International Symposium on Emerging and Re-emerging Pig Diseases
Barcelona 12-15 June, 2011

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Venue:
Palau de Congressos de Catalunya
Avida Diagonal, 661-671. 08028 Barcelona, Spain

Promoted by:
CReSA
Centre de Recerca en Santitat Animal

21st INTERNATIONAL PIG VETERINARY SOCIETY (IPVS) CONGRESS
July 18 to 21, 2010 - Vancouver, British Columbia, Canada
CReSA® Research Contributions
21st INTERNATIONAL PIG VETERINARY SOCIETY (IPVS) CONGRESS

July 18 to 21, 2010
Vancouver, British Columbia, Canada

MAJOR TOPICS

1. PIG HEALTH

Major Viral Pathogens
Porcine Reproductive and Respiratory Syndrome
Porcine Circovirus-associated disease
Swine Influenza Virus
Other viral diseases

Major Bacterial Pathogens
Mycoplasma hyopneumoniae
Lawsonia intracellularis
Actinobacillus pleuropneumoniae
Other bacterial diseases

Parasitic Pathogens
Parasitic infections
Respiratory diseases
Enteric diseases
Other diseases
Case studies
Pharmacology and therapeutics
Eradication/Control/Biosecurity
Diagnostics

2. PRODUCTION and MANAGEMENT

Housing and husbandry
Reproduction
Production and management
Nutrition and growth
Genetics
Economics

3. FOOD SAFETY, ANIMAL WELFARE and SOCIAL CHANGE

Food safety, zoonoses and antimicrobial resistance
Animal welfare and behaviour
Impact of pig farming on the environment
Dear colleagues,

The Centre de Recerca en Sanitat Animal (CReSA) is a foundation for animal health research created in 1999 through an initiative of the Universitat Autònoma de Barcelona (UAB) and the Institut de Recerca i Tecnologia Agroalimentàries (IRTA), a public organization established by the Catalonian government for agronomic research. The CReSA pools the potential of the animal health research teams of both founding institutions, and carries out its work in a new technologically advanced BSL3 facility for conducting research, grouping efforts and focusing new resources on this field. In general, the CReSA's main objectives are animal health research, technological development and technology transfer, in co-operation with the UAB, IRTA, other institutional partners and the private sector.

Most of the research that the center conducts is focused on swine diseases. By participating in the 21st International Pig Veterinary Society (IPVS) Congress we would like to share our experiences and the findings of our research projects and increase awareness of future trends. This booklet shows how CReSA will contribute to the 21st International Pig Veterinary Society (IPVS) Congress, and summarizes our research on swine health during the last months in 39 oral presentations and posters.

Yours sincerely,

[Signature]

Dr. Mariano Domingo
Director of CReSA
Dear colleagues,

On behalf of the 6th International Symposium on Emerging and Re-emerging Pig Diseases Organizing Committee, it is my great pleasure to welcome you to Barcelona, Spain. As in previous editions -starting in Minneapolis (USA) in 1991- the efforts of this International Symposium have been to bring together scientists and veterinarians from various disciplines working around the world in the passionate field of novel and economically significant diseases in swine.

Across its history, this International Symposium had different names and included different scientific topics. At first, the focus was on Aujeszky’s disease (AD) but in the last two editions the focus shifted to porcine reproductive and respiratory syndrome (PRRS), swine influenza (SI) and postweaning multisystemic wasting syndrome (PMWS). PRRS and PMWS are still two of the most relevant pig diseases worldwide and, in consequence, they will continue being the core of this Symposium. But SI should not be forgotten. The emergence of the novel pandemic human influenza virus A(H1N1) in 2009 awoken the fear of a global threat for human health.

Although the evolution of the influenza pandemic has not accomplished the most dramatic predictions, this episode reinforced the notion that pigs may play a substantial role in the generation of new influenza viruses. Therefore, SI will be again another of the issues of the present Symposium. In addition, and most importantly, the 2011 Symposium aims at fostering the coherence of its essence: emerging and re-emerging pig diseases. We wish practitioners and scientists from around the world coming to Barcelona to present the most recent and relevant information on emerging and re-emerging diseases of pigs and, accordingly, sessions for these presentations will be also held.

Spain is a magnificent and beautiful country and tourist/vacation possibilities are almost endless. Barcelona is a cosmopolitan city in which you will feel at home. You can choose from culture to environment and nature, from sea to mountains, from exhibitions to partying... you will surely meet up with your match. Discover, taste, experience and enjoy!

Hope to see you in Barcelona in 12-15 June 2011!

Yours sincerely,

Dr. Joaquim Segalés Coma
Investigator of CRESA and UAB Professor
Chair of the 6th International Symposium on Emerging and Re-emerging Pig Diseases Organizing Committee
CReSA: CENTRE DE RECERCA EN SANITAT ANIMAL

ABSTRACTS

SWINE INFLUENZA

ANALYSIS OF CROSS REACTIVITY OF PORCINE SERA IN THE HAEMAGGLUTINATION INHIBITION ASSAY USING SWINE H1N1 OR THE NEW PANDEMIC H1N1 INFLUENZA VIRUS

EXPERIMENTAL INFECTION WITH H1N1 EUROPEAN SWINE INFLUENZA VIRUS PROTECTS PIGS FROM A SUBSEQUENT INFECTION WITH THE 2009 PANDEMIC H1N1 HUMAN INFLUENZA VIRUS

LONGITUDINAL STUDY OF SWINE INFLUENZA VIRUS INFECTION IN A FARROW TO FINISH FARM
M. Simon-Grifé, G.E. Martín-Valls, M.J. Vilari, M. Mora, M. Martín, N. Busquets, E. Mateu, J. Casal

SEARCHING FOR THE UNIVERSAL H1N1 PEPTIDE-BASED VACCINE

SEROPREVALENCE AND RISK FACTORS ASSOCIATED TO TOXOPLASMA GONDII IN DOMESTIC PIGS FROM SPAIN
Ignacio García-Bocanegra, Meritxell Simon-Grifé, Jitender P. Dubey, Jordi Casal, Gerard E. Martín-Vall, Oscar Cabezón, Anselmo Perea, Sonia Álmera

PORCINE CIRCOVIRUS DISEASES

EVALUATION OF CIRCOVAC® ONE SHOT VACCINE APPLIED IN THREE-WEEK-OLD PIGLETS ON PRODUCTION PARAMETERS IN FARMS WITH AND WITHOUT A DIAGNOSIS OF POSTWEANING MULTISYSTEMIC WASTING SYNDROME (PMWS)
Lorenzo Fraile, Llorenç Grau-Roma, Manel Casals, Patxi Sarasol, Nuria Novos, Miquel Nofrarias, Rosa López-Jimenez, Sergio López-Soria, Marina Sibila, Mathieu Chevalier, Joaquim Segalés

EVIDENCE OF A GENOTYPIC SHIFT IN PORCINE CIRCOVIRUS TYPE 2 (PCV2) FROM PCV2A TO PCV2B AFTER PMWS OUTBREAKS IN TWO SPANISH FARMS
M. Cortey, E. Pileri, M. Sibila, J. Puigós, M. Balasch, J. Plana, J. Segalés

EXPERIMENTAL CO-INOCULATION WITH PORCINE CIRCOVIRUS TYPE 2 (PCV2) AND MYCOPLASMA HYOPNEUMONIAE IN CONVENTIONAL PIGS
M. Sibila, M. Fort, M. Nofrarias, A. Pérez de Rozas, I. Galindo-Cardiel, E. Mateu, J. Segalés

INFECTIOUS RISK FACTORS FOR POSTWEANING MULTISYSTEMIC WASTING SYNDROME (PMWS) DEVELOPMENT

INTERNATIONAL IMMUNOHISTOCHEMISTRY RING TRIAL FOR THE DETECTION OF PORCINE CIRCOVIRUS TYPE 2 (PCV2) ANTIGEN ON FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUES

NATURALLY PORCINE CIRCOVIRUS TYPE 2 (PCV2) EXPOSED PIGS GENERATE A SPECIFIC ANTIBODY RESPONSE ABLE TO NEUTRALISE DIFFERENT PCV2 ISOLATES OF DIFFERENT GENOTYPES AND GEOGRAPHIC ORIGINS
Sherry Kurtz, Martí Cortey, Marta Fort, Marina Sibila, Joaquim Segalés

PATHOLOGICAL CHARACTERIZATION OF NECROTIZING LYMPHADENITIS ASSOCIATED WITH PORCINE CIRCOVIRUS TYPE 2 INFECTION IN PIGS
Iván Galindo-Cardiel, Llorenç Grau-Roma, Mónica Pérez-Maillo, Joaquim Segalés

POSTWEANING MULTISYSTEMIC WASTING SYNDROME (PMWS) AGE-SHIFT PRESENTATION IS NOT SUPPORTED BY AN 11-YEAR RETROSPECTIVE STUDY IN SPAIN
Joaquim Segalés, Martí Cortey

