-2522
Fax: 507-372-4788 (Diagnostic Lab)

NEWPORT DIAGNOSTICS

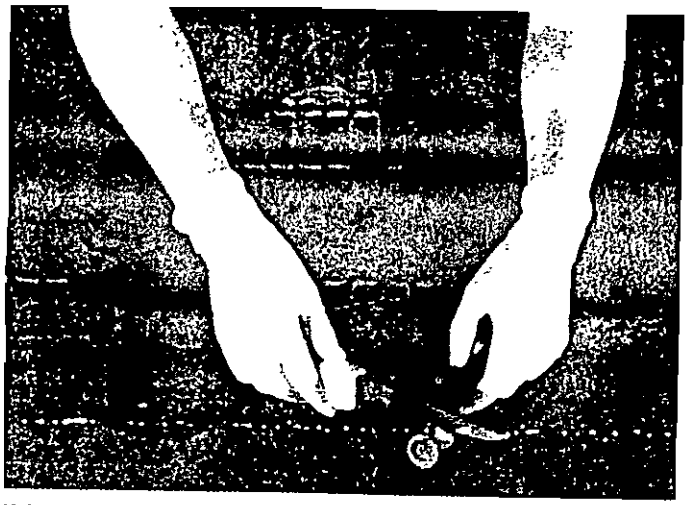

Cecum
Liver
Lung
Heart


Stomach
Spleen

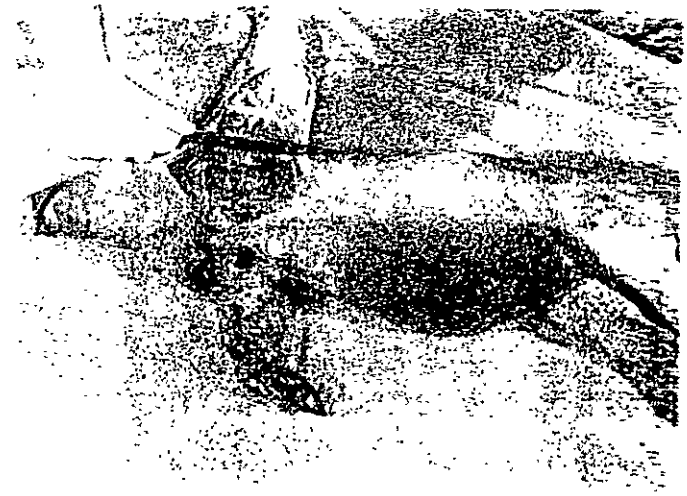

Bladder


Kidney

Nursery Pig - Necropsy Instructions



IMPORTANT: Start with a sharp knife.



Cut through both front legs at the armpits to separate the leg from the rib cage.

After cutting through the armpits, the pig will lie on its back.

Hook the knife under the sternum. Cut through the cartilage of the ribs to the sternum.

Continue this cut to remove the skin and body wall of the belly from the pig.

Most organs are now visible.

Grower/Finisher - Necropsy Instructions

Hold the lower foot down with your foot and pull up on the upper leg.

Use the knife to cut through the armpit to separate the leg from the rib cage.

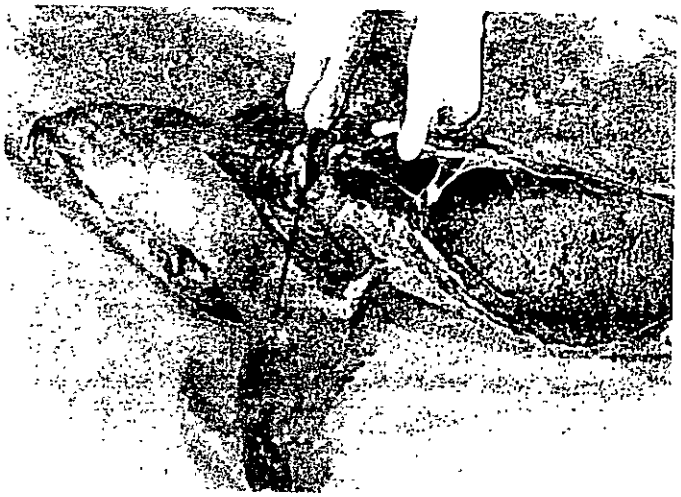
The hind leg is cut back as well. Use the same procedure as the front leg.

As you push the hind leg toward the floor behind the pig. the hip joint should snap open.

Cut under the skin from the back leg.

Continue to cut to the front leg.

Nursery Pig - Necropsy Instructions

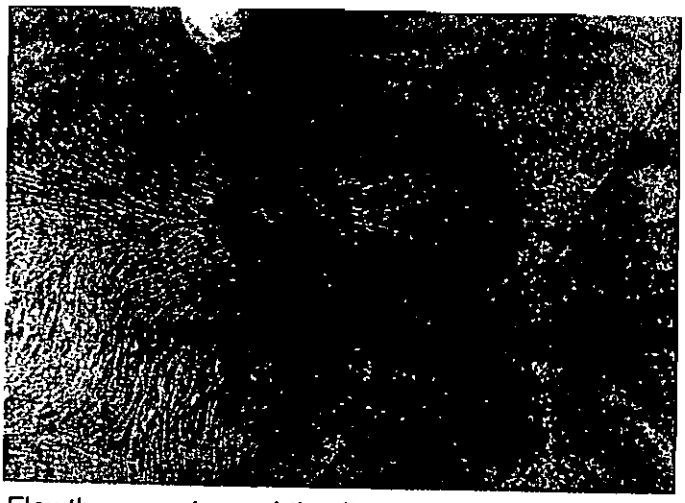


Cut between the ribs below the collar bone.

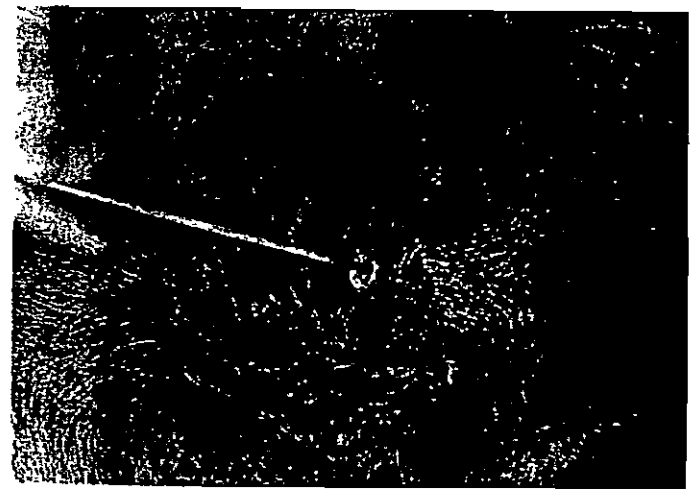
Spread the ribs open.

Organs are easily examined.

BRAIN SWABS: Cut through the skin and muscle behind the ears.



Flex the nose toward the floor and the ears down to open the space between the top vertebra and the skull. This will allow room to cut between them.

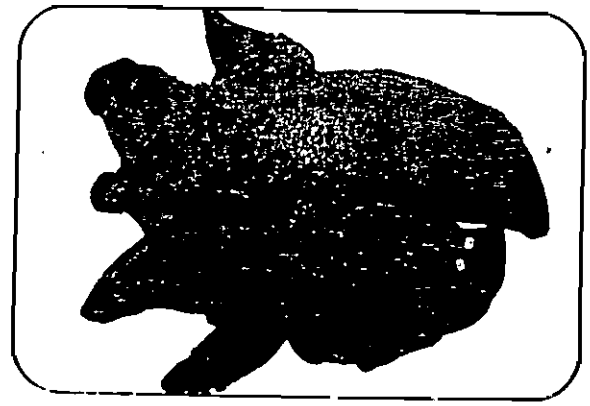


Place a swab in the exposed spinal cord toward the brain. This is an excellent way to test for strep.

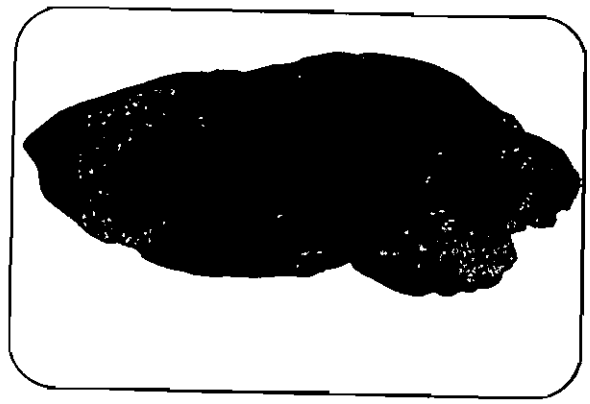
SIV

(Swine Influenza)

Normal Lung



Dorsal Surface of SIV Infected Lung



Diffusely Congested Lung Typical of SIV

Clinical Signs/History:

- Respiratory: Coughing, rapid breathing (thumping), depression, fever to 108^{O}F, anorexia, dyspnea, weakness, prostration and a mucous discharge from the eyes and nose
- Outbreak is characterized by sudden onset and rapid spread through the entire herd, often within 1-3 days

Stage of Production:

- Farrowing, nursery, grow-finish

Diagnosis:

Lesions:

- In uncomplicated infections, the lesions usually are confined to the lungs. The pneumonic areas are clearly demarcated, collapsed, and purplish red. They may be distributed throughout the lungs, but tend to be more extensive and confluent ventrally. The airways contain a copious mucopurulent exudate, and the bronchial and mediastinal lymph nodes are edematous and enlarged.

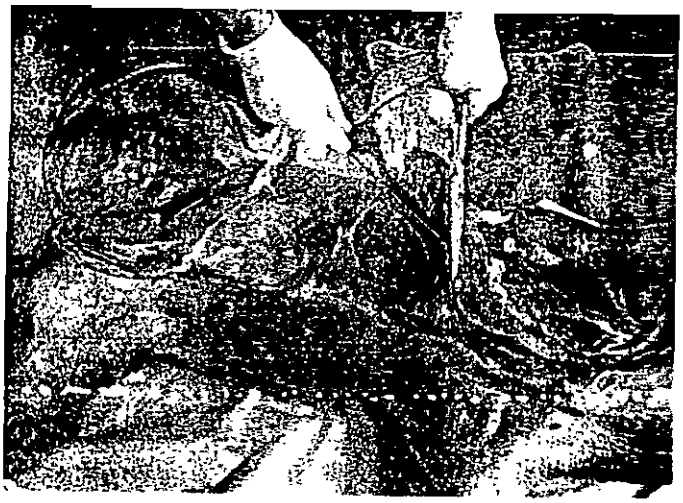
DX Tests: Histopathology, Virus Isolation (VI), Directigen™ ELISA, Serology (HI, ELISA)

Specimens To Submit:

- Lung, - fresh and formalin-fixed
- Nasal Swabs
- Serum - sow & pig

Grower/Finisher - Necropsy Instructions

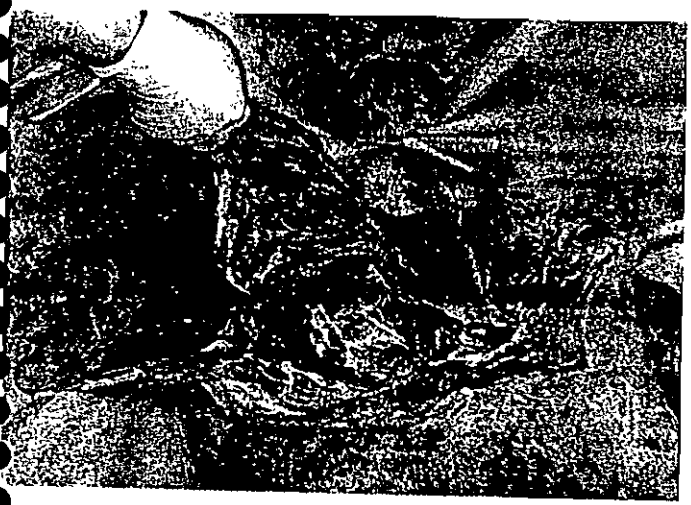
Cut the skin back from the abdominal muscles.



Continuing to cut the skin back from the abdominal muscles.

Carefully open the abdomen (without cutting the intestines) on the midline. Start between the hind legs and work up to the sternum.

Cut through the cartilage between the sternum and the ribs.



Cut between the ribs and break the ribs open 2-3 at a time.

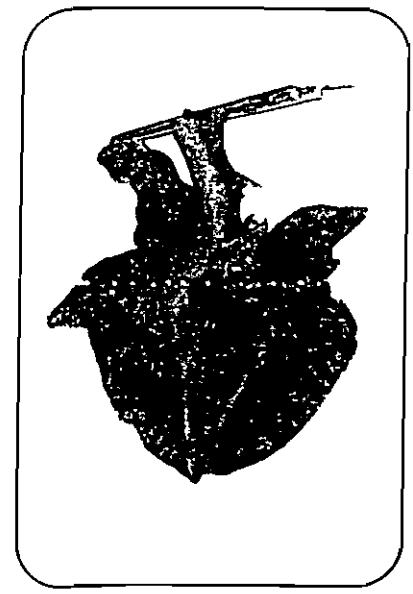
Organs are now exposed for examination.

PRRS

(Porcine Reproductive and Respiratory Syndrome)

Normal Lung

PRRS



PRRS

Clinical Signs/History:

- Respiratory: Cough, rapid breathing (thumping), unthrifty pigs
- Reproductive: Abortions, mummies, stillborns, weakborns

Stage of Production:

- Gestation, farrowing, nursery, grow-finish

Diagnosis:

Lesions:

- Lesions are not diagnostic; diagnosis is based on herd history and Virus Isolation (VI) or viral antigen testing.

DX Tests:

- The most commonly used serological tests are the ELISA or the (IFA) Indirect Fluorescent Antibody test.
- Tissue tests include: Virus Isolation (VI) and PCR.

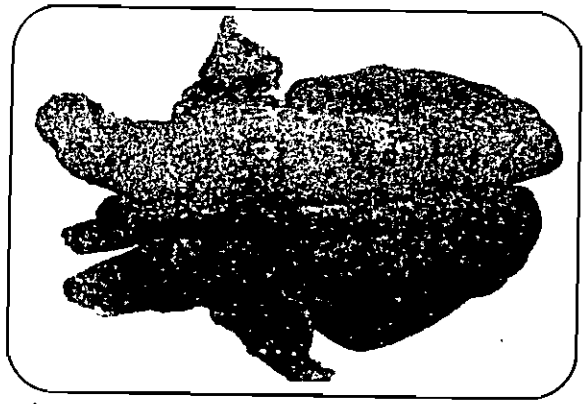
Specimens To Submit:

- Lung - fresh and formalin-fixed
- Serum - sow, pre-suckle pig, nursery, and grow-finish pig serum

PMWS

(Post Weaning Multi-Systemic Wasting Syndrome)

Wasting Pig



Lungs noncollapsed - heavy and firm or rubbery

Enlarged Mediastinal Lymph Nodes

Clinical Signs/History:

- A wasting syndrome known as post weaning multi-systemic wasting syndrome (PMWS).
- The most frequent clinical sign and the one required for diagnosis of PMWS is wasting or failure to thrive. In decreasing order of frequency, other signs include dyspnea, enlarged lymph nodes, diarrhea, pallor, and jaundice.
- All of the fundamental clinical signs are often not observed in a single pig, but most affected farms will present the majority, if not all of the signs, over a period of time.
- Other less common clinical signs including coughing, fever, gastric ulceration, central nervous disorders, and sudden deaths have also been reported, but to a lesser degree.

Stage of Production:

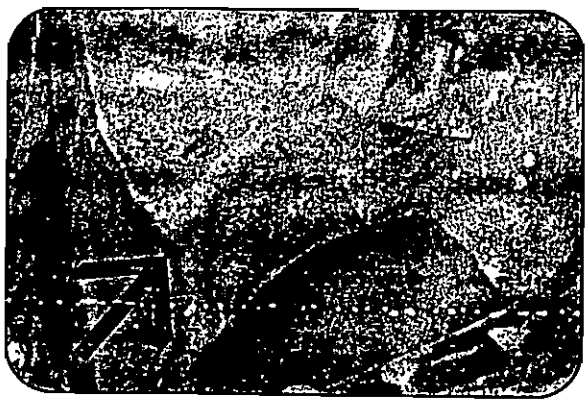
- Nursery, early grow-finish

Diagnosis:

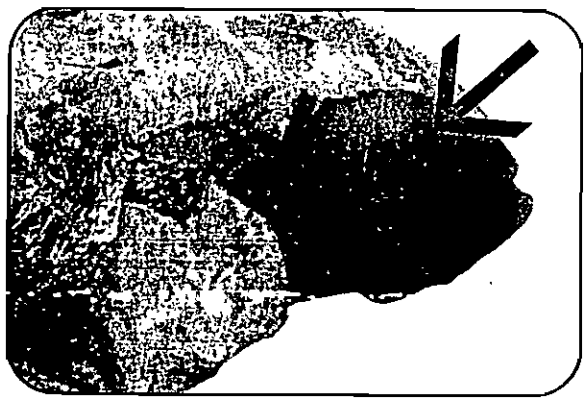
- Diagnosis of PMWS requires that a pig or group of pigs have a specific set of clinical signs, microscopic lesions, and the presence of PCV2 associated with the characteristic lesions.
- PMWS Diagnostic Criteria: 1. Clinical Signs: wasting/failure to thrive, +/- dyspnea, diarrhea, pallor, icterus. 2. Microsopic Lesions: depletion of lymphoid tissues and/or lymphohistiocytic to granulomatous inflammation in any organ (predominantly lung, lymphoid tissue, liver, kidney, intestine, pancreas). 3. PCV2 antigen or genetic material within characteristic lesions.

MYCOPLASMA

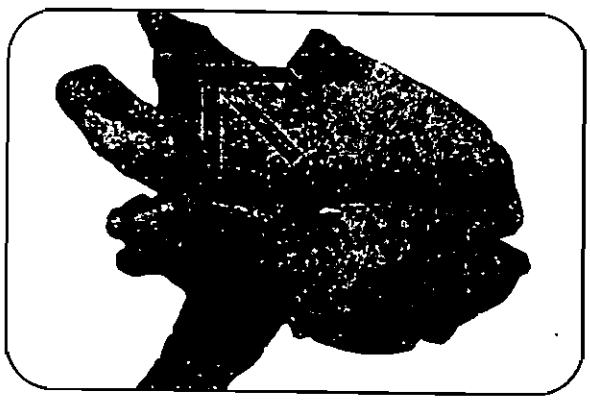
(Enzootic Pneumonia)



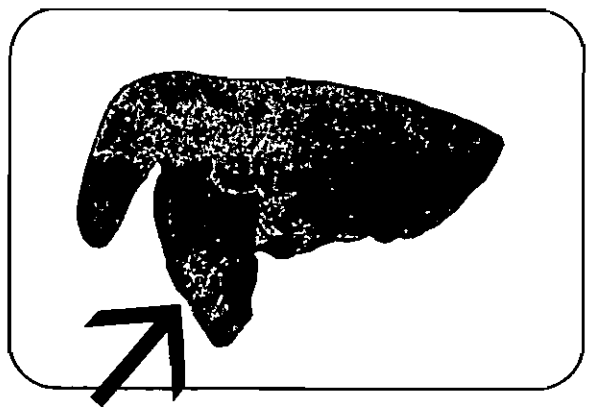
Normal Lung



Mycoplasma



Mycoplasma



Mycoplasma

Clinical Signs/History:

- Respiratory: Coughing is the most common sign and is most obvious when pigs are roused. Sporadically, individual pigs or groups develop severe pneumonia.

Stage of Production:

- Nursery, grow-finish

Diagnosis:

Lesions:

- Affected lung tissue is gray or purple, most commonly in the apical and cardiac lobes. Old lesions become clearly demarcated. The associated lymph nodes may be enlarged.

DX Tests: Histopathology, serology

Specimens To Submit:

- Lung - fresh and formalin-fixed
- Serum - submit 10 to 15 samples from each group: nursery, grower, finisher

PMWS

(Post Weaning Multi-Systemic Wasting Syndrome)

Diagnosis:

- Clinical signs alone are not diagnostic.
- Gross lesions alone are not diagnostic.
- Role of co-infections: Field observations and scientific literature suggest that PCV2, although essential for development of PMWS, may require other factors or agents to induce the full spectrum of clinical signs and lesions associated with advanced PMWS in conventional pigs:
    1. PRRS + Circovirus
    2. Mycoplasma + Swine Influenza + Circovirus

DX Tests:

- Histopathology, virus isolation (V.I.)

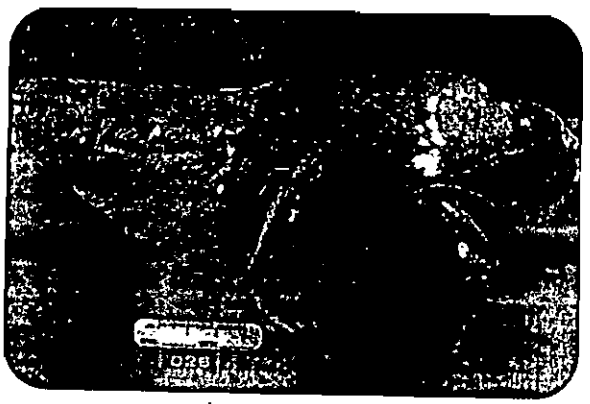
Specimens To Submit:

- Lung
- Lymphoid tissue - spleen, lymph nodes
- Kidney
- Liver
- Intestine
- Pancreas

PASTEURELLA

Normal Lung

Mycoplasma Lung



Mycoplasma and Pasteurella Lung

Clinical Signs/History:

- Respiratory: Cough, rapid breathing (thumping)

Stage of Production:

- Nursery, grow-finish

Diagnosis:

Diagnosis is based on necropsy findings and culture of Pasteurella from the lesions.

Lesions:

- Exudative bronchopneumonia, sometimes with pericarditis and pleuritis

DX Tests: Culture

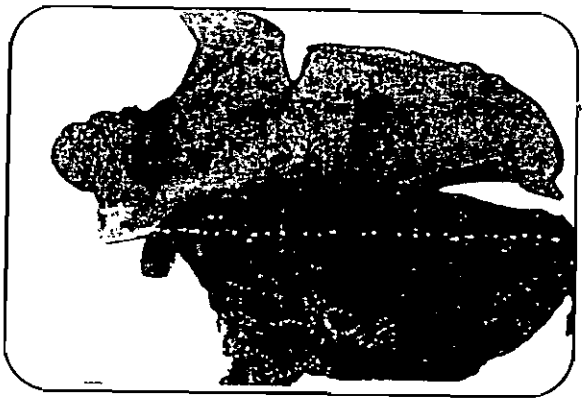
Specimens To Submit:

- Lung, - fresh and formalin-fixed
- Nasal Swabs

APP

(Actinobacillus pleuropneumoniae)

Normal Lung



APP

Clinical Signs/History:

- Respiratory distress is severe; there is thumping, and sometimes open-mouth breathing with a blood-stained frothy nasal and oral discharge, fever, anorexia and reluctance to move.

Stage of Production:

- Primarily a disease of growing pigs

Diagnosis:

An explosive disease onset is suggestive.

Lesions:

- The pneumonia is usually bilateral. The characteristic lesion is a severe fibrinonecrotic and hemorrhagic pneumonia with accompanying fibrinous pleuritis. In acute cases, the lungs are dark and swollen and ooze bloody fluid from the cut surface. Hemorrhagic, even necrotic bullae (cavities) of various sizes may be present. The trachea may contain blood-stained froth. In chronic cases, the lesions are more organized and localized.

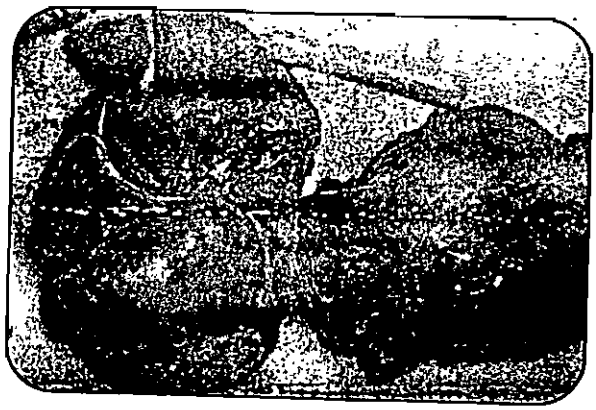
DX Tests: Culture, Histopathology, Serology

Specimens To Submit:

- Infected lung for isolation and identification of APP organism is most definitive.
- Serum samples (10 - 15) from nursery, grower, finisher for ELISA testing

HPS

(*Haemophilus parasuis*)



Fibrin on Heart
HPS

Polyserositis in Abdomen
HPS

Clinical Signs/History:

- Sudden death
- A temperature of 104º-107ºF develops, and there is anorexia, depression, and occasionally mild rhinitis and dyspnea with coughing. Some pigs become lame with painful, warm, swollen and fluctuating joints. Chronic arthritis and occasionally meningitis and convulsions may develop.

Stage of Production:

- Nursery

Diagnosis:

Based on history, clinical signs, and necropsy. Confirmed by culture of the organism from joint fluids, cardiac blood, or CSF.

Lesions:

- Polyserositis, polyarthritis and meningitis

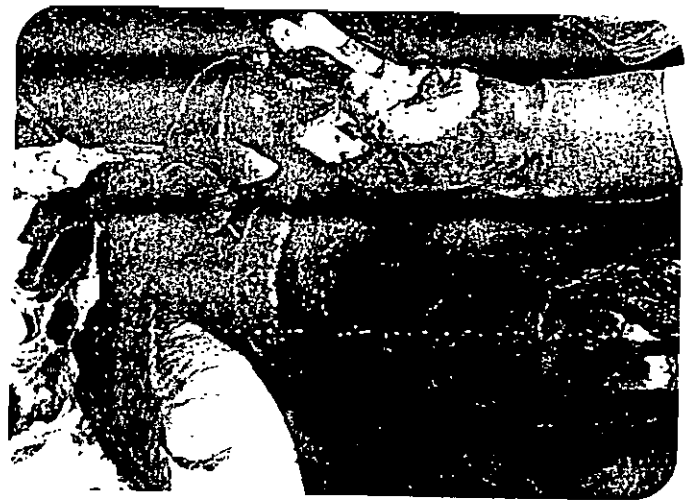
DX Tests: Culture

Specimens To Submit:

- All organs (except stomach & intestine) - fresh & formalin fixed
- Brain, cerebrospinal fluid (CSF)- fresh, Brain/CSF swabs

HBS

(*Hemorrhagic Bowel Syndrome*)



Blood Filled Intestine

Clinical Signs/History:

- Sudden death of grow-finish and young breeding pigs

Stage of Production:

- Grow-finish

Diagnosis:

Sudden death of previously healthy grow-finish pigs and characteristic post-mortem findings.

Lesions:

- Gross enlargement of small intestine with watery bloody fluid.

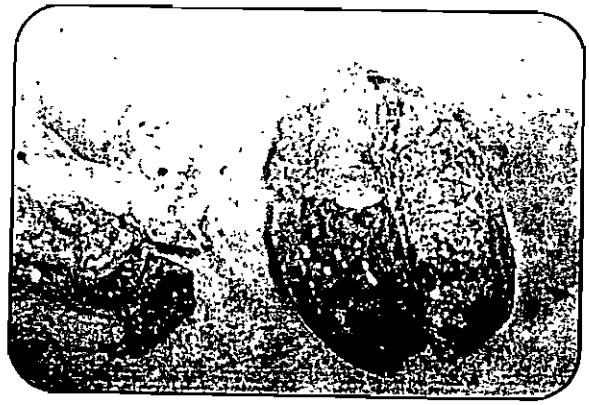
DX Tests: Post-mortem examination

Specimens To Submit:

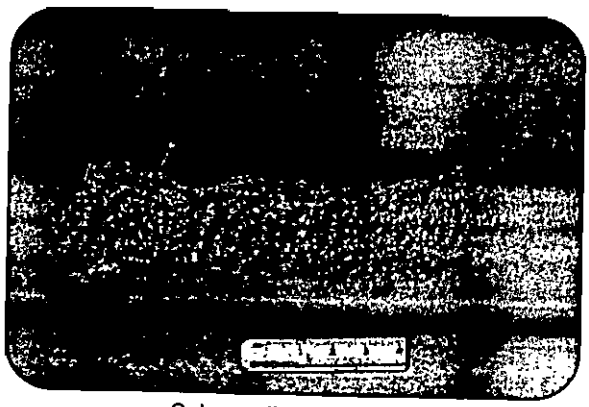
- Small intestine - fresh and formalin-fixed

SALMONELLA

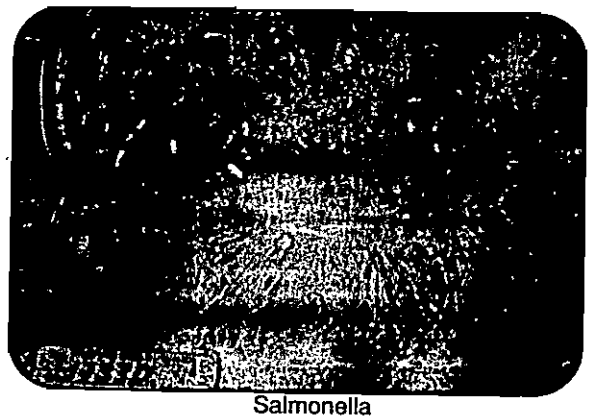
Salmonella choleraesuis



Salmonella choleraesuis



Salmonella typhimurium



Salmonella
Swollen Lymph Nodes

Clinical Signs/History:

- Septicemia is the usual syndrome in pigs up to 6 mo. of age. Illness is acute, depression is marked, fever (105-107^{O}F) is common and death occurs in 24-48 hours. Nervous signs may occur in pigs; these animals may also suffer from pneumonia. Mortality may reach 100%. Nursing pigs may develop diarrhea, but usually succumb to generalized septicemia. Weaning or grow-finish pigs are febrile and have liquid feces that may be yellow and contain shreds of necrotic debris.

Stage of Production:

- Farrowing, nursery, grow-finish

Diagnosis:

Depends on the clinical signs and on the laboratory examination (culture) of feces, tissues from affected animals, feed (including all mineral supplements used), water supplies, and feces from wild rodents and birds that may inhabit the premises.

Lesions:

- In pigs, a dark red to purple discoloration of the skin is common, especially at the ears and ventral abdomen.
- Also, a swollen spleen, liver and lymph nodes can be seen as well as congested hemorrhagic lungs and roughened necrotic intestinal mucosa with ulceration and accumulation of debris.

DX Tests: Culture, histopathology

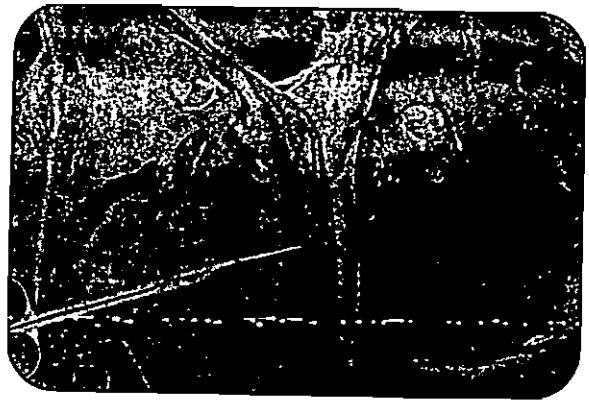
Specimens To Submit:

- Tissues (fresh and formalin-fixed) from affected animals, feed, water supplies, and feces from wild rodents and birds examination

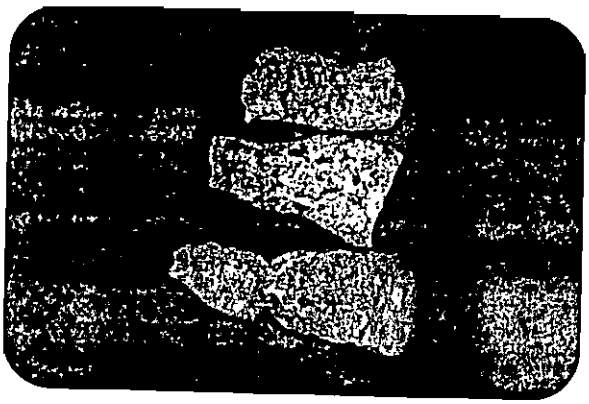
ILEITIS

(Lawsonia intracellularis)

Normal Intestine-can see fingers through lining



Thickened Intestine



Thickened Intestine

Thickened Mucosa

Clinical Signs/History:

- Diarrhea
- Ileitis can be either a chronic disease in growing pigs, or an acute hemorrhagic form in market weight and adult pigs.

Stage of Production:

- Grow-Finish

Diagnosis:

Confirmation is based on histologic observation of characteristic proliferation and inflammation of mucosal crypts.

Lesions:

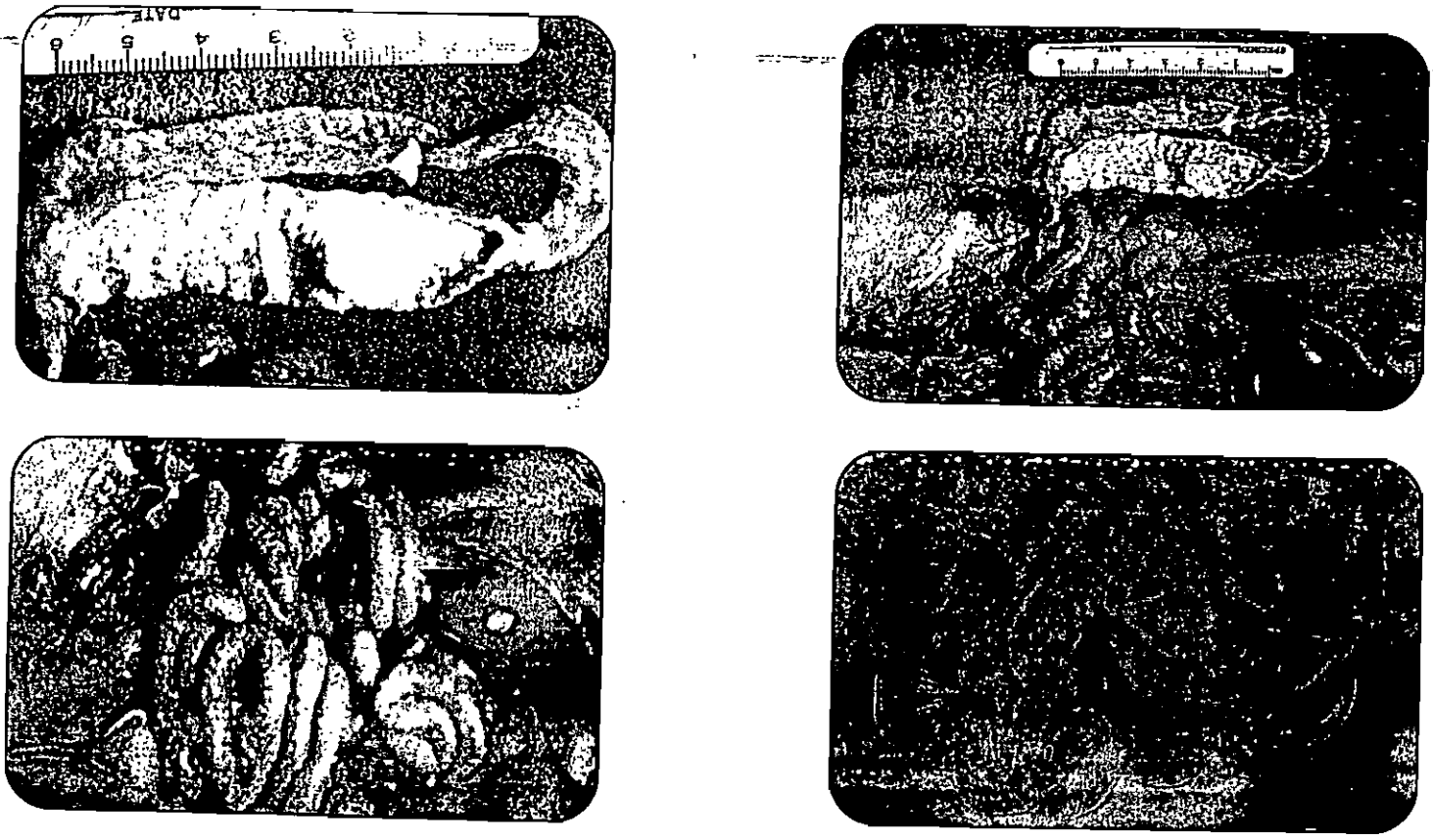
- Lesions may occur anywhere in the lower half of the small intestine, cecum, or colon, but are most frequent and obvious in the ileum. The wall of the intestine is thickened and the mesentery may be edematous.

DX Tests: Histopathology

Specimens To Submit:

- Small intestine (Ileum) - fresh and formalin-fixed

COCCIDIOSIS



Characteristic Appearance of Coccidiosis

Clinical Signs/History:

- Diarrhea
- Clinical signs of coccidiosis are due to destruction of the intestinal epithelium and, frequently, the underlying connective tissue of the mucosa. Infection is characterized by a watery or greasy diarrhea, usually yellowish to white and foul smelling. Piglets may appear weak, dehydrated and undersized; weight gains are depressed and sometimes piglets die.

Stage of Production:

- Farrowing and nursery

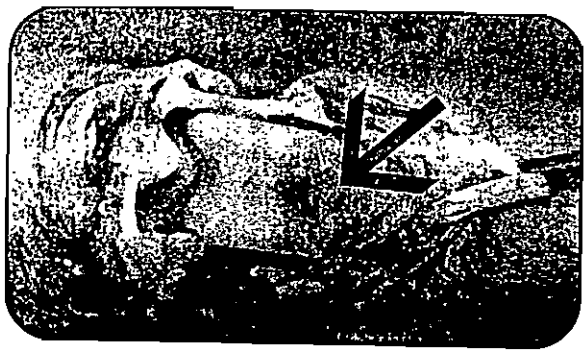
Diagnosis:

- Observation of oocysts which can be identified in feces by salt or sugar flotation methods.