THEORETICAL AND EXPERIMENTAL APPROACHES TO ESTIMATE THE USEFULNESS OF POOLED SERUM SAMPLES FOR THE DIAGNOSIS OF POSTWEANING MULTISYSTEMIC WASTING SYNDROME (PMWS)
M. Cortey, S. Napp, A. Alba, E. Pileri, L. Grau-Roma, M. Sibila, J. Segalés

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS)

CLINICAL PROTECTION PROVIDED BY AMERVAC®- PRRS AGAINST HETEROLOGOUS CHALLENGE WITH A HIGHLY VIRULENT ASIAN (GENOTYPE-II) STRAIN

EFFICACY OF AMERVAC®- PRRS AFTER HETEROLOGOUS CHALLENGE WITH A PATHOGENIC CHINESE-LIKE PRRS VIRUS

IMMUNE RESPONSE AGAINST PRRSV IN SOWS AFTER BOOSTER VACCINATION USING PROGRESSIS® (INACTIVATED) OR A MODIFIED LIVE VACCINE AFTER PRIMOVACCINATION WITH A MODIFIED LIVE VACCINE
M. Gimeno, I. Díaz, L. Darwich, E. Mateu, A. Callén, J. Venet, F. Joisel

PERFORMANCE OF AN IMMUNOENZYMATIC ASSAY BASED ON THE DOUBLE RECOGNITION ELISA FOR EARLY DETECTION OF PRRS VIRUS INFECTION
Ivan Diaz, Anna Coll, Angel Ventrec, Belén Rebollo, Enric Mateu, Antonio Sanz
<table>
<thead>
<tr>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>POTENTIAL POSITIVE EFFECT OF COMMERCIAL SPRAY-DRIED PORCINE PLASMA ON PIGS CHALLENGED WITH PRRS VIRUS</td>
<td>Ivan Díaz, Cristina Lorca, Ivan Galindo, Joy Campbell, Immaculada Barranco, Liudmila Kuzemtseva, Irene M. Rodríguez-Gómez, Joe Crenshaw, Louis Russell, Javier Polo, Joan Pujols</td>
</tr>
<tr>
<td>TORQUE TENO VIRUS INFECTIONS</td>
<td></td>
</tr>
<tr>
<td>GENETIC VARIABILITY AND PHYLOGENY OF SWINE TORQUE TENO VIRUS 1 (TTV1) AND 2 (TTV2) BASED ON COMPLETE GENOMES</td>
<td>Martí Cortey, Lisa Macera, Joaquim Segalés, Tuja Kekarainen</td>
</tr>
<tr>
<td>IMPACT OF TORQUE TENO VIRUS (TTV) ON PORCINE DENDRITIC CELL FUNCTION AND ACTIVITY</td>
<td>Laura Martínez-Guinó, Kenneth C. McCullough, Artur Summerfield, Joaquim Segalés, Tuja Kekarainen</td>
</tr>
<tr>
<td>ROLLING CIRCLE AMPLIFICATION FOR DETECTING ANELLOVIRUSES FROM PIG SERUM SAMPLES</td>
<td>Lisa Macera, Martí Cortey, Fabrizio Maggi, Joaquim Segalés, Tuja Kekarainen</td>
</tr>
<tr>
<td>SWINE TORQUE TENO VIRUS TYPE 2 (TTV2) IN SITU HYBRIDIZATION: FROM DURBAN 2008 TO VANCOUVER 2010</td>
<td>Mario Aramouni, Tuja Kekarainen, Maria Ballester, Gerard E. Martin-Valls, Joaquim Segalés</td>
</tr>
<tr>
<td>TORQUE TENO SUS VIRUS 1 AND 2 VIRAL LOADS IN POSTWEANING MULTISYSTEMIC WASTING SYNDROME (PMWS) AFFECTED AND NON-AFFECTED PIGS</td>
<td>Mario Aramouni, Marina Sibila, Gerard Martin-Valls, David Nieto, Martí Cortey, Joaquim Segalés, Tuja Kekarainen</td>
</tr>
<tr>
<td>TORQUE TENO SUS VIRUS TISSUE DISTRIBUTION BY A SEMI-QUANTITATIVE PCR METHOD</td>
<td>Mario Aramouni, Martí Cortey, Joaquim Segalés, Tuja Kekarainen</td>
</tr>
<tr>
<td>TORQUE TENO VIRUS (TTV) DETECTION IN ABORTED AND SLAUGHTERHOUSE COLLECTED FOETUSES</td>
<td>Laura Martínez-Guinó, Tuja Kekarainen, Jaime Maldonado, Mario Aramouni, Anna M. Llorens, Joaquim Segalés</td>
</tr>
<tr>
<td>MISCELLANY</td>
<td></td>
</tr>
<tr>
<td>A CASE ON CHRONIC SALT INTOXICATION IN GROWING PIGS</td>
<td>Nuria Llanes, Carmen Alonso, Joaquim Segalés</td>
</tr>
<tr>
<td>CAPSULAR POLYSACCHARIDE MODULATES STREPTOCOCCUS SUIS INTERACTIONS WITH SWINE DENDRITIC CELLS</td>
<td>Marie-Pier Leclerc, Marie Agnieszka Karczewski, Charles Surprenant, Tufária Mussá, Maria Montoya, Marcelo Gottschalk</td>
</tr>
<tr>
<td>CLINICAL EFFICACY OF ACETYLSALICYLIC ACID AS AN ADJUNCT TO THE ANTIBACTERIAL TREATMENT OF PORCINE RESPIRATORY DISEASE</td>
<td>Carles Villa, Tomás Alcalá, Sergio López, Miquel Nofrarias, Rosa López-Jimenez, Sonia Espín, Teresa Varela, Lorenzo Fraile</td>
</tr>
<tr>
<td>CLINICAL, PATHOLOGICAL AND LABORATORIAL INVESTIGATIONS OF A SEVERE OUTBREAK OF ACUTE ULCERS IN PIGS</td>
<td>Carmen Alonso García-Mochales, Nuria Llanes Bartó, Joaquim Segalés</td>
</tr>
<tr>
<td>DETECTION OF AFRICAN SWINE FEVER VIRUS (ASFV) DNA IN SEVERAL CELL-TYPES FROM FORMALIN-FIXED PARAFFIN-EMBEDDED INFECTED TISSUES USING A NEW IN SITU HYBRIDISATION (ISH) PROTOCOL</td>
<td>Maria Ballester, Iván Galindo, Carmina Gallardo, Jordi M. Argilaguet, Joaquim Segalés, Javier M. Rodríguez, Fernando Rodríguez</td>
</tr>
<tr>
<td>EFFECTS OF CARBOHYDRALATE SUPPLEMENTATION ON GUT MICROBIOTA AND IMMUNOLOGY OF GROWING PIGS</td>
<td>J. Willamil, I. González, D. Torralldonada, E. Devillard, P.A. Geraert, I. Badiola</td>
</tr>
<tr>
<td>MARBOFLOXACIN ACHIEVES HIGH CONCENTRATION IN PIG TONSILS ACCORDING TO A DOSE DEPENDENT FASHION</td>
<td>Carles Villa, Marc Schneider, Rosa López-Jimenez, José María Caballero, Marcelo Gottschalk, Lorenzo Fraile</td>
</tr>
<tr>
<td>PRESENCE OF HEPATITIS E VIRUS IN SLAUGHTER PIGS</td>
<td>Manel Casas, Raquel Cortés, Sonia Fina, Bibiana Peralta, Martí Cortey, Enric Mateu, Jordi Casal, Marga Martín</td>
</tr>
<tr>
<td>POTENTIAL USE OF LONG PENTRAKIN 3 (PTX3) AS A BIOMARKER IN PIGS</td>
<td>Elsa Cresci, Lorenzo Fraile, Sonia Valentino, Meribel Simon-Grifé, Gerard E. Martin-Valls, Tufária Mussá, Barbara Bottazzi, Alberto Mantovani, Maria Montoya</td>
</tr>
<tr>
<td>PRELIMINARY STUDY OF ACTINOBACCILLUS SUIS COLONIZATION IN UPPER AND LOWER RESPIRATORY TRACT IN NATURALLY INFECTED ANIMALS</td>
<td>M. Sibila, E. Huerta, L. Grau-Roma, V. Aragón, J. Segalés</td>
</tr>
<tr>
<td>ACUTE PHASE PROTEINS AS BIOMARKERS OF PLEURITIS AND CRANO-VENTRAL PULMONARY CONSOLIDATION IN SLAUGHTER-AGED PIGS</td>
<td>Yolanda Sacco, Lorenzo Fraile, Mercé Giménez, Ana Alegre, Rosa López-Jimenez, Martí Cortey, Joaquim Segalés, Anna Bassols</td>
</tr>
<tr>
<td>PRELIMINARY STUDY OF ACTINOBACCILLUS SUIS COLONIZATION IN UPPER AND LOWER RESPIRATORY TRACT IN NATURALLY INFECTED ANIMALS</td>
<td>M. Sibila, E. Huerta, L. Grau-Roma, V. Aragón, J. Segalés</td>
</tr>
<tr>
<td>T CELL AND NEUTRALIZING ANTIBODY RESPONSES TO SYNTHETIC DENDRIMERIC PEPTIDES DERIVED FROM E2 AND NS2-3 PROTEINS OF CLASSICAL SWINE FEVER VIRUS</td>
<td>J. Tarradas, M. Monsó, R. Rosell, M. Mora, M. Muñoz, I. Muñoz, R. Lopez, I. Galindo, L. Fraile, M. Domingo, B. G. De la Torre, D. Andreu, F. Sobrino, L. Ganges</td>
</tr>
</tbody>
</table>

WHO IS WHO: 50
CENTRE DE RECERCA EN SANITAT ANIMAL (CReSA)

Animal health, the primary objective

Created in 1999 by initiative of the IRTA and UAB, the CReSA is a private foundation which benefits from the synergies generated by the human potential of its founding institutions. The CReSA is focused on research, technological development, studies and education in the sphere of animal health, in collaboration with the UAB, IRTA, other academic and research institutions, and the private sector.