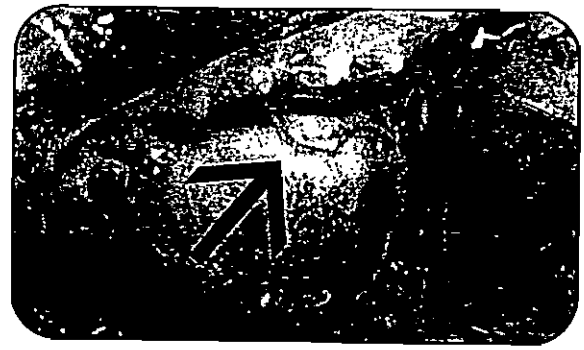
Specimens To Submit:

- Small and large intestine - fresh and formalin-fixed
- Fecal swab

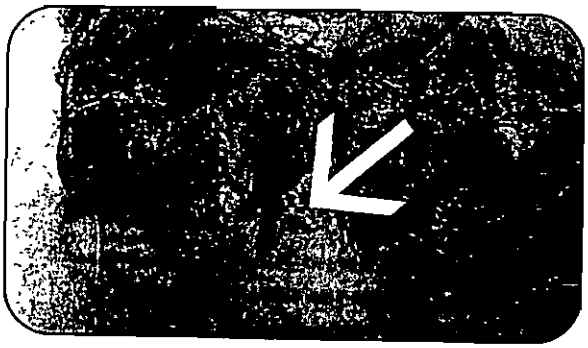
STREP SUIS



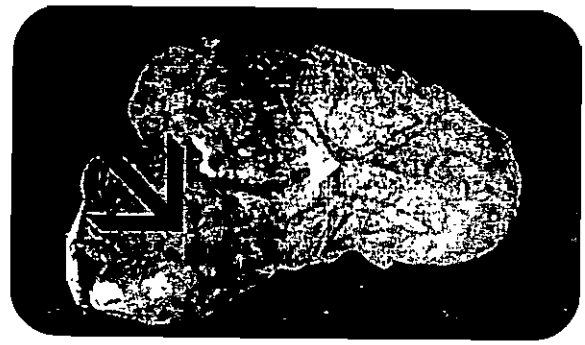
Joint Exudate



Fibrin Lesions on the Heart



Endocarditis



Hemorrhage on Brain

Clinical Signs/History:

- CNS/brain disease; down, paddling, dizzy, head tilt, convulsions, sudden death, arthritis with warm swollen joints, endocarditis (heart)

Stage of Production:

- Nursery, farrowing

Diagnosis:

Definitive diagnosis depends on isolation and identification of the causative organism. The disease can be confused with other streptococcal infections, other bacterial infections (such as Erysipelas, Salmonellosis, or acute Glässer's disease), water deprivation, or pseudorabies.

Lesions:

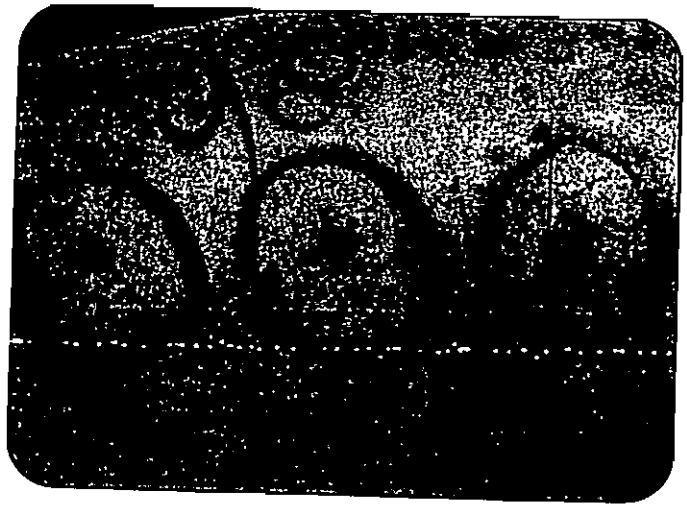
- The skin may be reddened in patches. Lymph nodes are often enlarged and congested, and fibrinous polyserositis is common. Joint capsules may be thickened and joints may contain excess clear or cloudy fluid. Affected lungs may show varying degrees of consolidation and fibrinopurulent bronchopneumonia.

DX Tests: Culture, Serotyping

Specimens To Submit:

- All organs (except stomach & intestine) - fresh and formalin-fixed
- Cerebrospinal fluid (CSF) - fresh brain/CSF swabs
- Brain - fresh and formalin-fixed

ERYSIPELAS



Skin Lesions

Clinical Signs/History:

- Acute septicemia, the skin (subacute) form, chronic arthritis and vegetative endocarditis may occur together or separately. Pigs with acute septicemia may die suddenly without previous signs. This occurs most frequently in finishing pigs weighing 100-200 lb. Acutely infected pigs are febrile (104°-108°F) walk stiffly and lie on their sternums separately rather than piling in groups. They squeal when handled and may shift weight from foot to foot when standing. Skin discoloration may vary from widespread erythema and purplish discoloration of the ears, snout and abdomen to diamond-shaped skin lesions almost anywhere on the body, but particularly the lateral and dorsal parts.

Stage of Production:

- Grow-finish

Diagnosis:

Acute Erysipelas is difficult to diagnose in pigs showing only fever, poor appetite, and listlessness. The typical diamond shaped skin lesions are diagnostic. Arthritis and endocarditis are difficult to diagnose in the live animal because other agents can cause similar syndromes.

Lesions:

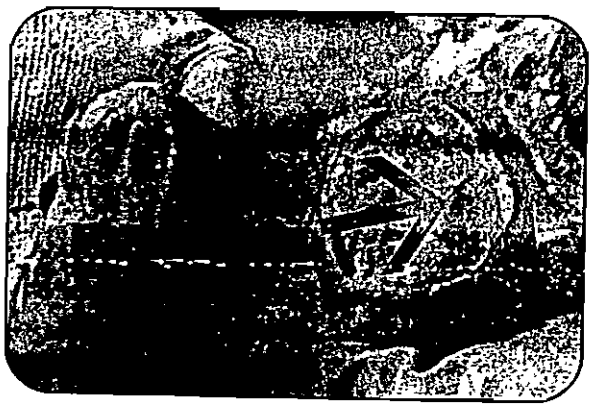
- In acute infection, in addition to skin lesions, lymph nodes are usually enlarged and congested, the spleen is swollen and the lungs are edematous and congested. Petechiae may be found in the kidneys, heart and occasionally elsewhere.

DX Tests: Culture

Specimens To Submit:

- Spleen, kidney, heart, joints - fresh
- Tonsils, lymph nodes - fresh

ULCERS



Ulcer

Blood in Intestine

Clinical Signs/History:

- Sudden death. In the acute form hemorrhage results in anorexia, weakness, anemia, and black tarry feces. Death can occur in hours or days. In the chronic form unthriftiness, anemia, and black tarry feces are characteristic.

Stage of Production:

- Grow-Finish

Diagnosis:

Appearance in a pen of one or two listless, anorectic pigs that show weight loss, anemia, dark feces, and sometimes dyspnea is suggestive of gastric ulceration, as is the sudden death of an apparently healthy pig.

Lesions:

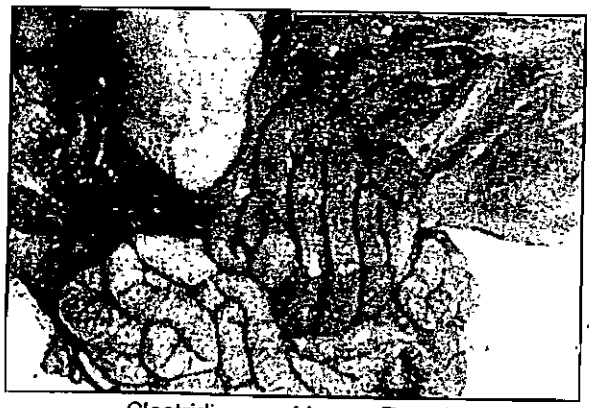
- The typical terminal ulcer lesion is found in the gastric mucosa near the esophageal opening (cardia) in the rectangular area of white, glistening, nonglandular, squamous epithelium. In acute hemorrhage, the stomach and upper small intestine contain dark blood.

DX Tests: Post-mortem examination

Specimens To Submit:

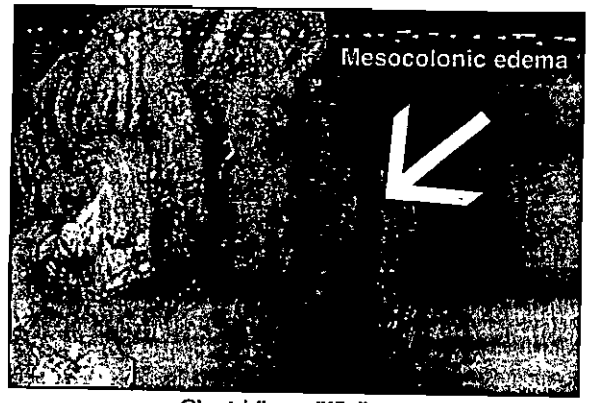
- Stomach - fresh

CLOSTRIDIUM



Clostridium perfringens Type A

Clostridium perfringens Type C



Clostridium difficile

Clinical Signs/History:

- Diarrhea is the sign most common to enteric clostridial infections. Sudden onset of hemorrhagic, diarrhea followed by collapse and death is characteristic in piglets 1-3 days old as a result of Clostridium perfringens Type C. Clostridium Type A and Clostridium dfficile most frequently cause diarrhea without hemorrhage in pigs 3-15 days of age.

Stage of Production:

- Farrowing, Nursery

Diagnosis:

Necropsy is usually sufficient to establish the diagnosis of Clostridium perfringens Type C in the peracute hemorrhagic form and in the acute form with jejunal emphysema. Histologic observation of villous necrosis with mucosal colonization by numerous large gram-positive rods is adequate for confirmation. Isolation and identification of the organism is necessary to diagnosis Clostridium perfringens Type A and Clostridium difficile.

Lesions:

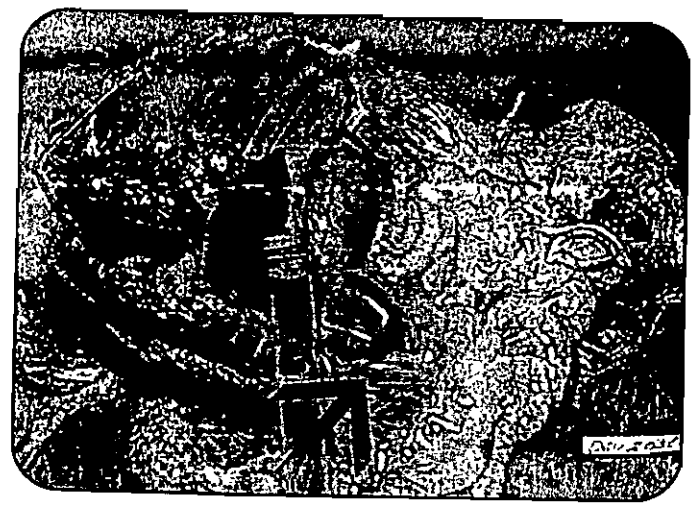
- Clostridium perfringens Type C - the small intestines are dark red, hemorrhagic, and filled with hemorrhagic liquid. Less acute cases at 3-5 days may have gas bubbles (emphysema) in the wall of the jejunum and necrosis of the mucosa of the jejunum and ileum.
- Clostridium Type A - lesions are much milder than seen with Clostridium perfringens Type C and are similar to those seen with E.coli.
- Clostridium difficile - mesocolonic edema can be seen in Clostridium difficile cases.

DX Tests: Culture and toxin PCR tests, histopathology

Specimens To Submit:

- Small and large intestine - fresh and formalin-fixed
- Fecal Swabs

E.COLI



Distension of the intestine
with yellowish, mucoid fluid

Clinical Signs/History:
- Diarrhea

Stage of Production:
- Farrowing, nursery

Diagnosis:

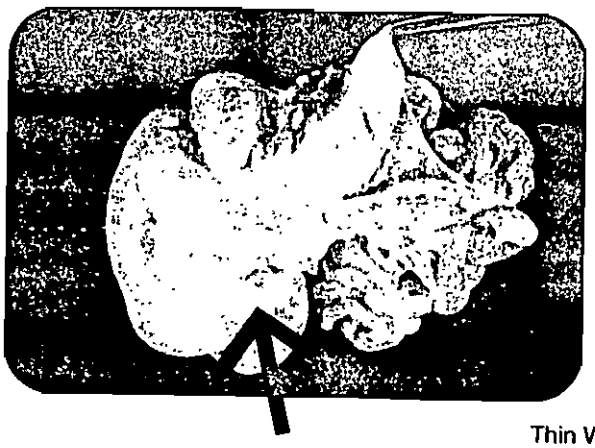
Confirmation is based on histologic observation of villous colonization and isolation of pathogenic E-coli.

Lesions:
- Dehydration and distention of the small intestine with yellowish, slightly mucoid fluid is characteristic. The colon contains similar fluid.

DX Tests: Culture, Histopathology, E.coli toxin and fimbriae PCR

Specimens To Submit:
- Small & large intestine - fresh and formalin-fixed
- Fecal swab

ROTAVIRUS



Thin Walled Intestine

Clinical Signs/History:

- Diarrhea
- Commonly, the infection is endemic in a herd. Sows have varying levels of antibody in the colostrum and milk which provides varying degrees of passive protection to nursery pigs. Diarrhea often begins in pigs 5 days to 3 weeks old, or immediately after weaning. Laboratory procedures are required for accurate diagnosis.

Stage of Production:

- Farrowing

Diagnosis:

Lesions:

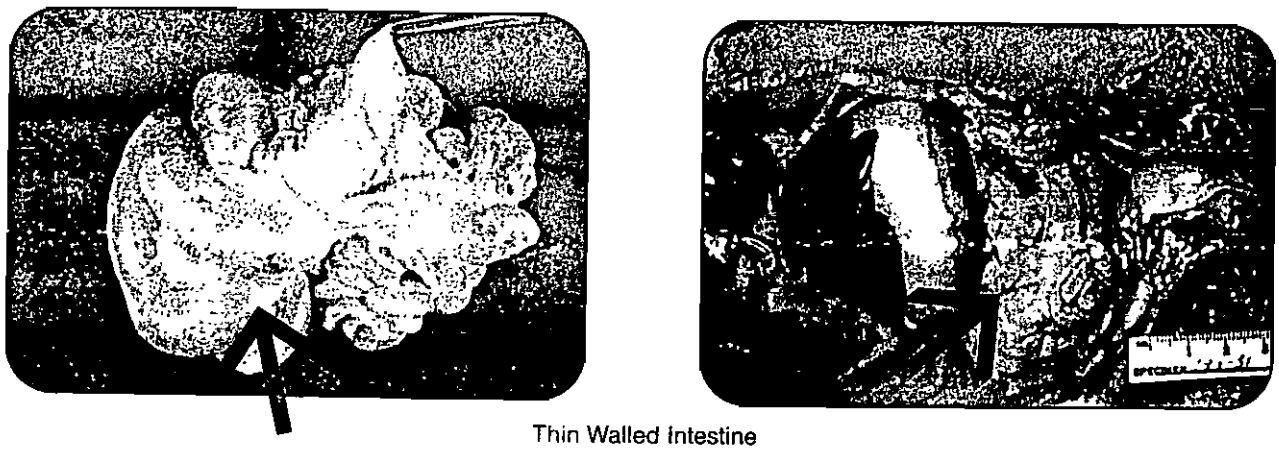
- The small intestine appears thin-walled and the cecum and colon contain liquid feces.

DX Tests: Histopathology, Rotavirus test

Specimens To Submit:

- Small and large intestine - fresh and formalin-fixed
- Fecal swab

ROTAVIRUS



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Stage of Production:

- Farrowing

Diagnosis:

Lesions:

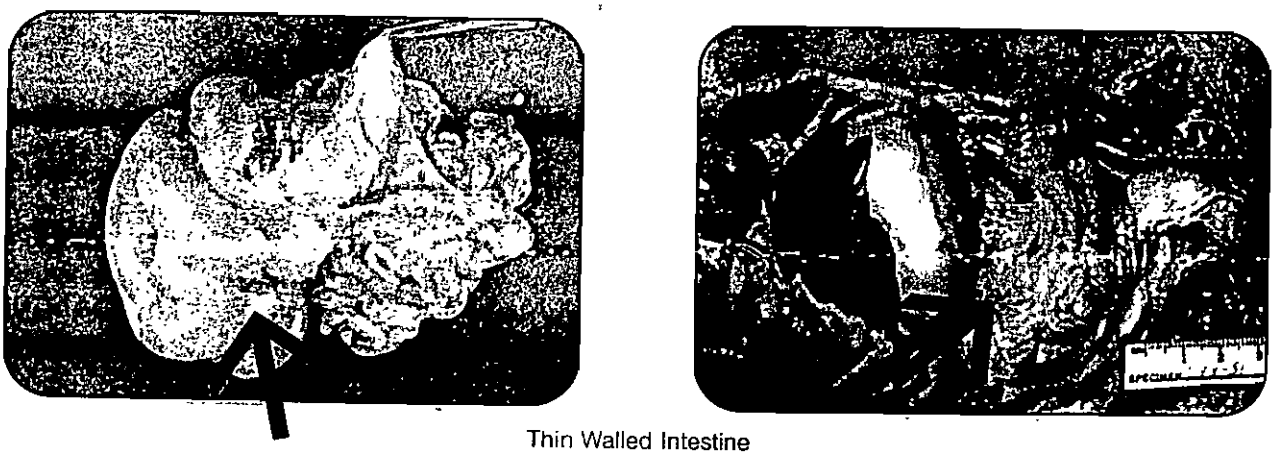
- The small intestine appears thin-walled and the cecum and colon contain liquid feces.

DX Tests: Histopathology, Rotavirus test

Specimens To Submit:

- Small and large intestine - fresh and formalin-fixed
- Fecal swab

ROTAVIRUS



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Stage of Production:

- Farrowing

Diagnosis:

Lesions:

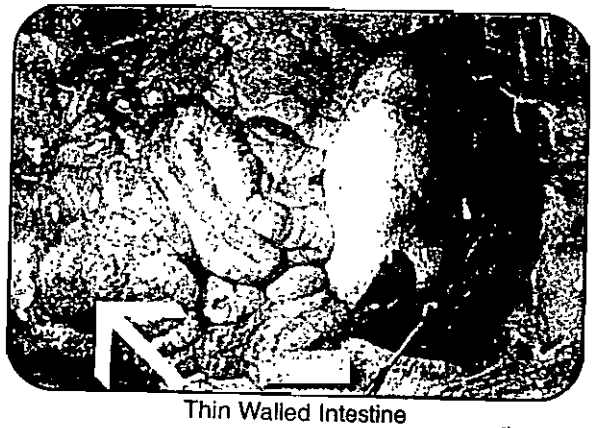
- The small intestine appears thin-walled and the cecum and colon contain liquid feces.

DX Tests: Histopathology, Rotavirus test

Specimens To Submit:

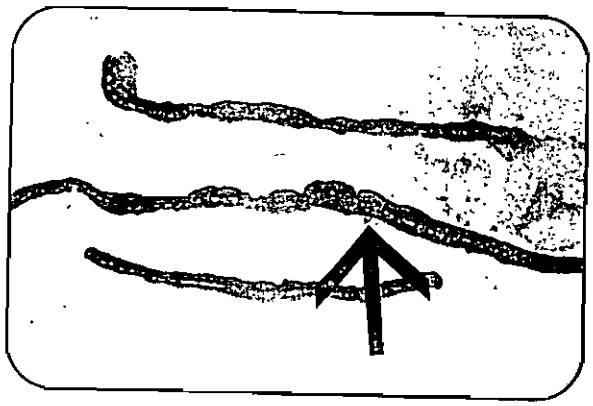
- Small and large intestine - fresh and formalin-fixed
- Fecal swab

TGE

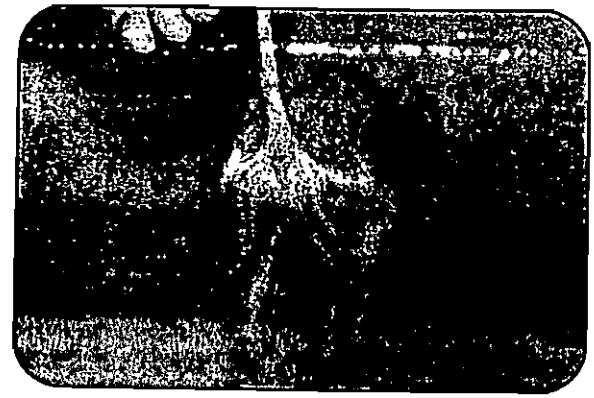


Thin Walled Intestine

Thin Walled Intestine



Thin Walled Intestine



Skin Covered with Liquid Feces

Clinical Signs/History:

- In susceptible herds, vomiting often is the initial sign, followed by profuse watery diarrhea, dehydration, and excessive thirst. Feces of nursing pigs often contain curds of undigested milk. Mortality is nearly 100% in piglets <1 week old, whereas pigs >1 month old seldom die. Gestating sows occasionally abort and lactating sows often exhibit vomiting, diarrhea and agalactia. Diarrhea in surviving nursing piglets continues for 5 days, but older pigs may be diarrheic for a shorter period.

Stage of Production:

- All

Diagnosis:

Clinical signs in the epidemic form of TGE usually provide a presumptive diagnosis. In the mild endemic form, laboratory procedures are required. Histologic and immunofluorescent examination of the small intestine to demonstrate typical lesions and the presence of TGE viral antigen provide confirmatory evidence.

Lesions:

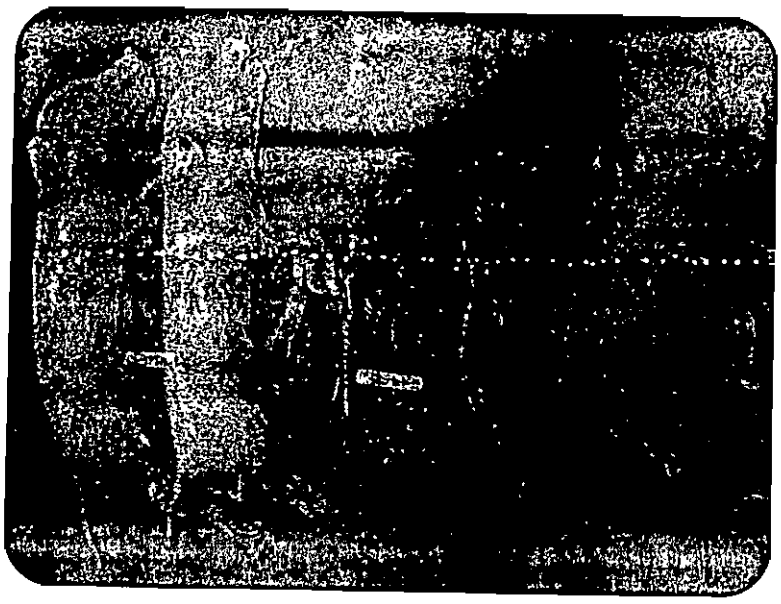
- Piglets dying of TGE are severely dehydrated and the skin is soiled with liquid feces. The stomach usually contains milk curd, but may be empty. The small intestine is thin walled, and the entire intestine contains greenish or yellow watery fluid and clumps of undigested milk.

DX Tests: Histopathology, Fluorescent Antibody (FA), Serology.

Specimens To Submit:

- Small and large intestine - fresh and formalin-fixed
- Fecal swab
- Serum

Reproductive Diseases: (PARVO/LEPTO, ETC)



Clinical Signs/History:
- Abortions, mummies, stillborns, weakborns

Stage of Production:
- Gestation, farrowing

Diagnosis:
- Porcine Parvovirus (PPV) is usually asymptomatic in adults. Sows infected before 70 days of gestation may have mummification or increased numbers of stillbirths. PPV is the most commonly identified cause of reproductive failure with associated mummification.
- Lepto can cause abortions occurring 2-4 weeks before farrowing and is the most common manifestation of leptospirosis in pigs.

Specimens To Submit:
- Aborted fetuses, mummies, stillborns, weakborns, sow and presuckle pig serum

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PCV2: Still so many questions! (Part 2)

By Robert Desrosiers

In Part 1 of this series, I mentioned that there appeared to be two main possibilities to explain why Quebec had suddenly experienced more PMWS problems lately: something new, other than PCV2, that would trigger the condition, or newer, more virulent isolates of PCV2. Although we did not have what is needed to conclude, it seemed that the first hypothesis was more likely because of a previous study conducted in our province in the past (Larochelle et al, Can J Vet Res, 2003, 67:114-120). In that study, isolates that were 100% homologous were found in herds with and without clinical signs of PMWS. However, recent work that has just been conducted in both Ontario and Quebec is making me wondering. This part of the series will look at possible differences between the virulence of PCV2 isolates.

Are there differences in the virulence of PCV2 isolates?

Five years ago a paper (Hamel et al, Can J Vet Res, 2000, 64:44-52) from Canada was reporting differences in the RFLP (Restriction Fragment Length Polymorphism) patterns of PCV2 isolates. Isolates could be included in one of 5 different patterns, called A, B, C, D and E, and 80% of the isolates belonged to pattern A. More recently, researchers (Wen et al, Vet Micro, 2005, 110:141-146) looked at 173 isolates from China and found that they belonged to 9 different RFLP patterns, called A to I. The nucleotide sequence identity ranged from 90.5 to 99.5%. Finding such genomic differences between isolates does not necessarily mean that they are of variable virulence, but let's just say that there are more chances of having virulence differences than if all isolates tested had been found identical in their genome.

Also recently, researchers in Belgium (Meerts et al, Arch Virol, 2005, 150:427-441) found that PCV2 isolates associated to PMWS or PDNS cases had different replication kinetics in PK15 cells, when compared to isolates found associated with abortion. This work suggests that on top of genetic differences, there could also be some biological differences between isolates of PCV2.

Similar to Quebec, Ontario has seen a sharp increase in the number of PMWS cases lately. In fact the number of cases identified in the Animal Health Laboratory went from about 25 in 2003, to approximately 60 in 2004 and to over 140 in the first 6 months of 2005 (Delay et al, 2005, Animal Health Laboratory Newsletter, 9:22). Concurrently with this increase in the number of cases, several additional lesions seem to be 'new' to Ontario pigs with PCV2 infections. Prominent pulmonary interlobular edema has been observed in many pigs. The same for thickening of the walls of ileum and colon, sometimes accompanied by mucosal erosion or ulceration, and reminiscent of porcine proliferative enteropathy (or ileitis) associated with *Lawsonia intracellularis*. In lymph nodes, histologic lesions of lymphoid depletion and histiocytic infiltration are reported to be more severe than previously seen. Very interestingly, this increase in the number and severity of cases seems to have occurred at the same time as a change in the PCV2 isolates found in these cases. While the predominant RFLP pattern (based on the use of 3 restriction enzymes on a 902bp portion of the genome) was a type 422 in 2004 and years before, it became a pattern 321 in 2005. When this 902bp portion of the genome was sequenced for 4 of these new type 321 viruses, they were found to have more than 99% sequence homology, and be only 91.6% similar to an older 422 isolate (Carman S, personal communication, 2005). Only one isolate tested had a 321 pattern in 2004, and 39 in the first 6 months of 2005. An obvious question is thus to ask if there is a connection between the recent increase in the number and

severity of cases in Ontario, and the fact that a different type of PCV2 isolates is now recovered from these cases.

Although preliminary and awaiting validation, some results from Quebec are also of great interest. The whole genome of 13 recent Quebec PCV2 isolates, obtained from cases with a history and histological lesions compatible with PMWS, was sequenced by researchers of the Faculté de Médecine Vétérinaire of the Université de Montréal and all isolates were found to be 99.9 to 100% homologous (Gagnon CA, personal communication, 2005). Although such a high level of homology seems odd, given the results that had been observed elsewhere and in Quebec in the past, this is apparently what was obtained. When compared to isolates that circulated in Quebec in the past, before significant problems were observed in the province, the homology found was only about 95-96%. When the RFLP pattern of recent Quebec isolates was deduced from the sequence, using the Ontario RFLP typing procedure, it was found to be the same (pattern 321) as the main one presently found in Ontario.

So if I summarize the recent Quebec\Ontario situation, we had a great increase in PMWS cases this year. In each province the isolates that are now isolated from sick pigs seem to be different than what they were before we had significant problems. Some of these recent isolates from each province were compared and found to be of the same 321 RFLP pattern. With all this, one can evidently be tempted to conclude that there must be a connection and that these new PCV2 isolates may simply be more virulent than the ones we had before. This is clearly a possibility, but what would be nice now is to have someone, somewhere, that could infect pigs experimentally with these newer isolates and with older ones, and see if virulence differences can be confirmed or not.

There is, to my knowledge, only one report (Hasslung et al, Vet Micro, 2005, 106:49-60) that compared the pathogenicity of different PCV2 isolates in pigs. In this paper an isolate of PCV2 obtained from a healthy pig, in a herd with no PMWS, in a country (Sweden) that remained 10 years without reports of PMWS after this isolate was obtained, was compared to a reference isolate from Canada. In general the pigs infected experimentally with the Swedish isolate showed more severe clinical signs of disease than pigs infected with the Canadian isolate, but pigs in both groups displayed gross and histological lesions consistent with PMWS. Because of the small number of animals involved and of possible differences obtained in this experiment compared to previous ones with the same isolates, the authors were cautious and concluded that the pathogenicity of the Swedish isolate was equal or higher than that of the Canadian isolate.

Dr. Gordon Allan and the Belfast team of researchers, in Northern Ireland, have used five different PCV2 isolates so far in their experimental model, including the Swedish isolate referred to above that was obtained from a healthy pig. All isolates tested up to now were capable of producing PMWS problems (Allan G, personal communication, 2005). This suggests that, under experimental infection conditions, many PCV2 isolates have the potential to produce PMWS problems. But more experimental work is needed to clarify whether or not some isolates may actually be more virulent than others, and if differences in isolates and virulence could at least partially explain outbreaks of PMWS in countries or areas which we know were positive to PCV2 way before these outbreaks occurred.

Just to complicate the picture a bit more, a recent study was conducted in a single Italian farm with PMWS. A total of 18 PCV2 isolates were recovered and sequenced. Nine were from PMWS-pigs and 9 from clinically healthy pigs. The sequences had a homology that varied from about 92 to 100%. Furthermore, there was no consistent homology of viruses in diseased animals (Allan G, personal communication, 2005). So there was way more variation found between isolates recovered from that same herd, than from isolates recently recovered from different herds of Quebec. Weird, I know, but oh so interesting.

What is my personal opinion on all that? Are there virulence differences between PCV2 isolates? Well, I certainly can't be sure at this point. But the list of swine pathogens for which virulence differences between isolates have been found is so long, and the list of swine pathogens for which these differences have not **yet** been found is so short, that the odds for now are in favor of virulence differences between PCV2 isolates.

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PCV2 : Still so many questions (Part 5)

By Robert Desrosiers

Apart from PMWS, to what other conditions has PCV2 been associated so far? (Continuation)

It has been reported that PCV2 could be responsible for some **sow reproductive problems** (e.g. abortions, mummified fetuses, stillbirths, sows not-in-pig). In many cases hearts from stillborn and/or neonatal pigs had severe lesions (non suppurative and necrotizing myocarditis) in which abundant PCV2 could be identified. But the early indications were that this was not frequent and was occurring mainly in gilts in startup herds (Sanford, IPVS 2002, Vol 1, 171). However, recently Kim et al (Vet Record, 2004, 155:489-492) detected PCV2 in 13.1% of aborted fetuses and stillbirth pigs from Korea. Similarly Ritzmann et al (Dtsch Tierarztl Wochenschr, 2005, 112:348-351) identified PCV2 in 27.1% of samples examined from aborted fetuses, mummified fetuses, stillborn and non viable neonatal piglets from Germany. Closer to us, Farnham et al (Can J Vet Res, 2003, 67:108-113) looked at stillborn fetuses from three US herds with prolonged history of reproductive problems. Twenty eight of 171 sera were positive for the presence of antibodies to PCV2. Of these 28 positive samples, 13 were also positive by PCR and it was possible to isolate the virus from 9 of them. Experimentally, Park et al (J Comp Path, 2005, 132:139-144) have shown that all seronegative sows infected intranasally with PCV2 three weeks before expected farrowing showed abortion and premature farrowing. The six infected sows delivered a total of 65 stillborn and 10 liveborn piglets. The virus could be found in the tissues of piglets and the authors concluded that the virus was capable of crossing the placenta and inducing reproductive failure. As described in Part 3 of this series reproductive troubles were caused when negative sows were inoculated with PCV2 in the uterus two minutes after insemination. Other studies suggested that PCV2 may play only a minor role in sow reproductive problems. Maldonado et al (Vet J, 2005, 169:454-456) were able to identify the virus in only one of 100 cases of aborted fetuses and stillborn pigs from Spain. In the same vein, Bogdan et al (Can Vet J, 2001, 42:548-550) could not detect PCV2 in any of the submitted tissues from 36 cases of abortion or reproductive failure in Saskatchewan and Alberta between 1995 and 1998. Since different studies are delivering different messages, we need to learn more on this particular issue before we can conclude on its relative importance in the field.

Recently, Opriessnig et al (J Swine Health Prod, 2006, 14:42-45) described a case where PCV2 virus was identified in the reproductive tissues and semen of a **boar with poor fertility.** The boar was also infected with *Mycoplasma hyopneumoniae* and *Pasteurella multocida* type D, and it was suggested that the boar may have become infected with PCV2 relatively late in life, and that the clinical signs observed before the autopsy was performed (fever, rapid breathing) were probably due to the concurrent infection with these pathogens.

Candotti et al (ssDNA Viruses of Plants, Birds, Pigs and Primates, Sept 2001, Saint-Malo, 90) reported cases of **necrotizing tracheitis** (inflammation of the trachea) in 70-80 kg pigs from Italy dying following an acute respiratory condition. The trachea showed a very reduced lumen and the only pathogen detected in the lesions, by PCR and immunohistochemistry, was PCV2.

Jolie et al (IPVS 2000, 639) described a condition in 9 week old caesarean derived and colostrum

deprived pigs that showed depression, **stiffness and reluctance to move**. The pigs did not respond to antibiotic therapy, and the mortality rate rapidly increased. Some of the pigs were yellowish at the time of necropsy. Many of the pigs had gastric ulcers, but the microscopic examination revealed that the pigs had lesions in, among others, the liver and muscles. These muscular lesions varied from **subacute to acute and severe degeneration or necrosis of skeletal muscles,** and to a lesser extent the myocardium (heart). Several tissue sections were tested for porcine circovirus by immunohistochemistry and found positive. Severe lesions to the skeletal muscles as noted in these pigs and associated with this organism had seemingly not been reported before.

As mentioned above, **heart lesions** associated to PCV2 have been observed in stillborn and neonatal pigs from herds with reproductive problems. Recently Opriessnig et al (J Comp Path, in press) described heart lesions in which abundant PCV2 could be identified in 4 to 7 week old pigs from herds with a history of sudden deaths. So heart lesions associated to PCV2 are not limited to stillborn or neonatal pigs. The authors also reported lesions to arteries in a 6 week old naturally infected pig, and in 13 and 14 week old pigs which had been experimentally infected. Their findings suggested that the cardiovascular system and endothelial cells in particular may play an important role in the pathogenesis of PCV2-associated diseases.

Harms et al (AAVLD 1999, 4) described an outbreak of acute disease in five of 26 **five week old, caesarean derived/colostrum deprived pigs**. Affected pigs developed jaundice and died within the next 3-5 days. There was **mild to massive necrosis of liver cells**, as well as moderate to marked lymphoid depletion in lymphoid tissues. Abundant PCV2 antigen was demonstrated by immunohistochemistry in lymphoid tissues, and small to large amounts were also present in the liver.

Drolet et al (Vet Rec, 2002, 150:139-143) looked at the **kidneys of pigs at slaughter** that were either normal, or had **a few to numerous white spots on them**, corresponding to lesions of **multifocal interstitial nephritis**. There was a significant association between the lesions and the presence of porcine parvovirus and PCV2 as detected by PCR. Kidneys that had both viruses detected were 22.7 times more likely to have these lesions. However, they could not be detected in the kidneys by immunohistochemistry and the authors concluded that the biological nature of the relationship between these two viruses and the lesions remained to be defined. Recently Spanish researchers (Martinez et al, Res Vet Sci, in press) looked at 29 'white spotted' and 15 normal kidneys from wasted pigs at slaughter. PCV2 nucleic acid was detected in only one kidney with lesions. The authors concluded that none of the studied agents (PCV2, parvovirus, PRRS virus, Leptospira spp., and bacteria) appeared to be specifically associated as being the potential cause of the renal lesions. Although the possible role of PCV2 in white spotted kidneys at slaughter remains to be elucidated, it should be mentioned that in pigs affected with PMWS in the field, the presence of white spots on the kidneys is certainly not constant, but when present it is one of the gross lesions that can be suggestive of the condition.

Kim et al (Vet Rec, 2005, 156:177-178) reported an association between PCV2 and lesions of **necrotizing lymphadenitis** (necrosis of lymph nodes) in 43 to 73 day old pigs, from seven herds. The virus was detected in the lymph nodes of affected pigs. The microscopic lesions observed in the necrotic lymph nodes were different from those seen in PMWS. Granulomatous inflammation and intracytoplasmic inclusion bodies, lesions considered characteristic of PMWS, were not observed in these pigs. According to the authors, clinical signs of PMWS were not seen in any of these herds.