The objectives of the CReSA are:

- To develop research programs within the field of animal health.
- To transfer the achieved scientific advances to the sector.
- To offer services within the field of research and development by arranging R+D programs.
- To advise agrifood companies and public administration and to provide technological support in the field of animal health.
- To organize scientific and technical training programs.

Combining scientific and productive interests

Located on the campus of Universitat Autònoma de Barcelona (UAB), next to the School of Veterinary Medicine, 20 km from central Barcelona, the structural organization of the CReSA reflects its origins and its close relationship with the production sectors. The maximum decision-making body is the Trust, which approves the statutes, annual reports, strategic plans, budgets and annual accounts. The president of the Trust is the UAB Rector and the vice-president is the General Director of the IRTA. The center is directed by Dr. Mariano Domingo Álvarez. The Advisory Board is comprised of companies from the agricultural and livestock sector and constitutes a direct connection with the interests of the sector. Likewise, the main funding sources come from subventions from the UAB, IRTA, DAR, DIUE, DS, from projects obtained by means of national and European competitive calls and through contracts with companies.

Advanced technology and level 3 biocontainment facilities

The CReSA has technologically advanced facilities for making the studies, with two clearly differentiated areas: level 2 biosafety laboratories and level 3 biocontainment unit. This biocontainment unit enable the research team to carry out research into pathogenic agents listed as diseases notifiable to the World Organization for Animal Health (OIE).

The level 2 biosafety laboratories

- Pathologic anatomy
- Bacteriology
- Molecular biology
- Cell culture
- Immunology
- Parasitology and entomology
- Virology
- Rooms with specific equipments (microscopy, electrophoresis, ultrafreezing, etc.)

The level 3 biocontainment unit

- Secure management of high-risk infectious agents
- Hermetic isolation systems
- Negative pressure gradients
- Absolute air filtration
- Treatment of liquids and solids
- 6 high security laboratories: virology, bacteriology, cell culture, equipments, molecular biology and prions
- 12 high security rooms for experimental inoculations to house pigs, poultry, cattle, sheep, goats and rabbits, among others
- Climatic chamber for studies of entomology (arthropod vectors of animal viruses)

A centre of reference

The CReSA works to improve animal health and the quality and safety of animal products destined for human consumption. The researchers carry out research into innovative and effective vaccines, they study epidemiology, immune response and pathogenic mechanisms, assess risks for human health and develop standardized models of infection and diagnostic techniques. The work of these highly qualified professionals is expressed in a technological offer and services that combined with the cutting edge infrastructures make up a standard-setting research centre.

Cutting-edge technological services

- Studies of in vitro and in vivo infection with animal pathogens
- Development of new bacteria and virus infection models
- Studies of immunology
- Study and development of new vaccine strategies
- Development and setting up of diagnosis techniques
- Bacteriological studies
- Viral studies
- Studies of the validation of veterinary products
- Epidemiological studies
- Studies of entomology (arthropod vectors of animal viruses)
- Studies of pathological anatomy in farm animals
- Studies of prioncic protein

General welfare as the final aim

CReSA supports public administration and other institutions and companies. Besides matters of scientific interest, the studies carried out at the CReSA have different implications for consumers, producers and regulatory institutions. For this reason, the CReSA also conducts actions for the Government of Catalonia. Commissioned by the Department of Agriculture, Food and Rural Action, the Centre has established plans for the monitoring and control of different diseases. It also collaborates with the Department of Health in the diagnosis of spongiform diseases and in the training and assessment of slaughterhouse vets, and with the Agency of Public Health of Barcelona in urban bird diseases.
Highly specialized services

- Epidemiological studies
- Monitoring, control and emergency disease plans:
  - West Nile fever
  - Avian influenza and Newcastle's disease
  - Tuberculosis
  - Bluetongue
  - Bovine neosporosis
  - Maedi-Visna, border disease and paratuberculosis
  - Transmissible spongiform encephalopathies
  - Epizootic diseases
- Efficacy and tolerance studies with veterinarian medicines:
  - Laboratorial trials
  - Field trials
- Studies of antimicrobials
- Efficacy of insecticides on farms
- Taxonomic diagnosis of insects
- Scientific counseling
- Collaboration in technical publications

Subprograms and lines of research

VETERINARY EPIDEMIOLOGY AND RISKS ANALYSIS:
- Veterinary epidemiology and risks analysis

M ICROBIOTA, INTESTINAL HEALTH AND ANTIBIOTIC RESISTANCE:
- Microbiota and intestinal health
- Epidemiology of markers of antimicrobial resistance

ZOOONOTIC INFECTIONS:
- Pathogenesis, prophylaxis and ecology of avian influenza
- Immunology and development of vaccines against swine influenza virus
- Pathogenesis and diagnostic of animal prionic diseases
  - Epidemiology, pathogenesis and control of zoonotic bacterial infections
  - Epidemiology and transmission of hepatitis E virus in domestic animals

TRANSBOUNDARY DISEASES:
- Biology and control of arthropods vectors of animal and human diseases
- Pathogenesis, diagnostic and control of viral zoonosis transmitted by vectors arthropods
- Pathogenesis and prophylaxis of Pestivirus infections
- Pathogenesis and prophylaxis of Asfavirus infections
- Epidemiology and control of Orbivirus infections

ENDEMIC VIRAL INFECTIONS:
- Pathogenesis, epidemiology and control of ssDNA virus infections
- Immunopathogenesis and correlates of protection against PRRS virus
- Role of micro-RNAs in viral infections
- Diagnostic, epidemiology and control of avian viral infections

ENDEMIC BACTERIAL AND PARASITIC INFECTIONS:
- Epidemiology, pathogenesis and control of Haemophilus parasuis
- Epidemiology, pathogenesis and control of swine respiratory bacterial infections
- Pathogenesis and transmission of reproductive protozoa infections
- Mechanisms of bacterial pathogenesis

International projection

The leadership of the CReSA is also demonstrated through coordination and participation in research networks on a national and international level.

CONSOLIDER-INGENIO 2010
PORCIVIR. Pathogenesis of porcine viral infections.

6th FRAMEWORK PROGRAMME (EU)
EUROFLU. Molecular factors and mechanisms of transmission and pathogenicity of Highly Pathogenic Avian Influenza Virus.
MEDREONET. Surveillance network of Reoviruses, Bluetongue and African horse sickness in the Mediterranean basin and Europe.

7th FRAMEWORK PROGRAMME (EU)
TB-STEP. Strategies for the eradication of bovine tuberculosis.

NADIR. The network of animal infectiology facilities.
PORKSCON. Porcine reproductive and respiratory syndrome (PRRS): new generation, efficient and safe vaccine, new control strategies.

COST ACTIONS (EU)
B2B. Array technologies for BSL3 and BSL4 pathogens EuroPRRSNet. A European network for understanding and combating porcine reproductive and respiratory syndrome in Europe.

CYTED PROGRAMME
Latin American network for controlling sanitary risks of intensive and extensive pigs raising. Implications for consumers.
Introduction

In April 2009, a new influenza A/H1N1 strain circulating in the human population was reported in Mexico. The new H1N1 virus was a triple swine/human/avian reassortant that afterwards spread pandemically. Fortunately, the virulence of the pandemic H1N1 (panH1N1) seems to be low but this episode made clear the need for a better surveillance of influenza A viruses infection. Swine influenza is most often caused by subtypes A/H1N1, A/H1N2 and A/H3N2. Hemagglutination inhibition test (HI) still is the serological assay of reference and it is assumed to be subtype-specific. The main objective of the present work was to assess to what extent the use of different strains of a same subtype may affect the outcome of HI when examining swine serum samples.