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PCV2: Still so many questions! (Part 7)
By *Robert Desrosiers*

How can we control PMWS ? (Continuation)
In part 6 of this series I mentioned five points which, in my book and at this time, are among those that seem to offer the best chances of success or improvement when PMWS is a problem. I did not mention depopulation and repopulation, because it constitutes an option for virtually all diseases, but since it is a question that is frequently asked, I will say a few words about it. Hassing et al (IPVS 2004, Vol 1, 13) reported that of six Danish herds that were depopulated, cleaned, disinfected and left emptied for 3-4 weeks, then repopulated with animals from herds without PMWS, five got rid of the problem. In the last herd it reappeared about three months after the program, but in that case the supplier of pigs was the same as before the depopulation/repopulation. Gresham et al (Vet Record, 2003, 153:400-403) also reported that PMWS had not recurred in three farms after complete depopulation and re-stocking with pigs from unaffected farms. Thus successes in the control of PMWS have been obtained in the past with depopulation/repopulation, and it could constitute an alternative. It should be reminded though that since we still don't fully understand the epidemiology of that condition, and the ways by which it can become a problem, caution should be exercised when deciding to take a costly decision such as depopulation and repopulation. We had very few herds in Quebec with significant PMWS problems before late 2004, we have many since early 2005 and we're not sure yet why.

In this part of the series, we will look at why I believe that **genetics** can have a significant impact on the occurrence of this disease, and on the losses associated with it.

Let's start with something that seems quite straightforward. A herd has persistent problems with PMWS in the Landrace breed, but not in the Large White animals. Since these animals are in the same herd and buildings, environmental, management, nutritional and microbial factors are the same, so genetics appears to play a role in this particular case. In a paper by Harding et al (AD Leman Conf, 1999, 252-254) the authors mentioned that long and lean pigs of a Landrace-type conformation appeared to be more affected. Recently, Opriessnig et al (IPVS 2004, Vol 1, 12) experimentally infected Duroc (23), Landrace (19) and Large White (21) pigs with PCV2 at 5-7 weeks of age. One Landrace pig developed PMWS and two others had characteristic gross lesions of the condition. No pigs of the other two breeds showed clinical signs or gross lesions of PMWS. The three Landrace pigs with gross lesions also had severe microscopic lesions considered typical of this disease (lymphoid depletion, granulomatous inflammation). The authors concluded their results suggested that the Landrace pigs were predisposed to PCV2-associated lymphoid depletion and PMWS. This, however, does not necessarily mean that all Landrace pigs on the planet will show a predisposition to this disease. It could be that only certain lines of Landrace, or of other breeds, may be more susceptible to this condition than others.

On the opposite, it could also be that animals of some breeds or genetic lines might be more resistant than others to PMWS. For example, a lot of discussions are presently taking place

both of these definitions, a single animal does not constitute an outbreak of PMWS. In one suspect case in Australia only a single animal that had histological lesions and abundant PCV2 was identified and the herd was cleared of any diagnosis of PMWS.

As the number of PCV2 associated diseases increases, the difficulty of making a diagnosis will only increase. The recent outbreaks in Quebec and North Carolina highlight problems with control. Attempts to stamp out PMWS in the Auckland area were supported by the industry but not the government. The eradication effort was nearly complete when what appears to be a second incursion of the disease was recently detected in the South Island. In Australia, it is unlikely that governments would financially support a stamping out approach. The syndrome is not listed by OIE as an exotic disease and under the Australian Cost Sharing Deed of Agreement would not be eligible for government funding should the industry attempt or want eradication.

PMWS or PCV2 associated diseases are listed as notifiable diseases in all Australian states or territories. This doesn't mean that actions will automatically be taken to control or eradicate the condition. Any control or eradication efforts will be driven by the pork industry and it may well be that the industry will choose not to undertake a stamping out effort. The use of vaccines will be a contentious issue for a couple of reasons. First of all, it would appear that the vaccines currently under development all are based on PCV2. If, in fact, Agent X is the definitive cause of PMWS, then the vaccines may not be effective. If, however, the vaccines prevent the clinical signs that could be attributed to the effect of PCV2 even if Agent X is involved then they may have value. Accessing new vaccines is a tedious and time consuming process within Australia and the disease may have become endemic before the vaccines are permitted to enter Australia. Part of the Australian registration process requires that local efficacy data be generated to support the registration process. While this is not strictly correct the reality is that local data will be required.

In summary, the confirmed outbreaks in New Zealand and suspect cases in Australia have raised several issues. For New Zealand the suspected role of garbage feeding highlights the need for strict control and enforcement measures. For two separate outbreaks to be potentially linked to garbage feeding is an indictment of the regulatory processes. In Australia the difficulties associated with the lack of a definitive cause of PMWS and a diagnostic test means that the diagnosis is based on the interpretation of clinical findings. Histological findings of typical lesions, especially the presence of botryoid inclusions and abundant PCV2 antigen are less open to interpretation. Whether or not PMWS will be diagnosed in Australia is a matter of conjecture at this time.

Robert Desrosiers:
First I must thank Bill for his interesting piece. It's nice to hear about what's going on 'down under'. His contribution also opens the door to a big question: Is it PCV2 or Agent X? There are three main reasons why, in my opinion, PCV2 can hardly be dissociated from PMWS. The first one is that at least seven different teams of researchers, from four different countries, have been able to experimentally reproduce clinical signs, characteristic PMWS histological lesions, and mortality, using PCV2 alone. The second reason is that at least 19 different studies, from nine different countries have reported a direct correlation between the quantity of PCV2 found in the blood and tissues, and the severity of PMWS. The more PCV2, the more problems. Finally, the results obtained so far with a vaccine that contains only a PCV2 antigen suggest that there are many situations where it has a positive impact. Now don't get me wrong, this does not mean that agents X, Y or Z cannot or are not playing any role in field situations. But for the reasons mentioned above to totally exclude PCV2 from the equation and consider Agent X as the sole cause of this condition would not, at this time, seem logical to me.

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PMWS in the Antipodes

By William Hall

Until recently, PMWS was not a syndrome that was present in either New Zealand or Australia. However, in the last 2 years, New Zealand has experienced 2 outbreaks; the most recent being in the last 6 months in the Christchurch region on the South Island and involves a number of outdoor herds. Australia, on the other hand, remains the only major pig producing country that claims freedom from PMWS. This has not been without challenge, as in 2005 two suspect cases were investigated in the states of New South Wales and South Australia. Currently Australia and New Zealand import pork products from PMWS affected countries viz; Denmark, Canada and the USA. Following the completion of an Import Risk Assessment (IRA) by BioSecurity Australia, the US was added to the list of countries able to export pork to Australia.

Pork may be exported to Australia provided that major peripheral lymph nodes are removed; the product is de-boned, and does not come from the neck region of the pig. Upon arrival in Australia the product must be cooked to a minimum temperature of 70C for a minimum of 11 minutes (or a combination of a lower temperature and a longer time). This is the protocol for the inactivation of PRRS virus and these temperature and time combinations do not inactivate PCV2 virus. Preliminary research conducted in Australia would suggest the temperature required for inactivation of PCV2 is much higher. This may seem an insignificant observation, but if PCV2 is the true cause of PMWS then it has significance for the continued importation of pork into Australia. If, however, there is an Agent X that is the sole cause of PMWS, as postulated by a number of researchers throughout the world, it may be inactivated by the above cooking protocols.

The evidence for Agent X is based on the widespread occurrence of PMWS in countries where PCV2 is endemic, for example Denmark, the United Kingdom, Quebec and most recently North Carolina. PCV2 is widespread in New Zealand and Australia. Both countries are free of PRRS and Swine Influenza but parvovirus and *Mycoplasma hyopneumoniae* are widespread. At the International Conference on Circoviruses held in Belfast in September 2005, Dr-Gordon Allan identified a number of other viruses found in cases and non-cases of PMWS and concluded that these viruses were not Agent X.

The outbreaks in the Auckland area of New Zealand are thought to be associated with the lapse of regulations governing the feeding of garbage between 1998 and 2001. The more recent outbreak may also be associated with garbage feeding. Garbage feeding is not permitted in any state in Australia.

The lack of a definitive cause for PMWS means that a case definition is by necessity vague. Initially the Sorden criteria (*Swine Health Prod* 8 (3):133-136) were used in New Zealand to identify the first cases; however the need for a herd definition soon became obvious and a definition that included the presence of clinical signs, changes in mortality rates, typical histopathological findings, and the presence of abundant PCV2 antigen was developed. This definition formed the basis of the Australian case definition. It is important to note that under

severity of cases in Ontario, and the fact that a different type of PCV2 isolates is now recovered from these cases.

Although preliminary and awaiting validation, some results from Quebec are also of great interest. The whole genome of 13 recent Quebec PCV2 isolates, obtained from cases with a history and histological lesions compatible with PMWS, was sequenced by researchers of the Faculté de Médecine Vétérinaire of the Université de Montréal and all isolates were found to be 99.9 to 100% homologous (Gagnon CA, personal communication, 2005). Although such a high level of homology seems odd, given the results that had been observed elsewhere and in Quebec in the past, this is apparently what was obtained. When compared to isolates that circulated in Quebec in the past, before significant problems were observed in the province, the homology found was only about 95-96%. When the RFLP pattern of recent Quebec isolates was deduced from the sequence, using the Ontario RFLP typing procedure, it was found to be the same (pattern 321) as the main one presently found in Ontario.

So if I summarize the recent Quebec\Ontario situation, we had a great increase in PMWS cases this year. In each province the isolates that are now isolated from sick pigs seem to be different than what they were before we had significant problems. Some of these recent isolates from each province were compared and found to be of the same 321 RFLP pattern. With all this, one can evidently be tempted to conclude that there must be a connection and that these new PCV2 isolates may simply be more virulent than the ones we had before. This is clearly a possibility, but what would be nice now is to have someone, somewhere, that could infect pigs experimentally with these newer isolates and with older ones, and see if virulence differences can be confirmed or not.

There is, to my knowledge, only one report (Hasslung et al, Vet Micro, 2005, 106:49-60) that compared the pathogenicity of different PCV2 isolates in pigs. In this paper an isolate of PCV2 obtained from a healthy pig, in a herd with no PMWS, in a country (Sweden) that remained 10 years without reports of PMWS after this isolate was obtained, was compared to a reference isolate from Canada. In general the pigs infected experimentally with the Swedish isolate showed more severe clinical signs of disease than pigs infected with the Canadian isolate, but pigs in both groups displayed gross and histological lesions consistent with PMWS. Because of the small number of animals involved and of possible differences obtained in this experiment compared to previous ones with the same isolates, the authors were cautious and concluded that the pathogenicity of the Swedish isolate was equal or higher than that of the Canadian isolate.

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Economic losses associated with post weaning multi-systemic wasting syndrome

Joseph F. Connor, DVM

PCV2 is a common virus identified in tissues and serum submitted from herds. Clark and Harding first identified PMWS in a Western Canadian herd in 1991. In a retrospective study Sorden et. al. identified tissues from pigs in the United State consistent with PMWS in September 1993. (Figure 1) Clinical expression of the agent has been defined in PMWS (post-weaning multi-systemic wasting syndrome), respiratory disease, reproductive disorders, enteritis, and porcine dermatitis and nephropathy syndrome (PDNS). In herds we service, all these clinical expressions occur, but most commonly the identification of PCV2 is an incidental finding and not the primary reason for the original submission. PCV2 appears to be not the only component in PMWS.

Figure 1. Trend in types of PCV2 – associated diseases in field cases submitted to the ISU-VDL[6]

	2000	2001	2002	2003	2004	Jan – July 2005
Pneumonia	404	379	557	407	343	155
PMWS	209	255	346	283	224	181
Systemic infections	49	94	179	129	113	86
Enteritis	2	11	25	23	21	14
Abortion	1	10	9	3	2	1
PDNS	7	8	12	7	16	12

This is case definition currently utilized for a positive herd diagnosis of PMWS.

Clinical Criteria

To satisfy the clinical criteria a herd must have an on-site investigation by a veterinarian and the veterinarian must have determined there is current or historical evidence of the following:

- A herd syndrome characterized by wasting and ill thrift in 6-18 week-old pigs which is non-responsive to treatment and a fatality rate of >50% in affected pigs.
  AND
- Group mortality that satisfies at least one of the following criteria:
  - Acute phase mortality associated with non-responsive wasting in 6-18 week old pigs >15% or at least twice the pre-acute phase mortality for more than one month duration
    OR
  - In the post-acute phase, continued non-responsive wasting resulting in elevated mortality that is at least 3% higher than pre-acute phase levels in 6-18 week old pigs.

  AND
- Gross lesions that may include mottled, non-collapsed lungs and moderate to severe lymph node enlargement (lymphadenopathy).

Histopathological Criteria

To satisfy the histopathological criteria, tissues obtained from sampled pigs must be assessed by a standardized examination undertaken by the PIC-NA Reference Pathologist, Dr. Jim Collins at the University of Minnesota. The PIC-NA Reference Pathologist must have determined that the

* co-infections present in 97 to 95 % *

lymphoid tissues obtained from one or more pigs from the herd have evidence of depletion of lymphoid cells as well as one of the following lesions:

- Disseminated granulomatous inflammation in at least 3 of 10 separate tissues. (Tissues to submit for examination are spleen, thymus, ileum, lymph nodes (sternal, bronchial, inguinal and mesenteric) lung, kidney, liver and tonsil.)

OR

- A strongly positive (3+ or higher on a scale of 1-4) IHC for PCV2 antigen associated with the lymphoid depletion lesion or botryoid basophilic inclusion bodies consistent with PVC2 in H&E stained sections.[7]

PMWS is typically a history of wasting and in our cases almost always concurrent with porcine respiratory and reproductive syndrome virus (PRRSV), SIV, and/or *Mycoplasma hyopneumoniae*, and bacteria. Major clinical signs are wasting, dyspnea, icterus, and anemia. In an Ontario Study, the clinical signs observed on farms visited showed a prevalence of 0.41% jaundice, 3.17% pale, 4.85% wasting, 5.91% diarrhea, 1.60% coughing. (N=10,260 nursery pigs) Herds with an epidemic of PMWS showed these prevalences: 2.23% - jaundice, 6.39% - pale, 12.94% - wasting, 7.78% - dyspnea, 15.18% - diarrhea, and 1.31% - coughing.[1] Most commonly, the clinical cases have been associated with low mortality and morbidity. However, there is an occasional occurrence in which mortality has been above 25% and morbidity above 50% in an individual population of pigs in a barn or site. In several of these cases we have changed the pig flow in an attempt to eliminate PRRSV or pigs have been commingled from multiple sources with undoubtedly varying PRRSV status. Typically in these cases, PCV2 and PRRSV are occurring concurrently in pigs 12 to 16 weeks of age. If the PCV2 antigen is associated with the characteristic lung lesion it is considered involved in the respiratory disease. In cases in which PRRSV elimination was unsuccessful, the populations usually return to the typical PRRSV infection in the pigs 6 to 10 weeks of age (nursery) and PCV2 infection at 12 to 14 weeks of age with mortality and morbidity returning to the herd's baseline. In cases with commingled pigs from multiple sources, emptying of the site or change of sources has been successful. It is my opinion that the cases of PCV2 decrease dramatically in PRRSV negative pigs and is only an occasional clinical finding.[2] Nearly 75% of the cases fall in the age range of 7-16 weeks of age.

PCV2 enteritis has been an infrequent finding in enteritis cases we have submitted. It is almost exclusively been in cases that resemble sub-acute or chromic ileitis. Frequently, PCV2 enteritis has been identified in the ileitis cases in which typical ileitis prevention and control is unsuccessful emphasizing that initial diagnostics of any clinical case can be valuable.

PCV2 associated with reproductive failure is intriguing and its actual role in failure remains cloudy at best. By serology, the majority of the farms we service are PCV2 positive[3] and thus reproductive failure or loses should occur only sporadically. In cases we investigated most thoroughly, the herds have been PRRSV negative and considered high health.[4] In these cases, there have been undiagnosed abortions, and increases in mummified fetuses. The diagnosis has been confirmed by demonstration of PCV2 antigen in myocardial lesions.

PCV2 associated with PDNS is rare in our practice. Of most interest is that I have seen this clinical syndrome sporadically in pigs since I entered practice, but was mistaken of the cause until PCV2 was identified and reported.

To date we have not identified PCV2 in boar semen, but have identified it from lungs of boars submitted with an acute and chronic death from several cases.

Classically, based on the original description of PMWS in western Canada in the early mid-1990s, postweaning losses increased fourfold or more on affected farms. Losses attributable to PMWS resolved

Joe · Currently see mortality 7-25% + morbidity 7-50% but recov pigs finish OK.
Conner) 2004 - 12 to 16 wks of age
→ see mort+mob settle back down to ½ of what it was (5½ to 6
Steve Henry: peaking mortality 7/8 PEDV into farm. Upped vax 2-103

in 14-16 months on many affected farms, continued at some level above historical losses, or have emerged or are emerging since then. Apparently, PMWS was not a significant problem in eastern Canada at that time (mid-1990s). Today, available reports indicate that classical PMWS is becoming an emerging significant disease problem in Ontario and Quebec. A (PMWS) scenario very similar to that in western Canada was initially reported in some parts of the European Union simultaneously with the emergence or recognition of PMWS in Canada, for example in Spain and northern France (Normandy). In other parts of the European Union, such as the "low countries," Scandinavia, and the United Kingdom, there was no apparent or reported problem with PMWS or PCVD. In the last few years, however, there have been "epidemics" of PMWS in the United Kingdom, Denmark, and Sweden. Since these epidemics were reported to follow a point source pattern of spread, in other words, the pattern of a more "conventional" infectious disease, it has been proposed by some that an unidentified "agent X," and not PCV2, is the actual cause of PMWS. Belgium apparently still has a very low prevalence and incidence of PCVD, for reasons unknown. In the United States, reports of PMWS and PCVD have been less frequent. Available epidemiological data, primarily from the group at Iowa State, suggest that expression of PCV2 infection has been more commonly associated with the porcine respiratory disease complex, rather than with PMWS. Another way to approach the question of apparent intercontinental differences in the prevalence, incidence, and expression of PCVD is to consider what factors aside form the biology of PCVDs could contribute to perceived differences. Certainly, underreporting of PCVD could lead to the belief that there are significant differences between these diseases in North America and the European Union. Several factors could contribute to underreporting of any disease, including failure to look (properly), bias due, perhaps in part, to the "not invented here syndrome," and decisions based on the cost/benefit ratio of obtaining, sometimes costly, definitive diagnoses in a "soft" pig market; in other words, factors related to human nature rather than the nature of disease.[8]

Costs d/t - mortality effect - F. Conn - txmt cost ($2 pig initially but 1x mt cost Jul: $5.00 before → after.

Veterinarians in Denmark report increases in mortality from 2-4% to 5-15%, markedly lower average daily gain, higher feed conversion, and a 50-100% increase in feed antibiotic usage with an outbreak of PMWS. batch system (every sow) appears to be beneficial.

Generally speaking, hog operations in North America are large, totally confined, and hog dense. In contrast, in the European Union, although large confinement operations certainly exist, there is a higher prevalence of smaller, "traditional" farms where pigs are allowed to root and wallow out of doors. These are obvious differences that could be, and have been, implicated as critical factors in disease expression. However, the almost simultaneous emergence of PMWS in western Canada (large operations) and Spain and northern France (with more small and mixed operations) suggests that size (of operation) does not necessarily matter. This is not to say that hog density and the myriad of factors related to that, such as the population dynamics of infectious co-factors of PCVD, are not critical in disease expression. But such hog-density-related issues on affected premises are probably not a continent-dependent factor. Other differences in management, such as diet, weaning practices, vaccinations, and antibiotic use and misuse, which are likely to be factors in expression of PCVD, are also unlikely to be purely a function of intercontinental differences.[8]

farrow 'n' move clinical (wks).

From the first isolation and molecular characterization of PCV2 and continuing until today, at least two observations have been consistent: (1) PCV2 is not a "new" virus, since it has been shown to be present since at least the mid-1980s, prior to the emergence of widespread PCVD; (2) As a group, PCV2s, isolated form clinically normal and diseased pigs, are very closely related group of agents with genetic homology usually ranging from 96 to >99%. These consistent and international observations do not, however, preclude the possibility of inter-isolate variability in virulence that could be present within the context of limited genetic diversity. The experience with parvoviruses in carnivores, which comprise agents with genomes similar in size and coding potential similar to that of PVCs, provides a clear biologic precedent for significant differences in biological behavior that are the result of very small changes in the genome. Nevertheless, since PCVDs have been experimentally reproduced with PCV2 isolates with

R/C. FDRS seen

apparently low or no virulence from countries that had not reported PCVD, it is unlikely that mutations in PCV2 fully explain apparent geographical differences in the prevalence and severity of PCVD. Moreover, although the role of mutations in PCV2 in the pathogenesis and differential expression of PCV2 infection remains to be fully elucidated, it would seem that intercontinental and consistent differences in virulence among PCV2 isolate would not explain real or perceived differences in PCVD on different continents.[8]

Control measures are based on the assumption that the herds we service are PCV2 positive. The typical measures include:

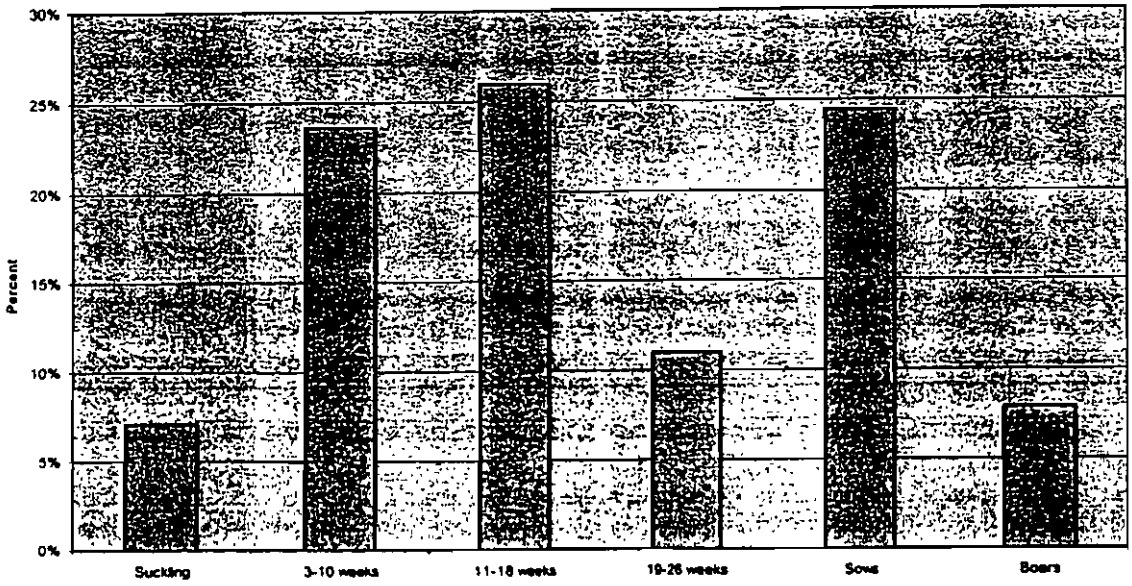
1. Minimize age spread in a population.
2. Minimize fallback pigs from farrowing.
3. Flow facilities all in/all out.
4. Allow facilities to dry and disinfect between populations. (Roccal-D+, Environ 1-Stroke)[5]
5. Reduce stocking densities.
6. Administer vaccines before infection if possible.
7. Manage the environment particularly humidity.
8. Maintain excellent hygiene.
9. Provide good nutrition.
10. Control concurrent infections.
11. Control secondary infections.
12. Maximize colostral intake.

For reproductive management of PCV2 in PRRSV negative herds:

1. Feedback mummified fetuses or weak piglets to developing gilts prior to breeding.
2. Grow the replacement gilts in close contact with the sow herd.

In summary, it is my opinion that PCV2 is a major component in PRDC and is particularly problematic with PRRSV, SIV, and *Mycoplasma*. PCV2 cause of reproduction failure occurs and may be a major factor of reproduction losses even in high health herds. Current control processes are helpful, but not completely successful.

Figure 2



Attachment 1

Case definition: PMWS

Circovirus associated disease: Post-weaning multisystemic wasting disease (PMWS)

Clinical description

PMWS is a condition of nursery and grower pigs characterized by the failure to grow at the same rate as penmates. Emaciation in affected pigs is progressive with failure to respond to antibiotic therapy. In addition, affected pigs may display dyspnea, which is also progressive and non-responsive to antibiotics.

Clinical case definition

An illness characterized by the following:

- Wasting, ill thrift, and failure to thrive in greater than 2% of pigs from six to eighteen weeks of age with or without other clinical signs. Commonly, anemia, icterus, progressive dyspnea and emaciation are observed. Gross lesions may include mottled, non-collapsed lungs and moderate to severe lymph node enlargement.

Laboratory criteria for diagnosis

- Detection of PCV2 by VI, PCR, and/or PCV2 antigen by immunohistochemistry and associated with the lesion (required).
- Depletion of lymphocytes from lymphoid tissues (required). Histiocytic inflammatory infiltration.

Case classification

Confirmed: a clinical compatible case that is laboratory confirmed

Comment

Laboratory testing should be performed or confirmed based in qualified laboratories. A respiratory syndrome, commonly known as Porcine Respiratory Disease Complex (PRDC), can cause similar clinical signs and may or may not meet the laboratory criteria for PMWS diagnosis.

PMWS/PCVD: DIAGNOSIS, DISEASE, AND CONTROL: WHAT DO WE KNOW?

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Introduction

Postweaning multisystemic wasting syndrome (PMWS) is now recognised as a global, epizootic disease that causes significant economic losses to pig producers. A "new" circovirus of pigs, porcine circovirus type 2 (PCV2) is now recognised as being the essential infectious agent of PMWS (1). However, in addition to PMWS, strong evidence from field and experimental studies has also linked PCV2 infections to reproductive problems in pigs and early (post weaning) and late porcine respiratory disease complex (PRDC), (2). PMWS is now seen as the most important clinical manifestation of a range of porcine circovirus diseases (PCVDs). The global explosion of PCVDs in the last 5 to 10 years has raised many questions regarding the source and nature of the disease epizootic. Retrospective testing of sera and tissue samples from pigs has shown that PCV2 infections occurred in pigs from at least 1969 (3) and sporadic cases of classical PCVD have now been identified in Spain and England from 1986 onwards (4).

PCV2 is not a "new" virus and PCVD is not a "new" disease. However, in contrast, field observations and epidemiological studies in the UK and Denmark strongly suggests that the spread of PCVD since 1999 has been consistent with the introduction of a "new" infectious agent into a naïve population. To date research into PCV2 and PCVD has provided some answers to specific questions. However, the results generated by different research groups have been equivocal. This short article will highlight some of the areas where results of research and field studies have generated more questions than answers.

PMWS: Diagnosis

The individual pig: We have to start somewhere! PMWS is now recognized as the major clinical manifestation of PCVD. The disease in individual animals of all age groups is characterized by clinical signs which can include growth retardation, dyspnoea, enlargement of inguinal lymph nodes, diarrhoea, and/or occasionally jaundice (2). However, not all of these clinical signs will be seen in all individual pigs affected with PMWS. At necropsy, the most frequent lesions seen are enlargement of lymph nodes and non-collapsed, tan-mottled lungs (5). The main histological lesions consist of a variable degree of lymphocyte depletion with loss of follicles together with histiocytic and multinucleate giant cell infiltration in the lymphoid tissues, and lymphohistiocytic inflammatory infiltrations in a wide range of tissues (5). The association of moderate to high amounts of PCV2 virus antigen and/or nucleic acid with these lesions is an essential criterion for the diagnosis (6). It has been suggested that the definition of a PMWS-affected pig should not include the inclusion of PCV2 association with lesions. It is difficult to understand why the presence of PCV2 associated with lesions should not be acceptable as an essential criterion for diagnosis of a PCVD, and, if this is not to be included, what diagnostic criteria should be used in determining if an individual pigs has PMWS/PCVD? Certainly thin or wasted pigs alone do not fulfil the criteria for PMWS/PCVD (7) and a constellation of histological lesions have been recorded in cases of PMWS/PCVD, all of which do not always appear in individual affected pigs.

The herd case: An attempt to clear muddy waters! The criteria for a herd diagnosis of PMWS/PCVD are still under debate. Essentially this debate revolves around the fact that some herds can have occasional individual deaths, which fulfil the criteria of PMWS outlined above. This situation is undoubtedly similar to what was seen prior to the global explosion of PMWS in the mid 1990s when individual cases of PMWS occurred on some farms and were misdiagnosed. In an attempt to clarify the definition of a herd case of PMWS, the EU multidisciplinary consortium currently working on PCVD (Control of Porcine Circovirus Diseases (PCVDs): Towards Improved Food Quality and Safety. [pcvd.org]) has proposed their definition of a PMWS herd case. An abridged version of this definition is presented below.

1) The occurrence of PMWS is characterised by an excessive increase in mortality and wasting post weaning compared with the historical level in the herd. There are two options (1a and 1b) for recognising this increase, of which 1a should be used whenever possible:

1a: If the mortality has been recorded in the herd, then the increase in mortality may be recognised in either of two ways:

Current mortality mean of historical levels in previous periods + 1.66 x SD2 or statistical testing of whether or not the mortality in the current period is higher than in the previous periods by the chi-square test. In this context, mortality is defined as the prevalence of dead pigs within a specific period of time. The current time period is typically one or two months. The historical reference period should be at least three months.

1b: If there are no records of the mortality in the herd, the following approach may be used: An increase in mortality exceeding the national or regional level by 50% is considered indicative of PMWS.

2) Pathological & histopathological diagnosis of PMWS: Autopsy should be performed on at least five pigs per herd. A herd is considered positive for PMWS when the pathological and histopathological findings, indicative for PMWS, are all present at the same time in at least one of the autopsied pigs. The pathological and histopathological findings are:
2.1 Clinical signs including growth retardation/wasting. Enlargement of inguinal lymph nodes, dyspnoea, diarrhoea and jaundice may be seen.
2.2 Presence of characteristic histopathological lesions in lymphoid tissues: Lymphocyte depletion together with histiocytic infiltration and/or inclusion bodies and/or giant cells.
2.3 Detection of PCV2 in moderate to massive quantity within the lesions in lymphoid tissues of affected pigs (detection in tissue by IHC or ISH).
This definition is not perfect and problems can be anticipated (defining the national/regional level of mortality and the necessity to have only 1 of 5 selected animals in a herd sample that fulfil the individual diagnostic criteria). However, it is the considered opinion of a team from North America and the EU who have had extensive field experience of diagnosing and working with PMWS and provides a platform for further debate. It should be noted that an increase of mortalities on a farm triggered by altered management practices that was reduced by changing the altered management practices and controlling the bacterial infections with antibiotics should *not form* a basis for declaring the farm or country free of PMWS. The use of these "criteria" to maintain PMWS freedom is to be discouraged.

PCVDs: Diagnosis
Although PMWS is currently considered the major disease presentation of PCV2 infection, a number of other disorders have been linked to infection with this virus and some of these should be considered under the umbrella of porcine circovirus diseases (PCVDs). PCV2 is now recognised as a causal agent of reproductive disorders in pigs (8). The case definition for PCV2-associated reproductive problems should include three main criteria:
1) Abortions and/or stillbirths and/or mummified foetuses
2) The presence of foetal heart lesions characterized by extensive fibrosing and/or narcotizing myocarditis
3) The presence of PCV2 in the myocardial lesions and other foetal tissues
PCV2 antigen has been demonstrated in abundance in lung lesions from pigs with proliferating and necrotising pneumonia (2), in tissues from sows with sow abortion and mortality syndrome (SAMS) (9) and PCV2 is also considered a contributor to porcine respiratory disease complex (10, 11). Recently PCV2 has been associated with acute respiratory disease in fattening pigs in the UK (Jake Waddilove: personal communication). It is currently not possible to definitively outline the role of PCV2 infection in some of these disease complexes as experimental reproduction of the diseases has not been carried out with an inoculum containing PCV2 virus. Porcine dermatitis and nephropathy syndrome (PDNS) is a disease that may affect nursery and growing pigs, and, sporadically, adult animals (12). An increased prevalence of PDNS has been reported in association with outbreaks of PCVD (13) which has led some workers to propose that PDNS should be recognised as a PCVD. However, to date, PDNS has not been reproduced experimentally following infection with PCV2 virus nor is PCV2 antigen consistently found in typical PDNS-histological lesions. Although many epidemiological studies and field observations suggest a link between the occurrence of PMWS and PDNS, it is still too early to class PDNS as a PCVD.

PCVD: The Disease
Although multiple attempts to experimentally reproduce PCVD have been published in the literature, to date, the disease progression of PCVD following experimental infection or natural infection in the field has not been fully elucidated. Indeed, very little is actually known about the pathogenic process of PCV2 infection in pigs leading to clinical PCVD.

Transmission of PCV2 infection and PCVD: Still a debate! The oro-nasal route is considered the most likely and frequent route of PCV2 transmission (14, 15, 16, 17, 18). Under commercial farm conditions, the majority of pigs seroconvert to PCV2 between 2 and 4 months of age (19, 20), indicating that horizontal transmission of PCV2 between pigs is very efficient.
Transmission of PCVD following co-mingling of affected pigs from a diseased farm with un-affected pigs from a non-diseased farm (21) has been demonstrated. A repeat of this experiment which included better controls has recently been completed with a low rate of disease transmission being recorded (P. Baekbo, personal communication), indicating that the disease is not highly contagious. Similar studies on co-mingling of pigs at our institute have confirmed these findings in that only one of ten in-contact animals developed disease (un-published observation). Although it has been proposed that PCVD can be introduced into a farm through feeding untreated swill containing "contaminated" pig products (22), to date, there is no scientific evidence that supports this proposal. It is not known if PCV2 infection and/or PCVD can be transmitted through feeding untreated products from PCVD-affected animals to pigs. Indeed, early attempts at experimental reproduction of PCVD using tissue homogenates from PCVD-affected animals consistently fail ed to reproduce clinical disease (15).
Reproductive disease associated with PCV2 infection has been described under field conditions (8, 23). Some authors were successful in infecting foetuses and in producing reproductive problems by intranasal inoculation of pregnant sows

or by intrauterine inoculation immediately after artificial insemination (24, 25, 26, 27). Transplacental transmission of PCV2 has been demonstrated following intranasal experimental infection of both seropositive and seronegative pregnant sows (26, 27).

PCVD: PCV2 replication and lymphoid depletion. Still a puzzle! Still unresolved is the identification of the main target cells in the pig that support PCV2 replication. The large amount of PCV2 found in lesions in macrophages and dendritic cells of diseased pigs appears to be the result of accumulation of viral particles (28, 29) and not the result of active virus replication in these cells. However, it is still possible that PCV2 replicates in a small, as yet unidentified sub-population of these cell types. A recent *in vitro* study (30) has reported the significant up-regulation of PCV2 replication in lipopolysaccharide (LPS) stimulated porcine alveolar macrophages (PAMs). This is in contrast to the results reported by others (28, 29, 31), and although a clear differences in susceptibility of PAMs from different pigs to PCV2 infection has been reported (31) the authors failed to demonstrate evidence of production of new infectious PCV2 virus in these studies. PCV2 replication has been demonstrated *in vitro* in porcine aortic endothelial cells, porcine gut epithelial cells and porcine fibrocytes (32).

Recent *in vitro* studies have identified that PCV2 enters cells of the porcine monocyte line 3D4/31 via clathrin-mediated endocytosis and requires an acid environment. Additionally it was demonstrated that PCV2 can use a heparin sulfate and chondroitin sulfate B glycosaminoglycan as a receptor for cell attachment (33). In contrast, it has also been reported (32) that PCV2 does not bind to heparin sulfate receptors on porcine-derived dendritic cells. Further studies on receptor sites for PCV2 on a range of primary cell types, derived from pigs, are required.

A number of experimental infection studies have been reported that have attempted to determine the primary sites of replication of the virus in the host and the disease progression of PCVD (14, 9, 34). In these studies PCV2 has been demonstrated in diseased pigs in a wide range of lymphoid tissues, liver, lung, myocytes, endothelial and epithelial cells. PCV2 antigen has been demonstrated in small numbers of porcine B and T lymphocytes in tissues from field cases of diseased pigs (35), however the presence of the virus in these cells types following experimental infection is not a common finding (14, 17, 34, 36). To date none of these studies have convincingly identified early replication sites for PCV2 in infected pigs nor have they elucidated the mechanisms for establishment of the primary lesions of lymphoid depletion and granulomatous inflammation infiltration of cells containing, but not replicating PCV2.

An ongoing study at Veterinary Sciences Division, Belfast is attempting to identify the early replication sites of PCV2 in experimentally infected pigs. In this study preliminary results indicate that PCV2 can be recovered on day 1 post infection (PI) from the small and large intestine, mesenteric lymph node, tonsil, bone marrow and nasal mucosa. At 3 days PI virus can be recovered from the large intestine, bronchial and mesenteric lymph nodes, nasal mucosa and trachea. From day 5 PI PCV2 was recovered from lungs, large intestine, nasal mucosa, bronchial mucosa, all 6 lymph nodes sampled and oesophagus of all sacrificed pigs. By day 14 PI, PCV2 was recovered from all tissue samples taken. Although others have reported PCV2 viremia (PCV2 DNA) at ÿ 3 days following experimental infection (37), in the current experiment infectious PCV2 was not recovered from serum or PBMCs until day 7 days PI. Further studies on these samples have yet to be completed.