Material and methods

Ninety-four serum samples were selected from a sera bank (2007-2009) corresponding to a cross-sectional study of swine influenza in Spain where sera had been tested in HI using an European avian-like A/swine/Neth/Best/96 strain (NLH1N1) (GD laboratories, Deventer, Netherlands). The group of selected sera included negative and positive anti-NLH1N1 sera (≤1:320) that were re-tested in HI using the Spanish avian-like H1N1 A/swine/Spain/53207/2004 strain (ESH1N1) and the novel pandemic H1N1 (A/Catalonia/S2169/2009) strain (panH1N1) isolated from a human sample. Similarity of the predicted amino acid sequences of H1 were 89.3% between NLH1N1 and ESH1N1; 75.1% between NLH1N1 and panH1N1 and 71.5% between ESH1N1 and panH1N1. Sera were also examined using A/H1N2 and A/H3N2 (A/swine/Gent/7625/99 and A/swine/Neth/St.Oedenrode/96) (GD laboratories, Deventer, Netherlands) strains. Statistical analysis was done with Statdirect 2.7.5.

Results

Figure 1 show the comparative distribution of HI titres using the different H1N1 strains. Comparison of results of sera analyzed with NLH1N1 or ESH1N1 showed that both strains produced fairly similar titres (r=0.79; CI95%:0.70-0.85). In contrast, regression of log-transformed titres for NLH1N1 or ESH1N1 and panH1N1 was non significant, suggesting that antibodies towards panH1N1 did not cross-react with other H1N1. No cross-reactivity between none of the A/H1N1 and either H1N2 or H3N2 was observed (data not shown). Interestingly, in 4 farms animals seropositive (≥1:40) against panH1N1 were found. In one of those farms (analysed in the first quarter of 2009) 8 animals were positive (Table 1); some of them reacted with other swine influenza strains.

Table 1. Results by HI with different A/influenza strains of the 8 pigs seropositive to panH1N1.

<table>
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<tr>
<th>Pig</th>
<th>panH1</th>
<th>NLH1</th>
<th>ESH1</th>
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Discussion

The present results indicate that panH1N1 has little or no cross-reactivity in terms of HI with other contemporary swine H1N1 viruses. However, the results also showed that, most probably, H1 viruses cross-reacting with the panH1N1 strain already existed before or at the same time of the emergence of that new pandemic strain. Considering the limited virulence of current panH1N1 it can not be discarded that similar or cross-reacting H1N1 virus circulated completely unnoticed in pig herds. Also, raise the question of how to interpret the HI results obtained with a given influenza strain.

References

EXPERIMENTAL INFECTION WITH H1N1 EUROPEAN SWINE INFLUENZA VIRUS PROTECTS PIGS FROM A SUBSEQUENT INFECTION WITH THE 2009 PANDEMIC H1N1 HUMAN INFLUENZA VIRUS

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Introduction
The recent pandemic human influenza virus A(H1N1) 2009 contains gene segments from ancestors in North American and Eurasian swine lineages as well as from avian and human lineages. The emergence of this A(H1N1) 2009 virus poses a potential global threat for human health. Importantly, the fact that it can infect other species, like pigs, favors a possible encounter with other influenza viruses circulating in swine herds. In Europe, H1N1, H1N2 and H3N2 subtypes of swine influenza virus (SIV) currently have a high prevalence in commercial farms. To achieve a better assessment of the risk posed by the A(H1N1) 2009 virus in the actual situation of swine farms, it was analyzed whether a previous infection with a circulating European avian-like swine influenza virus generated cross-protective immunity against a subsequent infection with the new human influenza virus A(H1N1) 2009.

Material and methods
Two Influenza A virus isolates were used in this study: a circulating European avian-like swine influenza virus strain (A/Swine/Spain/53207/2004 (H1N1)) (GenBank accession number CY010587 and hereafter referred to as V1) and the new human influenza virus A(H1N1) 2009 (A/Catalonia/63/2009 (H1N1)) (GenBank accession numbers GQ464405-GQ464411 and GQ168897, hereafter referred to as V2). Twenty-two snatch-farrowed, colostrum-deprived Large White x Landrace piglets were obtained from a herd with a standard health status. At the age of 40 days, pigs were randomly distributed into four groups, namely C/C (n = 6), V1/C (n = 4), C/V2 (n = 8) and V1/V2 (n = 4) balanced by sex and weight. Group V1/C animals were intranasally inoculated with 3.5 ml of 10^7.04 tissue culture infectious doses 50% (TCID50) per ml of V1 on day 0. Pigs from group C/V2 received 3.5 ml of 10^6.15 TCID50 per ml of V2 on day 21. Finally, pigs in group V1/V2 were inoculated with both viruses, V1 on day 0 and V2 on day 21, whereas C/C pigs served as control animals.
Pigs were clinically monitored daily until the necropsy day (28 days post-inoculation). Blood and nasal swabs were taken regularly during the experimental period. Anti-influenza A virus nucleoprotein (NP) antibodies (IgG in serum and IgA in nasal swabs) levels were also measured. Samples were analyzed by ID Screen® Influenza A Antibody Competition ELISA (ID-Vet, France). Primers and probes were modified to improve the European avian-like swine and new human influenza virus A(H1N1) 2009 detection based on sequences database and previous reports (1). V1 and V2 viral loads in nasal swabs and lung tissue were assessed by a TaqMan quantitative RT-PCR. Inhibition of hemagglutination on serum was performed following standard procedures (2). The test was standardized at four hemagglutinin units and the starting dilution was 1:20.

Results
Pigs only infected with V2 had mild to moderate gross lesions (broncho-interstitial pneumonia). However, pigs inoculated with V1 virus and subsequently infected with V2 had very mild lung lesions, apparently attributed to the residual lesions caused by V1 infection. These later pigs also exhibited boosting levels of specific IgA antibodies in nasal swabs and IgG in sera. This boosting effect was also apparent for inhibition of V1 and V2 hemagglutination levels. Finally, in all animals from group V1/V2, neither virus shedding nor viral load in lungs or in nasal swabs were detected after challenge with V2, indicating a cross-protective effect in which specific anti-influenza antibodies seem to play a crucial role.

Discussion and conclusions
A previous infection with one of the circulating European avian-like SIV H1N1 in the field was able to confer protective immunity to pigs against a challenge with the new variant influenza virus A(H1N1) 2009 virus. These data pave the way for understanding the intimate immune responses generated between different influenza viral infections, within the same subtype. Further characterizations are required to understand the whole picture and all the mechanisms involved in protection.

References
LONGITUDINAL STUDY OF SWINE INFLUENZA VIRUS INFECTION IN A FARROW TO FINISH FARM


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Introduction
Pigs can be infected with avian, swine and human influenza A viruses and, historically, swine was proposed to be the mixing vessel where reassortant influenza strains arise; however the H5N1 incidents showed that avian influenza viruses could be transmitted directly to humans. The recent emergence of a human pandemic influenza A virus harbouring genes thought to be originally of swine influenza viruses (SIV) fuelled again the interest in the epidemiology of influenza in pigs. Swine influenza is a common respiratory pathogen of pigs causing a disease clinically similar to human influenza although, as suggested by others (1), subclinical infections might be common, particularly when considering the high seroprevalences (2) and the relatively low incidence of confirmed influenza outbreaks. It is worth to note, that the precise dynamic of SIV infection within a farm is scarcely known beyond the situation of an epidemic clinical outbreak. The present study was aimed to determine the infection dynamics of SIV in a farrow-to-finish farm.

Material and methods
A farrow-to-finish farm previously known to be positive for SIV antibodies was selected for the present study. The farm had 300 sows in stock and the annual replacement percentage in average was a 55%. Farrowing batches were scheduled every 3 weeks. A whole weaning batch (n=121) of 3-week-old piglets was selected for the study. Nasal swabs and sera were taken weekly between the 3rd and the 13th week of age and afterwards, pigs were sampled again at 15, 17, 20 and 24 weeks of age. In each visit to the farm a clinical inspection of pigs was performed. Serum samples were tested for antibodies against swine influenza nucleoprotein using a commercial indirect ELISA (CIVTEST influenza, HIPRA, Spain). Samples were considered to be positive when the sample-to-positive control (S/P) ratio ≥ 0.2. SIV shedding was determined in nasal swabs by means of a TaqMan one-step qRT-PCR using a Fast7500 equipment (Applied Biosystems, Foster City, CA). Primers and probe were adapted from a previous report (3) and the design was based on sequences of European SIV strains retrieved from Genbank to improve the SIV matrix (M) gene detection.

Results
No clinical signs of influenza were observed for the duration of the study. However, seropositive animals were detected by ELISA at all sampling times (Figure 1). Initially, (3 weeks of age) seroprevalence was of 56.2% (68/121) declining until a low of 10.4% (12/115) at 6-weeks of age. Afterwards and until 13 weeks of age, seroprevalence showed ups and downs. From 15 weeks of age, all remaining seronegative animals seroconverted. Based on RT-PCR results, shedder pigs were observed at 3, 4, 7, 13, 15, 17, 20 and 24 weeks of age with point prevalences ranging from 1 to 25%. As shown in Figure 1, four waves of viral circulation were observed: weaning (3-4 weeks of age), 7 weeks of age, 13 weeks of age and 20 weeks of age. Interestingly the same animal could be shedding SIV in different infection waves.