The mechanism by which lymphoid depletion occurs in PMWS-affected animals remains to be elucidated. Potential mechanisms for a viral induced lymphoid depletion include a direct consequence of virus replication in immune cells or an indirect consequence of virus replication such as interference with antigen presentation, apoptosis induction, altered cytokine expression of immune cells or inhibition of the complement (38). However, PCV2 infection of lymphocytes has not yet been conclusively demonstrated, suggesting that lymphocyte depletion is more likely to be an indirect effect of PCV2 infection such as cytokine imbalance, apoptosis or alteration of migration pathways (39). Systemic lymphoid depletion following PCV2 infection has been attributed to apoptosis (39) who concluded that lymphocyte depletion with apoptotic death of B lymphocytes was caused by PCV2. However in a later experimental study in gnotobiotic pigs (40) and a study of field cases of PCVD (41) both groups concluded that apoptosis was not the primary mechanism of lymphoid depletion in PCVD. Recently (42) have reported that, following transfection of PK/15 cell cultures, the PCV2 protein coded for by ORF 3 is involved in PCV2-induced apoptosis by activating caspase-8 and caspase-3 pathways. Conversely (29) failed to demonstrate any apoptotic effects following infection *in vitro* of monocytic cells with PCV2 virus nor in lymphocytes co-cultured with PCV2-infected monocytes. The significance of apoptosis as a possible mechanism of lymphoid depletion in PCVD-affected pigs needs further study.

To date, studies on immune functions, specifically related to cytokine profiling, following PCV2 infection and progression towards PCVD have proven non-conclusive. Details of these studies will not be reviewed in this article but can be found elsewhere (29, 43, 44, 45, 46, 47, 48, 49, 50, 51). A recent study on cytokine profiles in blood samples from 50 pigs from 5 different litters on a PCVD-affected farm in Northern Ireland has shown no significant differences in levels of IL10, IFN gamma or IFN alpha in sequential blood samples taken over a seven week period from pigs that did or did not develop PCVD (52).

Clearly the results generated to date by different workers on possible mechanisms of lymphoid depletion in PCVD-affected pigs and cytokine profiling differ considerably and further controlled studies are required.

PCVD: Control

Control without PCV2 vaccination. Sometimes it works, sometimes it does not! Until recently effective control measures for PCVD have focused on the understanding of the co-factors and triggers involved on individual farms and the control or eradication of these triggers.

Management measures and the implementation of what is today known as the Madec' s 20-point plan has significantly decreased the percentage of mortality in some severely affected farms (53). These measures were designed to reduce "infection pressure" in regard to PCV2 and any other infections, improve hygiene and to reduce stress at the different production stages (54, 55). Significant positive results have been obtained when these measures are applied and significant improvement in loss rates are achieved when the rate of compliance with the recommended measures is higher (56). However these measures have not produced satisfactory results in all situations and their application in the field is sometimes difficult or unpractical depending on the system and existing buildings that have to be worked with.

The control of concurrent viral infections in the postweaning period has also been used in an attempt to decrease the incidence of PCVD. However, in this respect, attempts to control the clinical severity of PCVD in an experimental model by the use of PPV vaccination to protect young piglets from infection with this virus were unsuccessful (57). Although it is quite clear that co-infections with PRRS virus can make PCVD problems worse, to date, no published results are available on the control of PRRSV infection (by vaccination or other systems) to mitigate the effects of PCVD. However, it is known that experimental co-infection of pigs with PCV2 and a modified live PRRSV vaccine up-regulates PCV2 replication leading to more severe PCV2-associated histological lesions, when compared to PCV2 infection alone (58). Indeed because it has been demonstrated both experimentally and in the field that certain commercial vaccines can potentiate PCV2 replication, leading to disease, producers with PCVD-affected herds should consider determining the approximate timing of PCV2 infection, with the objective to re-schedule vaccination to minimize the disease (59).

Injection of PCV2 hyperimmune sera from slaughterhouse age pigs (serum therapy) in suckling or nursery pigs has been reported as successfully reducing mortality in several PCVD-affected farms (60, 61). The success of this procedure has been variable. The mode of action of serum-therapy has not been elucidated.

Field observations from farmers and veterinarians have suggested that certain genetic lines of pigs, specifically in relation to boar lines, are more or less susceptible to PCVD. This observation has been supported by recent experimental studies where Landrace pigs were experimentally shown to be more susceptible to develop PCVD lesions than Duroc and Large White pigs (62). Other studies have shown contradictory results with the use of Pietrain boar line; while the use of this genetic line did not seem to have any effect on the offspring in one study (63), another study showed lower general postweaning and PCVD-associated mortalities (64). Field trials on selected farms in Northern Ireland using different boar lines have also indicated a highly significant difference in mortalities due to PCVD between offspring. These findings with regard to the role of genetics in susceptibility/resistance to PCVD need to be expanded.

PCV2 vaccination. Hope for the future (at last)? PCVD is not usually observed in pigs younger than 4 weeks of age (5). This may be associated with protective maternal immunity as suggested by field and experimental studies (65, 66, 67). In contrast, other field studies have demonstrated that high levels of colostrum-derived serum antibodies to PCV2 had no significant protective effect against PCVD (68, 69) although a statistically significant relationship between the levels of colostrum-derived antibodies to PCV2 in young piglets and the time of appearance of clinical PCVD, with piglets with higher titres of maternally-derived antibodies developing disease later in life was demonstrated (69). The protective effect of maternal-derived passive immunity on PCVD development is supported by the fact that disease occurs once these titres have declined (19, 70), and as such, measures that increase maternal immunity may diminish PCVD impact on piglet mortality. An inactivated, adjuvanted PCV2 vaccine for use in sows and gilts that potentially offers protection from PCVD through passive transfer of PCV2 antibodies is now commercially available (Merial: Circovac). This vaccine has been shown experimentally and in field trials to reduce the incidence of PCVD on affected farms (71, 72) and the efficacy under extended field conditions is currently being elucidated. The vaccine is licensed for use in parts of France, Germany, Denmark and Canada and preliminary results and feedback from producers and field veterinarians are encouraging.

Other commercially available inactivated PCV2 vaccines (Fort Dodge and Intervet) are being produced for use in young pigs and are, or probably soon will be, available for use in North America and elsewhere. One of these vaccines (Fort Dodge; Suvaxyn PCV2-One Dose) is based on a chimeric infectious DNA clone containing the immunogenic ORF2 capsid gene of PCV2 cloned into the non-pathogenic PCV1 genetic backbone (73) and is designed for single dose use in 3-4 week old piglets. Several challenge studies done in Europe and the USA demonstrate that pigs vaccinated with Suvaxyn PCV2-One Dose prior to PCV2 infection show a significant decrease in viremia and histopathologic lesions vs. unvaccinated pigs. The vaccine was proven safe in USA safety trials including at least one site that was known to be PCV2 positive with gross clinical signs of PCVD (Johanne Elsener, personal communication). The PCV2 vaccine produced by Intervet is based on a baculovirus expressed PCV2 protein. To date, no information on efficacy or safety of these vaccines has been placed in the public domain, nor has it been made available for this article. However it should be noted that both these adjuvanted vaccines are to be used in young piglets and the proven potentiation of PCV2 replication following administration of some commercial vaccines to young pigs (74) should be evaluated with respect to these 2 products.

PCV2: Agent X, "genotypes", "strains" and PCVD

PCV2 virus isolates, genotypes and strains: More confusion and misnomers! Recent retrospective epidemiology studies the UK, Denmark and New Zealand have concluded that the outbreaks of PCVD in those countries were the results of an incursion of a "new" infectious agent (Agent X) into a naive population. Following the study in the UK, it has been proposed that Agent X (probably a virus) spread slowly through Britain and also spreads slowly once on a farm (75). The agent spread by pig to pig contact and survived in the environment to be spread by humans and/or wildlife. These authors further suggested that PCV2 virus is associated with PCVD but is probably not the cause and the "risk factors" associated with a herd breaking down with PCVD included purchasing replacement gilts, closeness (3 to 5 miles) to an affected farm and permitting visitors who were not 3 days pig-free onto the farm. Similar conclusions with respect to Agent X have been drawn following a retrospective study in Denmark (76, 77). In these studies the authors concluded that recent outbreak and spread of PCVD in Denmark was consistent with what would be expected from an incursion of a "new" virus or a new highly virulent strain of PCV2 into a naïve population. However, in contrast to the findings reported in the UK study (75) no risk association of disease breakdown was found in relation to the use of external AI, distance to other PCVD positive herds and number of pig herds within a zone with a radius of 3 km". A retrospective epidemiology study in New Zealand into an outbreak of wasting disease in pigs in backyard farms on the North island concluded that an "exotic" agent was introduced into their country, probably in the late 1990's in untreated pig swill, resulting in an outbreak of PCVD which was spread by contact of other farms with contaminated equipment and/or pigs. To date, it has not been determined if PCVD can be spread by feeding untreated meat products from PCVD-affected animals to pigs.

Although epidemiological studies in Brittany, France on the initial outbreak of PCVD in Europe concluded that with PCVD they did not observe any epidemic "wave" similar to what was seen in 1981 with swine influenza (H1N1) and ten years later when porcine reproductive and respiratory syndrome (PRRS) struck (Francois Madec, personal communication), certainly, in the case of the UK and Denmark the data generated from retrospective questionnaires would seem to give some support to the new Agent X hypothesis. However, the dilemma that still remains un-resolved between some epidemiological results and laboratory-based experimental studies is that clinical PCVD can be produced experimentally in colostrum-fed (78), colostrum-deprived (2) and gnotobiotic pigs (40) using PCV2 as the only infectious agent.

Recent studies in Sweden and Eastern Canada can perhaps help to square this circle. It would appear from genomic sequence data generated on PCV2 isolates from PCVD-affected and non-affected farms in Sweden that two distinct genotypes (Swedish genotypes 1 and 2) of PCV2 are now circulating in this country (79). It is of interest that, to date, Swedish genotype 1 has only been detected on farms without epizootic PCVD and Swedish genotype 2 predominates on farms (16 of 16 farms tested) with epizootic PCVD in Sweden. However Swedish genotype 2 virus has also been detected on 4 of 11 farms in Sweden without epizootic PMWS. Additionally, although recent experimental infection studies at our institute using Swedish genotype 2 virus did produce clinical PCVD in the inoculates the severity and extent of the disease produced was similar to that produced in the same model following experimental infection with Swedish genotype 1 virus (unpublished). Similarly, a dramatic increase in the number and severity of PCVD outbreaks in Quebec and Ontario has recently been reported. In Ontario this increase seems to have occurred at the same time as a "change" in the PCV2 isolates found in these cases. (80). Using RFLP typing, Canadian PCV2 isolates from recent cases of PCVD have been shown to be different (RFLP type 321) to PCV2 isolates found in previous years (RFLP type 422). It is not known however if these differences in RFLP patterns, which appear to fit chronologically with the onset of serious problems in that province, are truly significant in respect of diseases severity. To date, the genomic sequences of these new Canadian viruses have not been published, however genomic analysis of 4 isolates from diseased pigs from 4 different farms in Eastern Canada in our laboratory and in the laboratory of our Swedish colleagues have shown strong similarities to the Swedish genotype 2 viruses. The hypothesis that "virulent" isolates of PCV2 with distinct genotypes may exist and are associated with diseases outbreaks is not supported by other studies (81, 82). In both these studies, one in The Netherlands and one in Canada, the authors failed to recognise any consistent genomic differences in PCV2 viruses recovered from pigs with and without PCVD. Indeed (82) concluded from their study on over 70 isolates of PCV2 that viruses associated with PMWS were scattered throughout the phylogenetic tree, often in groupings including PCV2 viruses identified from cases other that PCVD such as, porcine reproductive and respiratory syndrome (PRRS), generalized tremors, porcine dermatitis and nephropathy syndrome (PDNS), arthritis, nervous signs, erysipelas and even healthy pigs. Nevertheless, recently it has been reported that when comparing the virulence of two different isolates in a colostrums-fed (CF) experimental model, one of them was found to be more virulent than the other (83). Although differences in virulence between PCV2 isolates might play a role in the variability and severity of clinical presentations associated with this organism, it is important that further controlled laboratory studies are carried out in different experimental models before definitive answers can be given to this question.

Subsequent to the clinical observations and genomic sequence data findings in Canada and the occurrence of new outbreaks of PCVD on the South Island of New Zealand it has been proposed by some workers that these "new" PCV2 viruses are different "strains" of PCV2. The premature classification of the "new" PCV2 viruses into strains should be discouraged until biological differences between these viruses and "old" PCV2 viruses have been clearly demonstrated. Certainly the use of the terminology "strains of PMWS" should be avoided. It is entirely possible that biologically distinct strains of PCV2 do exist, however, to date we do not have data to support the use of this terminology "strain" in relation to different PCV2 isolates. Currently we *do have* PCV2 isolates, we *may have* distinct and conserved PCV2

genotypes, which *may equate* to distinct PCV2 strains and this *may be* important in the disease progression and epidemiology of PCVD.

PCV2 and PCVD: What we do not know

The circus comes to town! In conclusion, it is clear that a lot of questions regarding the epidemiology, pathogenic processes and control of PCVD have not all been answered and controversy still even surrounds the criteria for diagnosis of these diseases, the "causal agent" and even nomenclature to be used in describing disease outbreaks (the AASV have now changed the name porcine circovirus diseases (PCVDs) to porcine circovirus-associated diseases (PCVAD), which seems to be a not unexpected re-invention of the wheel). Hopefully answers will be provided in the coming years by international, multidisciplinary collaborative research incorporating field veterinarians and producers and it is also hoped that the PCV2 vaccines currently appearing on the international market will go some way to alleviating the losses being incurred by producers around the world due to PCVDs. Some researchers and laboratory-based diagnosticians have suggested that PCVD is no longer a major disease concern for the global pig industry. This is an assumption that needs to be challenged. Although the official figures for laboratory diagnosed outbreaks of PMWS have declined, this does not necessarily mean a decline in the incidence of the disease in the field as field veterinarians and producers are now "self-diagnosing" the disease. Additionally, it is notable that when PCVD-positive farms have "recovered" chronic losses and flare ups of disease still occurs, leading to loss of production. If you are a farmer then residual mortality, especially in the mid fattening due to underlying PCV2 circulation and PCVD combined with an increase of secondary infections, an increased use of antibiotics with poor results and heterogeneity of the pig batches, runts and low value pigs are all severely impairing your profit margin. The requirement for multidisciplinary, focused and trans-national (and hopefully trans-Atlantic) research on PCV2 infections and PCVD's is as important as ever.

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References

1. Ellis, J., Hassard, L., Clark, E., Harding, J., Allan, G., Willson, P., Strokappe, J., Martin, K., McNeilly, F., Meehan, B., Todd, D., Haines, D. (1998) Isolation of circovirus from lesions of pigs with postweaning multisystemic wasting syndrome. Can Vet J, 39:44-51
2. Allan, G. M., Ellis, J A. (2000) Porcine circoviruses: a review. J Vet Diagn Invest, 12:3-14
3. Staebler, S., Sydler, T., Buergi, E., McCullough, K., McNeilly, F., Allan, G., and Pospischil, A. (2005) PMWS: an emerging disease identified in archived porcine tissues. Vet J, 2005. 170(1): p. 132-4
4. Rodriguez-Arrioja, G. M., Segales, J., Rosell, C., Rovira, A., Pujols, J., Plana-Duran, J., and Domingo, M. (2003). Retrospective study on porcine circovirus type 2 infection in pigs from 1985 to 1997 in Spain. J Vet Med B Infect Dis Vet Public Health, 2003. 50(2): p. 99-101.
5. Segalés, J., Domingo, M. (2002) Postweaning multisystemic wasting syndrome (PMWS) in pigs. A review. Vet Q, 24:109-124
6. Sorden, S. D. (2000) Update on porcine circovirus and postweaning multisystemic wasting syndrome. J Swine Health Prod, 8:133-136
7. Staebler, S., Buergi, A., Pospischil, A., McCullough, K., Allan, G., Sydler, T. (2004) Circovirus infections in Switerzerland. In Proc of the 18th IPVS Congress, Hamborg, P 63
8. West, K. H., Bystrom, J. M., Wojnarowicz, C., Shantz, N., Jacobson, M., Allan, M, Haines, D. M., Clark, E. G., Krakowka, S., McNeilly, F., Konoby, C., Martin, K., Ellis, J. A (1999) Myocarditis and abortion associated with intrauterine infection of sows with porcine circovirus 2. J Vet Diagn Invest, 11:530-532
9. Harms, P. A., Sorden, S. D., Halbur, P. G., Bolin, S., Lager, K., Morozov, I., Paul, P. S. (2002b) Experimental reproduction of severe disease in CD/CD pigs concurrently infected with type 2 porcine circovirus and PRRSV. Vet Pathol, 38:528-539
10. Thacker, B., Thacker, E. (2000) The PRDC battle continues. Pig Progress, June:16-18
11. Harms, P. A., Halbur, P. G., Sorden, S. D (2002a) Three cases of porcine respiratory disease complex associated with porcine circovirus type 2 infection. J Swine Health Prod, 10:33-38
12. Drolet, R., Thibault, S., D'Allaire, S., Thomson, J. R., Done, S. H. (1999) Porcine dermatitis and nephropathy syndrome (PDNS): An overview of the disease. J Swine Health Prod, 7:283-285
13. Gresham, A., Giles, N., Weaver, J. (2000) PMWS and porcine dermatitis nephropathy syndrome in Great Britain. Vet Rec, 147:115
14. Allan, G. M., Kennedy, S., McNeilly, F., Foster, J. C., Ellis, J. A., Krakowka, S. J., Meehan, B. M., Adair, B. M. (1999) Experimental reproduction of severe wasting disease by co-infection of pigs with porcine circovirus and porcine parvovirus. J Comp Pathol, 121:1-11