Discussion
Results of the present study showed that SIV may circulate subclinically in an endemic farm. Maternal-derived antibodies (MDA) probably persisted for at least 6 weeks and, most probably, very early infections became softened because of those MDA. The results also show different consecutive viral waves that were not translated to an explosive spread in the farm (general seroconversion) except for the last two waves, suggesting the introduction and circulation of a strain previously absent in the population in late stage fatteners and finishers. In summary, the present data suggests an endemic and subclinical circulation of SIV in consecutive viral waves.

References
**SEARCHING FOR THE UNIVERSAL H1N1 PEPTIDE-BASED VACCINE**

**Introduction**

Pigs may act as “mixing vessels” and generate recombinant viruses of unknown pathogenicity for animals and humans, as reflected with the last human H1N1 influenza pandemic. One of the main problems to obtain efficient vaccines against flu is the high variability of the virus, limiting the protection to highly homologous viruses.

In an attempt to identify potential cross-reactive epitopes within the H1 haemagglutinin (HA), a conformational spectrum analysis was done using sequences available in the GenBank database. One highly conserved peptide of 34 amino acids mapped in the flanking region of the HA binding site from human A/South Carolina/1/18 (H1N1) (NF-34 peptide), was selected. The objective of this study was to test the protective effect of the synthetic HA-peptide based vaccine in vivo.

**Materials**

A total of eight 8 week-old conventional crossbreed pigs seronegative against swine influenza viruses (SIV) were randomly divided into two groups (four pigs each). Each group was intramuscularly injected three times either with the NF-34 peptide (10-15 µg per dose) (referred to as G1) or with phosphate buffer saline (referred to as G2) two weeks apart. One month after last dose, pigs were intranasally inoculated with 10^6 tissue culture infectious doses 50% (TCID50) of the human H1N1 pandemic virus (A/Catalonia/63/2009 strain). Six days later, pigs were sacrificed. Serum and peripheral blood mononuclear cells (PBMCs) taken before each immunization, before challenge and at 6 days post-challenge (necropsy day) were used to detect specific humoral and cellular responses, respectively. Specific antibodies were detected using a peptide-based ELISA or by the haemagglutination inhibition assay, while cellular responses were determined by IFN-γ-ELISPOT after overnight stimulation with either the NF-34 peptide or with inactivated virus. After challenge with pandemic H1N1 virus, pigs were sacrificed at day 6 post-challenge and viral loads checked by a real-time qRT-PCR in bronchoalveolar lavage (BAL).

**Results**

Pigs immunized with the NF-34 synthetic peptide developed peptide-specific antibodies. Also, G1 animals showed an antibody boost at day 6 post-challenge (lines in figure 1).

Despite the haemagglutination inhibitory antibodies induced after vaccination were relatively weak (data not shown), 50% (2/4) of G1 pigs showed lower viral load in the BALs compared with G2 pigs (Figure 2).

**Discussion**

Current vaccines against influenza (including those against seasonal influenza in humans) are restricted to the predicted circulating strain at a given time in a specific world location. It is important to notice that even though only partial protection was afforded in the present pig model, this was obtained against one of the most divergent H1N1 strains, with an HA differing in 3 aminoacids from the peptide used to vaccinate. We suggest that this strategy could be appropriated to immunize against divergent strains by incorporating to the vaccine epitopes able to induce neutralizing antibodies enabling to obtain safe and efficacious subunit flu vaccines.

**Acknowledgements**

Authors would like to thank Maria Ballester, Mercedes Mora, Joan Pujols, Virginia Aragón and Tuija Kekarainen from CReSA for their help and support and also for their invaluable discussions.

**References**

Seroprevalence and Risk Factors Associated to Toxoplasma gondii in Domestic Pigs from Spain

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Introduction

Toxoplasmosis is a worldwide zoonotic disease caused by Toxoplasma gondii, which infects most warm-blooded animals. In fact, human prevalence has been found worldwide in nearly one-third of the population (Tenter et al., 2000). The consumption of raw or undercooked pork meat containing tissue cysts is considered among the principal source for human infection (Dubey, 2009). Pigs can get infected with T. gondii through ingestion of sporulated oocysts in soil, vegetation or water, by ingestion of cysts in tissues of infected animals such as rodents, birds and other pigs, or congenitally (Dubey, 2009).

The aim of the present study was to provide recent information on epidemiology and prevalence of T. gondii and the risk factors associated to this protozoan parasite in domestic pigs from the main swine producing regions of Spain.

Materials

A cross-sectional study was conducted in 100 swine farms (83 farrow-to-finish and 17 piglet production farms) located all over Spain between 2007 and 2009. Blood samples were collected from a total of 2,970 pigs. Fourteen sows and 20 fattening pigs were randomly selected in each farrow-to-finish farm and samples from 14 sows were obtained from the piglet production farms. Presence of antibodies to T. gondii was tested by modification agglutination test (MAT). The serum from each pig was tested at dilutions from 1:25, 1:50 and 1:500. Epidemiological data were collected through a structured filled-in questionnaire in order to provide information on exposure levels to potential risk factors. The effect of the exploratory variables on the response variable (seropositivity to T. gondii) was investigated by the method of generalized estimating equations (GEE). The statistical analyses were performed using SPSS v15.0.

Results

Antibodies (MAT 1:25 or higher) against T. gondii were detected in 492 (16.6%; CI95%; 15.2-18.0) of 2,970 pigs tested, with titers 1:25 in 287 (58.3%), 1:50 in 171 (34.8%) and ≥1:500 in 34 (6.9%). Farm prevalence was 85.0% (CI95%; 78-92) and within-farm prevalence ranged from 2.9% to 92.8% (median= 17.6%). Significantly higher seroprevalences were detected in Valencia Community (27.3%), Extremadura (23.3%) and Catalonia (21.2%) with respect to the other sampled regions. Sows with parity number higher than three had significantly higher seropositivity to T. gondii compared to younger sows.

The GEE showed that age sows compared to fattening pigs (OR= 2.9; CI95%=1.8 – 4.5), no rodent control (OR= 1.9; CI95%= 1.04 – 3.60) and presence of cats (OR= 1.6; CI95%= 1.12 - 2.34) were risk factors potentially associated to T. gondii seroprevalence.

Discussion

The results showed widespread exposure to T. gondii among domestic pigs in Spain, with highly variable prevalence levels among farms and regions. The high seroprevalence rates indicate that raw or undercooked pork could be a source for human infections in Spain and the need of consumption of properly cook pork, especially in pregnant women. Higher exposure was related to age of pigs, with the highest levels observed in sows, and to poor management practices related to lack of rodent control and to the presence of cats in the farms. Therefore, in order to reduce the risk of infection by T. gondii in pig farms in Spain, adequate rodent control programs and restriction of cats in the farms should be advised, and especial emphasis of avoiding infection should be taken care in the sows.

References

EVALUATION OF CIRCOVAC® ONE SHOT VACCINE APPLIED IN THREE-WEEK-OLD PIGLETS ON PRODUCTION PARAMETERS IN FARMS WITH AND WITHOUT A DIAGNOSIS OF POSTWEANING MULTISYSTEMIC WASTING SYNDROME (PMWS)

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Introduction

Porcine circovirus type 2 (PCV2) vaccines have demonstrated to be very efficient to control postweaning multisystemic wasting syndrome (PMWS) under experimental and field conditions (1,2,3). Vaccination of sows and gilts increases PCV2 antibody titres in serum or colostrum providing protection of piglets against disease development (4). CIRCOVAC® is registered in many countries to be used in sows and gilts with the former indication. The objective of the present study was to evaluate the efficacy of the above-mentioned vaccine in farms infected with PCV2 and with or without a PMWS diagnosis.

Material and methods

CIRCOVAC® was tested in 17-26 day-old conventional pigs of 2 different Spanish farms. Both farms were selected by their previous recent PMWS history. In each farm, about 300 pigs received one single dose of 0.5 mL of CIRCOVAC®, while 300 control animals received the same amount of placebo. Also in each farm, 50 pigs from each group were bled and a rectal swab was also taken. PCV2 viral load in serum and rectal swab (real-time PCR) were investigated at 3, 6, 10, 14, 17, 20, 23 weeks of age and before slaughter (S). All animals were weighed and scored for physical condition at 3 and 10 weeks of age and S. Average daily gain (ADG) for the whole postweaning period was calculated. All pigs were necropsied and sampled for a PMWS diagnosis assessment (histopathology and PCV2 in situ hybridization, ISH). Analysis of variance with Bonferroni multiple comparisons (for normally distributed variables) and Kruskal-Wallis and Mann-Whitney tests (for non-normally distributed variables) were used to compare ADG, PCV2 DNA loads in sera and faeces between vaccinated and control animals in each sampling time.