15. Balasch, M., Segalés, J., Rosell, C., Domingo, M., Mankertz, A., Urniza, A., Plana-Durán, J. (1999). Experimental inoculation of conventional pigs with tissue homogenates from pigs with post-weaning multisystemic wasting syndrome. J Comp Pathol, 121:139-148
16. Ellis, J., Krakowka, S., Lairmore, M., Haines, D., Bratanich, A., Clark, E., Allan, G., Konoby, C., Hassard, L., Meehan, B., Martin, K., Harding, J., Kennedy, S., McNeilly, F. (1999) Reproduction of lesions of postweaning multisystemic wasting syndrome in gnotobiotic piglets. J Vet Diagn Invest, 11:3-14
17. Krakowka, S, Ellis, J. A., Meehan, B., Kennedy, S., McNeilly, F., Allan, G. (2000) Viral wasting syndrome of swine: experimental reproduction of postweaning multisystemic wasting syndrome in gnotobiotic swine by coinfection with porcine circovirus 2 and porcine parvovirus. Vet Pathol, 37:254-263
18. Rovira, A., Balasch, M., Segalés, J., Garcia, L., Plana-Duran, J., Rosell, C., Ellerbrock, H., Mankertz, A., Domingo, M. (2002) Experimental inoculation of conventional pigs with porcine reproductive and respiratory syndrome virus and porcine circovirus 2. J Virol, 76:3232-3239
19. Larochelle, R., Magar, R., D'Allaire, S. (2003) Comparative serologic and virologic study of commercial swine herds with and without postweaning multisystemic wasting syndrome. Can J Vet Res, 67:114-120
20. Sibila, M., Calsamiglia, M., Segales, J., Blanchard, P., Badiella, L., Le Dimna, M., Jestin A., Domingo, M. (2004) Use of a polymerase chain reaction assay and an ELISA to monitor porcine circovirus type 2 infection in pigs from farms with and without postweaning multisystemic wasting syndrome. Am J Vet Res, 65:88-92
21. Kristensen, C. S., Bækbo, P., Bille-Hansen, V., Hassning A-G, Bøtner, A. (2004) Transmission of PMWS. In Proc of the 18th IPVS Congress, Hamburg, p. 77
22. Lawton, D. E., Morris, R. S., and King, C. M., (2004). Pmws IN New Zealand Part 2: Epidemiological evidence for a novel agent. In Proc Congr Int Pig Vet Soc. P 128.
23. O'connor, B., Gauvreau, H., West, K., Bogdan, J, Ayroud, M., Clark, E. G., Konoby, C., Allan, G., Ellis, J. A. (2001) Multiple porcine circovirus 2-associated abortions and reproductive failure in a multisite swine production unit. Can Vet J, 42:551-553
24. Cariolet, R., Blanchard, P., Le Dimma, M., Mahé, D., Jolly, J. P., De Boisseson, C., Truong, C., Ecobichon, P., Madec, F., Jestin, A. (2001a) Experimental infection of pregnant SPF sows with PCV2 through tracheal and muscular routes. In Proc ssDNA Viruses Plants, Birds, Pigs and Primates (ESVV). p.128
25. Cariolet, R., Blanchard, P., Le Dimma, M., Mahé, D., Keranflech, A., Julou, P., Beaurepaire, B., De Boisseson, C., Truong, C., Jestin, A. (2001b) Consequences of PCV2 experimental infection of non immune SPF sows using the intra uterine route. In Proc ssDNA Viruses Plants, Birds, Pigs and Primates (ESVV). p.129
26. Nielsen, J., Ladekjaer-Mikkelsen, A. S., Ville-Hansen, V., Lohse, L., Botner, A. (2004) PCV2-associated disease following intrauterine infection. Proc Congr Int Pig Vet Soc, p.14
27. Park, J. S., Kim, J., Ha, Y., Jung, K., Choi, C., Lim, J. K., Kim, S. H., Chae, C. (2005). Birth abnormalities in pregnant sows infected intranasally with procine circovirus 2. J. Comp Path, 132:139-144
28. Gilpin, D. F, McCullough, K., Meehan, B. M., McNeilly, F., McNair, I., Stevenson, L. S., Foster, J. C., Ellis, J. A., Krakowka, S., Adair, B. M., Allan, G. M. (2003) In vitro studies on the infection and replication of porcine circovirus type 2 in cells of the porcine immune system. Vet Immunol Immunopathol, 94:149-161
29. Vincent, I. E., Carrasco, C. P., Herrmann, B., Meehan, B. M., Allan, G. M., Summerfield, A., McCullough, K. C. (2003) Dendritic cells harbor infectious porcine circovirus type 2 in the absence of apparent cell modulation or replication of the virus. J Virol, 77:13288-13300
30. Chang, H-W., Pand, V. F., Chen, L-J., Chia, M-I., Tsai, Y-C., Jeng, C-R. (2006b) Bacterial lipopolysaccharide induces porcine circovirus type 2 replication in swine alveolar macrophages. Vet. Micro. In Press
31. Meerts, P., Misinzo, G., McNeilly., F., Nauwynck, H. J. (2005) Replication kinetics of different porcine circovirus 2 strains in PK-15 cells, fetal cardiomyocytes and macrophages. ArchVirol 150, 427-441
32. Steiner, E., Balmelli, C., Vincent, I., Summerfield, A., McCullough, K. (2006) Multipotent Cell Targeting by Porcine Circovirus Type 2. J. Virol (in preparation)
33. Misinzo, G., Delputte, P. L., Meerts, P., Lefebvre, D. J., Nauwynck, H. J. (2006) Porcine circovirus 2 uses heparan sulfate and chondroitin sulfate B glycosaminoglycans as receptors for its attachment to host cells. J Virol 80: 3487-3494
34. Bolin, S. R., Stoffregen, W. C., Nayar, G. P., Hamel, A. L. (2001) Postweaning multisystemic wasting syndrome induced after experimental inoculation of cesarean-derived, colostrum-deprived piglets with type 2 porcine circovirus. J Vet Diagn Invest, 13:185-194
35. Kiupel, M., Stevenson, G. W., Kanitz, C. L., Anothayanontha, L., Latimer K. S., Mittal, S. K. (1999) Cellular localization of porcine circovirus in postweaning pigs with chronic wasting disease. Euro J Vet Pathol, 5:77-82
36. Kennedy, S., Moffett, D., McNeilly, F., Meehan, B., Ellis, J., Krakowka, S., Allan, G. M. (2000) Reproduction of lesions of postweaning multisystemic wasting syndrome by infection of conventional pigs with porcine circovirus type 2 alone or in combination with porcine parvovirus. J Comp Pathol, 122:9-24
37. Ladekjaer-Mikkelsen, A. S., Nielsen, J., Stadejek, T., Storgaard, T., Krakowka, S., Ellis, J., McNeilly, F., Allan, G., Botner, A. (2002) Reproduction of postweaning multisystemic wasting syndrome (PMWS) in immunostimulated and non-immunostimulated 3-week-old piglets experimentally infected with porcine circovirus type 2 (PCV2). Vet Microbiol, 89 :97-114
38. Drew, T. W. (2000) A review of evidence of immunosuppression due to porcine reproductive and respiratory syndrome virus. Vet. Res. 31: 27-39

39. Shibahara, T., Sato, K., Ishikawa, Y., Kadota, K. (2000). Porcine circovirus induces B lymphocyte depletion in pigs with wasting disease syndrome. J.Vet. Med. Sc. 62: 1125-1131
40. Krakowka, S., Ellis, J., McNeilly, F., Meehan, B., Oglesbee, M., Alldinger, S., Allan, G. (2004) Features of cell degeneration and death in hepatic failure and systemic lymphoid depletion characteristic of porcine circovirus-2-associated postweaning multisystemic wasting disease. Vet. Path. 41: 471-482
41. sendes, A. R., Majo, N., Segales, J., Mateu, E., Calsamiglia, M., Domingo, M (2004). Apoptosis in lymphoid organs of pigs naturally infected by porcine circovirus type 2. Journal of General Virology 85: 2837-2844
42. Liu, J., Chen, I., Kwang, J. (2005) Characterization of a previously unidentified viral protein in porcine circovirus type 2-infected cells and its role in virus-induced apoptosis. J. Virol. 79: 8262-8274
43. Darwich, L., Balasch, M., Plana-Duran, J., Segales, J., Domingo, M., Mateu, E. (2003a) Cytokine profiles of peripheral blood mononuclear cells from pigs with postweaning multisystemic wasting syndrome in response to mitogen, superantigen or recall viral antigens. J Gen Virol, 84:3453-3457
44. Darwich, L., Pie, S., Rovira, A., Segales, J., Domingo, M., Oswald, I. P., Mateu, E. (2003b) Cytokine mRNA expression profiles in lymphoid tissues of pigs naturally affected by postweaning multisystemic wasting syndrome. J Gen Virol, 84:2117-2125
45. Sipos, W., Duvigneau, J. C., Willheim, M., Schilcher, F., Hartl, R. T , Hofbauer, G., Exel, B., Pietschmann, P., Schmoll, F. (2004) Systemic cytokine profile in feeder pigs suffering from natural postweaning multisystemic wasting syndrome (PMWS) as determined by semiquantitative RT-PCR and flow cytometric intracellular cytokine detection. Vet Immunol Immunopathol, 99.63-71
46. Stevenson, L. S., McCullough, K., Gilpin, D. F., Vincent, I., Summerfield, A., Nielsen, J., McNeilly, F., Adair, B. M., Allan, G. M. (2004) Cytokine and C-reactive protein profiles induced by porcine circovirus type 2 experimental infection in 3 week-old pigs. 2004. Cytokine profiles induced by PCV2 experimental infection in 3-week-old pigs. In Proc of the 18th IPVS Congress, Hamburg, P16
47. Hasslung, F., Wallgren, P., Jadekjaer-Mikkelsen, A. S., Botner, A., Nielsen. J., Wattrang, E., Allan, G. M., McNeilly, F., Ellis, J., Timmusk, S., Belak, K., Segall, T., Meilin, L., Berg, M., Fossum, C. (2004) Experimental production of postweaning multisystemic wasting syndrome (PMWS) in Swedish and Danish pigs using a Swedish isolate of porcine circovirus type 2. Vet Microbiol, submitted
48. Chang, H. W , Jeng, C. R., Lin, T. L., Liu, J. J., Chiou, M. T., Tsai, Y. C., Chia, M. Y., Jan, T. R., and Pang, V. F. (2006a) Immunopathological effects of porcine circovirus type 2 (PCV2) on swine alveolar macrophages by in vitro inoculation. Veterinary Immunology and Immunopathology 110: 207-219
49. Gilpin, D. F., Stevenson, L. S., McCullough, K., Krakowka, S., Meehan, B. M., McNeilly, F., Foster, C., Adair, B., Welsh, M., Allan, B. M. (2001) Studies on the in vitro and in vivo effect of porcine circovirus type 2 infection of porcine monocytic cells. In Proc ssDNA Viruses Plants, Birds, Pigs and Primates (ESVV). p.97
50. Vincent, I. E., Carrasco, C. P., Guzylack-Piriou, L., Herrmann, B., McNeilly, F., Allan, G. M., Summerfield, A., and McCullough, K. C. (2005). Subset-dependent modulation of dendritic cell activity by circovirus type 2. Immunology 115: 388-398
51. Segalés, J., Allan, G. M. And Domingo, M (2005), Porcine circovirus disease. Porcine circovirus diseases. Anim Health Res Rev. (2):119-42
52. Stevenson, L., Gilpin, D. F., McNeilly, F., Welsh, M., McNair, I., Caprioli, A., Adair, B., Alan, G. (2006) Cytokine and peripheral blood population profiles of pigs from a PMWS affected farm in Northern Ireland. In preparation
53. Madec, F., Rose, N., Eveno, E., Morvan, P., Larour, G., Jolly, J. P., Le Diguerher, G., Cariolet, R , Le Dimna, M., Blanchard, P., Jestin, A. (2001) PMWS. on-farm observations and preliminary analytic epidemiology. In Proc ssDNA Viruses Plants, Birds, Pigs and Primates (ESVV). p.86-87
54. Madec, F., Eveno, E., Morvan, P., Hamon, L., Blanchard, P., Cariolet, R., Amenna, N., Morvan, H., Truong, C., Mahé, D., Albina, E., Jestin, A (2000) Post-weaning multisystemic wasting syndrome (PMWS) in pigs in France: clinical observations from follow-up studies on affected farms. Livest Prod Sci, 63: 223-233
55. Madec, F., Waddilove, J. (2002) Control PCV2 or control other factors? Several approaches to a complex problem. In PMWS and PCV2 diseases· beyond the debate, Merial Symposium, Ames, IA, USA. p.45-53
56. Guilmoto, H., Wessel-Robert, S. (2000) Control of PMWS in Brittany: a mainly zootechnical approach. In PMWS: a new emerging disease of swine. Merial Symposium, Melbourne, Australia. p.45-55
57. Opriessnig, T., Fenaux, M., Yu, S., Evans, R. B., Cavanaugh, D., Gallup, J. M., Pallares, F. J., Thacker, E. L., Lager, K. M., Meng, X. J, Halbur, P. G. (2004b) Effect of porcine parvovirus vaccination on the development of PMWS in segregated early weaned pigs coinfected with type 2 porcine circovirus and porcine parvovirus. Vet Microbiol, 98:209-220
58. Allan, G. M., Caprioli, A., McNair, I, Lagan-Tregaskis, P., Ellis, J., Krakowka, S., McKillen, J., Ostanello, F., McNeilly, F. (2006) Porcine circovirus 2 replication in piglets following experimental infection and use of a modified live vaccine against porcine respiratory and reproductive syndrome virus. Veterinary Micro. (accepted)
59. Opriessnig, T., Meng, X. J., Halbur, P. G. (2004c) Effect of timing of vaccination with a commercially available bacterin on PCV2-associated lesions. In Proc Congr Int Pig Vet Soc. p.96
60. Ferreira, D., Sansot, B., Laval, A. (2001) Attempt to use serotherapy to control mortality in PMWS. In Proc ssDNA Viruses Plants, Birds, Pigs and Primates (ESVV). p.144
61. Waddilove, A. E. J., Marco, E. (2002) Assessing serotherapeutic control of PMWS in the field. Proc Congr Int Pig Vet Soc. p.34

62. Opriessnig, T., Anderson, M. S., Rothschild, M. F., Evans, R. B., Fenaux, M., Meng, X. J., Halbur, P. G. (2004a) Evaluation of differences in host susceptibility to PCV2-associated diseases. In Proc Congr Int Pig Vet Soc. p.12
63. Rose, N., Abherve-Gueguen, A., Le Diguerher, G., Eveno, E., Jolly, J. P., Blanchard, P., Oger, A., Houdayer, C., Jestin, A., Madec, F. (2003) A cohort study about clinical post-weaning multisystemic wasting síndrome (PMWS) in pigs of different genetic background. Proc. Symp Vet Epid Econ. p.723
64. Lopez-Soria, S., Segalés, J., Nofrarías, M., Calsamiglia, M., Ramírez, H., Minguez, A., Serrano, J. M., Marin, O., Callen, A. (2004) Genetic influence on the expression of PCV disease. Veterinary Record 155:504
65. Allan, G., McNeilly, F., McNair, I., Meehan, B., Marshall, M., Ellis, J., Lasagna, C., Boriosi, G., Krakowka, S., Reynaud, G., Boeuf-Tedeschi L., Bublot, M., Charreyre, C. (2002) Passive transfer of maternal antibodies to PCV2 protects against development of post-weaning multisystemic wasting syndrome (PMWS): experimental infections and a field study. Pig J, 50:59-67
66. Calsamiglia, M., Segalés, J., Fraile, L., Espinal, A., Seminati, C., Martin, M., Mateu, E., Domingo, M. (2004) Sow effect on litter mortality in a swine integration system experiencing postweagning multisystemic wasting syndrome (PMWS). Prev Vet Med, submitted
67. Ostanello, F., Caprioli, A., Di Francesco, A., Battilani, M., Sala, G., Sarli, G., Mandrioli, L., McNeilly, F., Allan, G. M., Prosperi, S. (2005) Experimental infection of 3-week-old conventional colostrum-fed pigs with porcine circovirus type 2 and porcine parvovirus. Veterinary Microbiology 108, 179-186
68. Hassing, A. G., Kristensen, C. S., Baebko, P., Wachmann. (2004) Effect of sow on the mortality of pigs after weaning in PMWS herds. In Proc Congr Int Pig Vet Soc. p.76
69. McNeilly, F., McNair, I., Green, L., Waldner, C., Armstrong, D., Stewart, G., Ellis, J., Krakowka, S., Allan, G. (2006) PMWS: Studies on disease progression in relation to serum antibody levels to PCV2 in sows and piglets and PCV2 viremia in young pigs. The pig Journal. In press
70. Rodríguez-Arrioja, G. M., Segalés, J., Balasch, M., Rosell, C., Quintana, J., Folch, J. M., Plana-Duran, J., Mankertz, A., and Domingo, M. (2000). Serum antibodies to porcine circovirus type 1 and type 2 in pigs with and without PMWS.Vet. Rec. 146:762-764
71. Reynaud, G., Beseme, S., Brun, A., Charreyre, C., Desgouilles, S., Jeannin, P., Rehbein, S. (2004a) Safety of a high dose administration of an inactivated adjuvanted PCV2 vaccine in conventional gilts. In Proc Congr Int Pig Vet Soc. p.87
72. Reynaud, G., Brun, A., Charreyre, C., Desgouilles, S., Jeannin, P. (2004b) Safety of repeated overdose on an inactivated adjuvanted PCV2 vaccine in conventional pregnant gilts and sows. In Proc Congr Int Pig Vet Soc. p.88.
73. Fenaux, M., Opriessnig, T., Halbur, P. G., Elvinger, F., Meng, X. J. (2004) A chimeric porcine circovirus (PCV) with the immunogenic capsid gene of the pathogenic PCV type 2 (PCV2) cloned into the genomic backbone of the nonpathogenic PCV1-induces protective immunity against PCV2 infection in pigs. J Virol, 78:6297-6303
74. Krakowka, S., Ellis, J. A., Allan, G., McNeilly, F., Waldner, C., Rings M. (2006) Mycoplasma hyopneumoniae bacterins and porcine circovirus 2 infection: Potentiation of PCV2 infection and induction of PMWS in a gnotobiotic swine model of PCV2 associated disease. Can Vet J. Submitted
75. Woodbine, K. A, Medley, G. F., Slevin, J., KilBride, A. L., Novell, E. J., Turner, M. J., Keeling, M. J., Green, L. E. (2006) Hazards for breakdown with post-weaning multisystemic wasting in pigs from a retrospective cohort study. Vet. Rec. Accepted
76. Vigre, H., Baekbo, P., Jorsal, S.E., Bille-Hansen, V., Hassing, A.-G., Enøe, C., Bøtner, A.. (2005). Spatial and temporal patterns of pig herds diagnosed with Postweaning Multisystemic Wasting Syndrome (PMWS) during the first two years of its occurrence in Denmark. Veterinary Microbiology, 110, 17-26.
77. Enøe, C., Vigre, H., Nielsen, E. O., Bøtner, A., Bille-Hansen, V., Jorsal, S.E., Bækbo, P. (2006). A Danish case-control study on risk factors for PMWS - bio security in the herd. In proceedings of the 19th International Pig Veterinary Congress, Copenhagen
78. Opriessnig, T., Yu, S., Gallup, J. M., Evans, R. B., Fenaux, M., Pallares, F., Thacker, E. L., Brockus, C. W., Ackermann, M. R., Thomas, P., Meng, X. J, Halbur, P. G. (2003). Effect of vaccination with selective bacterins on conventional pigs infected with type 2 porcine circovirus. Vet Pathol, 40:521-529
79. Timmusk, S., Wallgren, P., Belak, S., Berg, M., Fossum, C. (2005) Genetic analysis of PCV2 capsid protein sequences reveals two main groups of Swedish isolates. In Proc. ESVV. In. Con Animal Ciroviruses and Associated Diseases, Belfast. P82
80. Desrosiers, R. PCV2: Still so many questions! (Part 2). (2005). International Pigletter, 25, No 8a.
81. Grierson, S. S., King, D. P., Wellenberg, G. J., Banks, M. (2004) Genome sequence analysis of 10 Dutch porcine circovirus type 2 (PCV-2) isolates from a PMWS case-control study. Research in Veterinary Science 77: 265-268
82. Larochelle, R., Magar, R., D'Allaire, S. (2002) Genetic characterization and phylogenetic analysis of porcine circovirus type 2 (PCV2) strains from cases presenting various clinical conditions. Virus Res, 90:101-112
83. Opriessnig, T., McKeown, N. E., Meng, S. J., Halbur, P. G. (2006). Comparison of the pathogenicity of US PCV2 field isolates in an experimental pig model. Proc, AASV, 451-452