Results

PCV2 infection was demonstrated to occur in both studied farms (measured by qPCR and ISH in tissues). PMWS individual case definition was fulfilled only in farm 1, while no cases accomplishing the diagnostic criteria were found in farm 2. ADG was significantly higher in vaccinated versus control animals in farm 1 and 2. Moreover, PCV2 viral load in serum was significantly lower (1-2 log) in vaccinated compared to control animals in most sampling times after PCV2 exposure in both farms. A similar result was obtained regarding PCV2 viral load in faeces.

Discussion

Vaccination with CIRCOVAC® in piglets under field conditions was able to significantly improve production parameters and reduce significantly PCV2 viremia and faecal load. These results agree with other publications using piglet vaccination in PMWS affected farms by means of different vaccine approaches, such as a PCV1-2 chimera vaccine (5) and a subunit vaccine based on PCV2 ORF2 protein (1). The present results support the idea that production parameters are suitable indicators of PCV2 vaccine efficacy.

References


CIRCOVAC® is a registered trademark of MERIAL in Canada and elsewhere.
Introduction

PCV2 is considered to be the essential infectious agent of post-weaning multisystemic wasting syndrome (PMWS). Many studies have not found any relationship between PCV2 strains and the occurrence of disease (1-3). Nonetheless, a growing number of publications (4-8) reported a shift from PCV-2a to PCV-2b that may be related to the occurrence of PMWS outbreaks. Based on the existing evidence at different country levels, the main objective of this study was to follow up the evolution of PCV2 genotypes in two farms in Spain that undergone a PMWS episode, to examine the dynamics of PCV2 infection and its relationship with the appearance of PMWS at herd level.

Material and methods

A total of 51 samples (serum and PCV2 isolates from the PMWS outbreak) from Farm 1, collected from 1995 to 2009, and 87 samples from Farm 2 (serum samples), collected from 1996 to 2003, were analyzed. PMWS outbreak suspicion and diagnosis were confirmed on May 2007 and March-April 2001 in farm 1 and 2, respectively. Both were small experimental farrow-to-finish (40-60 sows) operations, free from most of common swine pathogens, including porcine reproductive and respiratory syndrome virus. A fragment of 652 located between the Rep and the Cap protein of the PCV-2 genome was sequenced on all available samples.

Results

A sudden genotype PCV2a to PCV2b overturn was observed on Farm 1: 34/37 sequences were PCV2a before the PMWS outbreak in 2007, whereas all sequences after the outbreak were PCV2b (Figure 1). On Farm 2, the frequency of PCV2a decreased steadily from 1996 to 2003 and the frequency of PCV2b increased (Figure 1). The chi-square analyses performed on both farm datasets yielded significant results, statistically supporting the change of genotype from PCV2a to PCV2b in both herds at the time of PMWS occurrence.

Discussion

The genetic shift linked to PMWS epizootics has been described in several countries from Europe (5,7,8) and North-America (4,6). However, this is the first report of a PCV2 genotype shift associated with PMWS appearance at individual farm level. Overall, the putative higher virulence of PCV2b strains seems to be supported by the present data. Both studies related the occurrence of PMWS epizootics to high frequencies of PCV2b and lower frequencies of PCV2a.

Acknowledgements

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References

Introduction
Porcine circovirus type 2 (PCV2) is the etiologic agent of postweaning multisystemic wasting syndrome (PMWS) in pigs. However, PCV2 co-infection with some viral and bacterial agents may potentiate PMWS development (1). Particularly, a previous infection with Mycoplasma hyopneumoniae (M.) has been shown able to trigger PMWS in subsequently PCV2 infected pigs (2). The objective of this work was to study the effect of concurrent PCV2 and MH co-inoculation in conventional piglets.

Material and methods
Thirty six 6-week-old male conventional piglets were divided into 4 groups: control (n=6, receiving 2 ml of PBS); PCV2 (n=6, intranasally challenged with 1 ml of 10^5 TCID50 PCV2b per nostril); MH (n=12, intratracheally inoculated with 5 ml of MH 10^7 CCU on two consecutive days); and PCV2+MH (n=12, same inoculum conditions as above). Clinical signs, rectal temperature and body weight were recorded during the study period. Blood samples, faecal and nasal swabs were collected on 0, 7, 14 and 21 dpi. At necropsy (21 dpi), tracheal and bronchial swabs were also taken. Extension of cranio-ventral pulmonary consolidation lesions (CVPC) was assessed (3). Moreover, lymphoid tissues and lungs were collected and fixed in 10% buffered formalin. Sera were examined for PCV2 (total and neutralising (4)) and MH antibodies. DNA extracted from serum samples, nasal and faecal swabs were analyzed by a PCV2 quantitative PCR (Q-PCR) (5). PCV2 in situ hybridization (ISH) from lymphoid tissues and lung was performed (6). MH DNA from nasal, tracheal and bronchial swabs was analyzed by Q-PCR.

Results
PCV2 infection remained subclinical although mild PMWS-like microscopic lesions were observed in two PCV2 pigs and in one PCV2+MH animal. PCV2 was detected by ISH in 3 PCV2 and in 4 PCV2+MH challenged pigs. No significant differences on mean body weight and rectal temperature were observed among the 4 groups. Total antibody and NA titres were similar in both PCV2-inoculated groups. Number of PCV2 Q-PCR positive pigs in serum was higher but not statistically significant in all samplings in PCV2+MH than in PCV2 inoculated pigs. No significant differences in viral loads on different sampling days and duration of viremia were detected among PCV2 inoculated groups. Coughing was observed in 3 piglets from the MH group and in 6 PCV2+MH pigs but it was absent in non-MH inoculated pigs. Proportion of MH Q-PCR positive pigs and mean bacterial DNA load in nasal cavity were similar in MH and PCV2+MH groups. On the contrary, mean MH DNA load at bronchial and tracheal swabs was higher in the PCV2+MH group compared to the MH one. No significant differences on percentage of MH seropositive pigs among MH inoculated groups were observed. Eight pigs from MH group and nine from PCV2+MH group showed CVPC. No significant differences in mean lung scoring between MH group and PCV2+MH group were observed.

Discussion
In a previous study, MH inoculation two weeks before PCV2 challenge resulted in an increase of PCV2-associated lesions severity, amount of PCV2 antigen and incidence of PMWS in pigs (2). In the present study, the concurrent inoculation of MH and PCV2 did not produce that synergic outcome. These divergent results may be explained by the different timing of infection but also by the source of the animals used. In the present study, the inoculated animals were seropositive to MH- and PCV2 while in the previous one (2) the challenged animals were seronegative against both pathogens. Taking into account that infection with both agents was successful, the present study suggests that timing and initial pig serological status may be the most important issues for the final outcome of the co-infection.

Acknowledgements
This work is based on data generated in a study commissioned by Intervet/Schering-Plough Animal Health. Part of these results was presented at a satellite symposium hold at the IPVS 2008, Durban (South Africa) organized by this company.

5. References
**Introduction**

Porcine circovirus type 2 (PCV2) is considered to be essential but not sufficient for postweaning multisystemic wasting syndrome (PMWS) development. Thus, several co-infections have been suggested as triggers for disease development in the field (1). The aim of the present study was to gain further insights on infectious agents as potential risk factors for PMWS development.

**Material and methods**

Two longitudinal case-control studies in 13 PMWS-affected farms from Spain and Denmark were performed as previously described (2). Blood samples from pigs (approximately 100 per batch coming from 10 sows) were longitudinally collected throughout their productive life. Pigs showing PMWS-compatible clinical signs were bled and euthanized together with healthy aged-matched pigs (ratio 2:1). PMWS was diagnosed and pigs were classified into three categories: i) PMWS cases; ii) wasted non-PMWS cases; iii) healthy pigs (2).

Serological analyses were performed in all collected serum samples to detect antibodies against *Porcine reproductive and respiratory syndrome virus* (PRRSV), *Porcine parvovirus* (PPV), *Swine influenza virus* (SIV) and *Lawsonia intracellularis* (Law) in both countries. Moreover, in Spain, antibodies against *Mycoplasma hyopneumoniae*, *Aujeszky’sdisease virus* and *Salmonella spp.* were also assessed.

A Cox proportional hazards model was used to investigate the simultaneous effects of seroconversion and maternal immunity against the studied pathogens in each country. Data were analysed within a survival analysis framework, where development of PMWS was considered as death/failure. All analyses were carried out using the Splus® software package, version 6.1.

**Results**

The number of studied pigs and their pathological classification is displayed in table 1.

Results from survival analysis showed that, for the Danish dataset, seroconversion against Law and PPV had a protecting effect. For animals that seroconverted against Law, the protective effect increased with the level of maternal immunity against this pathogen. Maternal immunity against PPV gave a protective effect in Spanish dataset. Moreover, high levels of maternal immunity against PCV2 had a protecting effect in both countries.

**Discussion**

Maternal PCV2 antibodies in both Danish and Spanish data showed a protecting effect against PMWS development, which is in agreement with previous studies (3). This situation would support the interest of PCV2 vaccination in sows in order to transfer a higher load of PCV2 antibodies to the offspring.

The protective effect of maternal immunity against PPV is in agreement with previous works suggesting PPV as a co-infectious trigger (4). Besides, the fact that seroconversion against PPV and Law resulted protective for PMWS development could seem contradictory. However, the present analysis was based on antibody levels, and not on direct evidences of pathogen infection. Since it is known that PMWS affected pigs are immunocompromised (5), seroconversion may indicate a well-developed immune system, which in turn will make the animal less likely to develop PMWS.

**Acknowledgements**

The authors acknowledge the financial support from the European Commission (project No. 513928).

**References**

Introduction
Porcine circovirus type 2 (PCV2) is the essential infectious agent of postweaning multisystemic wasting syndrome (PMWS). PMWS diagnosis is mainly established based on laboratorial criteria, including histopathology and detection of PCV2 in tissues (1). Therefore, the objective of the present abstract is to summarise the results of an international immunohistochemistry (IHC) ring trial performed in 18 different laboratories across the world.

Material and methods
The IHC ring trial was undertaken using 10, formalin-fixed, paraffin-embedded tissues, including lymph nodes (n=5), lung (n=3) and thymus (n=2) from pigs with and without PMWS. The referral laboratory (laboratory 1) submitted the 10 tissue sections in duplicate to each laboratory (two laboratories contributed twice to the results). Each laboratory performed the IHC stain and evaluated the results blindly using a scale of 0 (negative), 1+ (weak reactivity), 2+ (moderate reactivity), and 3+ (strong reactivity). Secondly, a written survey detailing 28 technical aspects of the IHC assays were also sent to all participants.

Results
Sixteen out of 18 laboratories had a mean number of discrepant results between 0 and 1 among slides; such number was 2 or higher in the other two laboratories. Of all participant laboratories, only 14 sent the technique details survey completed. When the IHC result and IHC technique details were taken into account jointly, 12 out of the 14 submitting laboratories showed no significant differences among them.

Discussion
Overall, the present study indicates that most of the participant laboratories reported congruent results. Technical differences were not a major source of result disagreement.

Acknowledgements
This work was funded by project No. 513928 from the Sixth Framework Programme of the EU Commission.

References
Introduction

Porcine circovirus type 2 (PCV2) vaccines are able to induce a strong humoral immune response. Their neutralizing antibody response is apparently cross-protective against both predominant PCV2 genotypes (PCV2a and PCV2b) (2). Also, PCV2a infection protects against a subsequent PCV2b challenge, presumably through cross-neutralizing antibodies (NA) (5). It is also known that there are several epitopes (detected by monoclonal antibodies) in the PCV2 Cap protein that are shared between different genotypes, as well as PCV1 (4,6), which represents a putative basis for cross-neutralization. However, there were several strains for which there was little or no neutralization using the same antibody panel. This suggests the existence of antigenic variability between PCV2 genotypes. However, these studies describing antigenic variation have been done using monoclonal antibodies, which are specific for unique epitopes. Therefore, the objective of the present study was to further insight on PCV2 antibody neutralization by using a pig sera obtained from field studies to assess the level of antigenic variation by cross-neutralization amongst different isolates of PCV2. Viral isolates used were from different geographical regions.

Material and methods

Serum samples. A total of 82 sera were used for the present study. A first group of animals corresponded to PMWS affected pigs (n=20), which where found to be infected by PCV2b strains (3). A second group was composed of age-matched healthy pigs (n=17) from the same farms, and a proportion of them also infected with PCV2b strains. Finally, in order to get sera samples from animals that would have been exposed to PCV2 in the past, randomly sampled slaughter age pigs (n=45) were taken.

PCV2 neutralization test. Neutralization assays were carried out as described (1) using two PCV2a (Burgos and Stoon-1010) and two PCV2b (MO/S-06 and Sp-10-7-54-13) isolates. Burgos and Sp-10-7-54-13 strains were isolated from postweaning multisystemic wasting syndrome (PMWS) affected pigs in Spain, while Stoon-1010 and MO/S-06 were isolated from PMWS in Canada and United States, respectively.

Total PCV2 antibody test. An immunoperoxidase monolayer assay (IPMA) technique to detect antibodies to PCV2 (1) was also performed with all available sera and compared to the different neutralization values obtained.

Results

Most of the sera were able to neutralise the four PCV2 strains used in the test, even they differed in their antibody titre. Globally, PCV2a genotype strains used in the test originated significantly higher neutralising antibody titres when compared to those against the PCV2b genotype. No differences in NA titres were observed between PCV2 strains within the same genotype. In addition, NA titres detected in healthy and slaughterhouse animals were significantly higher compared to PMWS affected pigs. No statistical significant differences were detected in the neutralizing activity of sera obtained from either healthy, PMWS, or slaughterhouse pigs when tested to PCV2 strains of different geographical origin. Finally, there was no direct correlation between IPMA and neutralizing titres detected in any of the animal categories.

Discussion

The present study sought to examine the role of antigenic variability in the context of a natural infection. Pig sera of different animal categories were able to neutralize PCV2 strains from different genotypes and geographical regions. This point seems to be extremely important, since would explain the field evidence that PCV2 vaccines available in the market (all based on PCV2a strains) show efficacy in controlling PMWS independently of the causing genotype (2). However, the differences observed in the NA titres of the tested sera when either PCV2a or PCV2b strains were used as antigen in the test suggest the existence of antigenic variability among those strains.

Acknowledgements

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References

PATHOLOGICAL CHARACTERIZATION OF NECROTIZING LYMPHADENITIS ASSOCIATED WITH PORCINE CIRCOVIRUS TYPE 2 INFECTION IN PIGS

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Introduction
Necrotizing lymphadenitis is a pathological feature observed in about 2% of postweaning multisystemic wasting syndrome (PMWS)-affected pigs. The pathogenesis of the lesion has been linked to a Porcine circovirus type 2 (PCV2)-related apoptosis-induced mechanism (1), but no further studies have been focused on this topic. Therefore, the goal of this retrospective work was to gain further insights into PCV2-associated lymphoid necrosis pathogenesis in PMWS cases.

Material and methods
Three groups of animals were selected: PMWS-affected pigs with necrotizing lymphadenitis (group 1, n=5), PMWS-affected pigs without necrotizing lymphadenitis (group 2, n=5) and healthy pigs with no PMWS-related lesions (group 3, n=5). Investigations performed included immunohistochemistry (IHC) of cleaved caspase-3 (to detect apoptosis) and von Willebrand factor (vWF, to detect this glycoprotein, which is involved in haemostasis), special stain for fibrin (Mallory stain) and in situ hybridization (ISH) to detect PCV2 genome.

Results
Table 1 includes individual results of histopathological findings. Fibrin deposition detected by Mallory stain was significantly higher in PMWS-affected lymph nodes with necrosis compared to those with PMWS or normal lymph nodes. Lymph nodes from PMWS-affected pigs had significantly higher PCV2 amount than those of healthy pigs. Otherwise, no significant differences were observed between the two PMWS-affected groups in regards intensity, location or amount of PCV2 genome detected by ISH. Staining pattern of vWF in the PMWS-affected pigs with follicular necrosis was endothelial (peri-membranous), subendothelial (collagen bound), cytoplasmic and intravascular (circulating). In regards apoptosis, although labelled cells from PMWS-affected pigs with follicular lymphoid necrosis had a strong intracytoplasm staining, CCasp-3 staining pattern did not show differences among the three groups of studied pigs.

Discussion
Necrotizing lesions in lymph nodes of severely PMWS-affected pigs usually show multifocal to coalescent areas of coagulative necrosis (in some cases, affecting the whole parenchyma of the lymph node) together with vascular thrombosis (3). This latter fact has been confirmed in the present study by the presence of fibrin thrombi detected by the Mallory stain. Taking into account that vascular injury seems to be the primary determinant of a thrombotic response (2), the existence of marked thrombosis in PMWS-associated necrotizing lymphadenitis suggests certain extension of vascular damage in these pigs. Such damage evidence was supported by the high endothelium venule hypertrophy and the over-expression of vWF in subendothelial (collagen bound), cytoplasmic and intravascular (circulating) locations. Such findings would account for the increase of intravascular fibrin deposition and, consequently, thrombosis. Therefore, obtained results would point towards a vascular accident with thrombi development and the subsequent follicular necrosis as the cause of necrotizing lymphadenitis in PMWS affected pigs.

References

Table 1. Results of histopathology, Mallory stain, PCV2 in situ hybridization, vWF and apoptosis scores in the three groups of studied pigs.
Introduction
Postweaning multisystemic wasting syndrome (PMWS) was firstly diagnosed in Spain in 1997 (3). Since then, the number of disease diagnoses increased significantly, reaching a peak between years 2000-01 (2), and then decreased over time. After 2007, the number of PMWS diagnoses started increasing again, basically due to the advent of the first porcine circovirus type 2 (PCV2) vaccine in the market. Therefore, it seems that the number of PMWS diagnoses in a given period depends on several factors. Firstly, the need of a diagnosis in front of a novel disease (mainly between 1997 and 2001). Secondly, once the disease was known and no practical solutions did exist, the interest of laboratorial diagnosis decreased (2002-2006). This latter situation was coupled with the evolution of severe disease outbreaks to a more enzootic, chronic form of the disease. However, with the introduction of the first PCV2 vaccine product, the interest of a precise diagnosis of PMWS raised again among veterinarians (2007 to date). In such framework, a general perception was that the initial acute disease largely died down over time and since then veterinarians and producers face with the chronic effects of PMWS. Such situation leaded to the opinion that PMWS resulting mortality occurred at older ages in the so-called “chronic form” of the disease (3). Therefore, the objective of the present study was to assess if such PMWS age-shift presentation took place over the years in Spain.

Material and methods
A total of 650 pigs diagnosed as PMWS at the Pathology Diagnostic Service at the Universitat Autònoma de Barcelona (Spain) with an unequivocally known age were included in this study. These animals were diagnosed as PMWS based on the three internationally accepted case definition criteria (4) between 1998 and 2008. The number of pigs available per year was variable, ranging from 9 in 2006 and 134 in 1999. The average number of pigs per year was around 60. Different ages were measured in months and for statistical purposes, a single number was given for all age groups included (i.e, age 1 corresponded to pigs between 1 and <2 months of age, age 2 corresponded to animals between 2 and <3 months of age, and so on). Differences in the PMWS age presentation among years, and between the overall and yearly average age were tested by means of several chi-square and Kluskal-Wallis tests. Statistical significance level was set at p<0.05. Confidence intervals (95%) for every year are presented in Fig.1

Results
The youngest age recorded corresponded to 11 1-month-old piglets, while the oldest animals diagnosed as PMWS included 13 6-month-old pigs. More than 80% of the pigs fall between 2 and <4 months of age (532 out of 650 animals). Fig. 1 shows the evolution of the average age of pigs diagnosed as PMWS between 1998 and 2008. Significant differences in age presentation were overall obtained, mainly due to the average age in two years: younger pigs in 2001 and older pigs in 2005. However, the average age evolution between 1998 and 2008 did not significantly vary.

Discussion
With the emergence of new diseases, it is expected that initial severe outbreaks are followed by a more chronic, not as severe form of the clinical problem. Such situation has been described for PMWS. It has been claimed that the development of the so-called “herd immunity” has been the main reason for disease evolution as well as the assumed shift in age presentation (from weaners and younger fatteners to mid/late fatteners) (1). However, the present data do not fit with this general opinion, and points to a relatively constant average PMWS age presentation throughout time.

References
THEORETICAL AND EXPERIMENTAL APPROACHES TO ESTIMATE THE USEFULNESS OF POOLED SERUM SAMPLES FOR THE DIAGNOSIS OF POSTWEANING MULTISYSTEMIC WASTING SYNDROME (PMWS)

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INTRODUCTION

Traditional postweaning multisystemic wasting syndrome (PMWS) diagnosis requires the necropsy of suspicious animals and the access to histopathological expertise (1). One of the major differences between PMWS-affected and non-affected pigs is porcine circovirus type 2 (PCV2) load in serum and tissues (2,3,4). Consequently, real-time quantitative PCR (qPCR) has been proposed as a potential diagnostic alternative. However, sensitivity and/or specificity of qPCR on individual basis are relatively poor (5). The main objective of the present study was to analyze the applicability of qPCR to detect PCV2 loads in pooled serum samples, with the purpose of increasing the diagnostic capacity of the qPCR technique. A second objective was to develop an easy-to-use PMWS qPCR-based diagnostic recommendation at the herd level, without the need to perform post-mortem examinations.

MATERIAL AND METHODS

Firstly, a theoretical model based on two qPCR viral load thresholds (3,4) to discriminate between PMWS affected and non-affected pigs was validated with an experimental pooling study considering pools of 3, 5, 10 and 20 randomly selected individuals. Secondly, pool sensitivities (PSe) and pool specificities (PSp) were estimated by means of Monte Carlo simulations. Such pooling included sera from diseased pigs with a PMWS diagnosis (PMWS), diseased pigs without fulfilling the diagnosis of PMWS (wasted-non-PMWS) and healthy (healthy) animals. Using only the PMWS diagnosed individuals and the wasted-non-PMWS, a second pooling approach considering targeted sampling (animals showing clinical signs compatible with PMWS) was simulated as an option to increase sensitivity at herd level. If targeted sampling is used, the probability of detecting the disease would be theoretically increased. The PSe and PSp obtained previously were used to estimate the sensitivities and specificities at herd level (HSe and HSp) for both approaches. Finally, we evaluated the capability of the model to discriminate between PMWS-affected and non-affected farms using qPCR real and simulated pools.

RESULTS

Several pools of 20 individuals did not report correct results in the experimental study, most probably caused by handling difficulties. Therefore, Monte Carlo simulations considered only pools of 3, 5 and 10 individuals. The best HSe and HSp estimations were obtained using a single pool of 10 individuals or two pools of 5 individuals under the random sampling scenario. Targeted sampling reported worst estimations than random sampling, mostly because wasted-non-PMWS individuals showed higher qPCR values than healthy ones, making in this case difficult the differentiation of truly affected and non-affected individuals. However, the model correctly discriminated and identified most of the PMWS-affected and PMWS-non-affected farms despite some false-positives and false-negatives were reported.

DISCUSSION

Assuming that the qPCR data used (3,4) are representative of the field situation, present results suggest that sample pooling may be a suitable strategy for PMWS diagnosis. Sera pooling offers several advantages compared with classical PMWS diagnostic methods. First, it represents a cost saving strategy, since more individuals can be analyzed using the same fixed analysis expenses. Second, the use of qPCR is performed in an easy-to-get sample and, therefore, it does not require histopathological expertise, indispensable with a necropsy based method.

ACKNOWLEDGEMENTS

This work was funded by the Projects No. 513928 from the Sixth Framework Programme of the European Commission, GEN2003-20658-C05-02 (Spanish Government) and Consolider Ingenio 2010 – PORCIVIR (Spanish Government). Martí Cortey holds a Beatriu de Pinós Postdoctoral Research Grant funded by the Government of Catalonia.

REFERENCES

Introduction
The emergence of highly virulent PRRS virus (PRRSV) strains in China in 2006\(^1\),\(^2\) was a milestone in the epidemiology of PRRS in Asia. Nowadays, genotype II strains of different virulence coexist with genotype-I strains\(^3\),\(^4\) in this continent. At present, AMERVAC\(^\circ\)-PRRS (a genotype-I vaccine) is licensed and marketed in many countries of Asia and, therefore, contact of vaccinated herds with highly virulent genotype-II strains can be expected. The present study was aimed to explore the safety and potential efficacy of AMERVAC\(^\circ\)-PRRS against highly virulent genotype-II strain.

Material and methods
Twenty-one 4-week-old, PRRSV-naïve pigs were randomly divided into three groups (G1, n=8; G2 n=8; and G3, n=5) and housed separately in biosafety level 3 facilities at CReSA. Pigs in G1 were immunized intramuscularly with AMERVAC\(^\circ\)-PRRS (2 ml of 10\(^{4.6}\) TCID\(_{50}\)/ml). G2 and G3 were kept as unvaccinated groups. At day 32 post-vaccination, G1 and G2 pigs were intranasally inoculated with 2 ml of (1x10\(^5\) TCID\(_{50}\)/ml) of the Asian highly virulent isolate PRRSV21 (99% similar in ORF5 to other highly virulent isolates of Asia). Pigs in G3 remained as uninoculated controls. For the following 21 days, pigs were clinically examined (C) and rectal temperatures (T) were taken. Blood samples were taken on days, 0, 3, 7, 10, 14 and 21 post-challenge (PC). RT-PCR was used to assess the virological outcome of the challenge. Pigs were euthanized and necropsied on day 21 PC. Statistics were done using StatsDirect 2.7.5 software.

Results
Vaccination did not produce any adverse or side effect. All inoculated pigs became infected as determined by RT-PCR and developed fever but differences were noticed among groups (Figure 1). Thus, G1 pigs suffered less days of fever (p<0.05) than G2 ones did. Also, naïve (G2) pigs developed evident respiratory signs from day 5 PC that often persisted until day 18 PC. Two G2 pigs died. In G1 pigs, signs were milder, of shorter duration and affected less number of pigs. At necropsy, lesions were more frequent and more severe in G2 pigs compared to G1 (Table 1). Findings compatible with bacterial complications (i.e., polyserositis and bronchopneumonia) were more common in G2.

Discussion
Vaccinated animals had clinical protection against the virulent challenge in spite of the different genotypes of the vaccine and the challenge isolate. This observation also suggests the existence of conserved epitopes among PRRSV genotypes.

References

Table 1. Main findings in necropsies of G1, G2 and G3 pigs.

<table>
<thead>
<tr>
<th>Lesion</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average pneumonia score (1-4)</td>
<td>1.0</td>
<td>2.2</td>
<td>0</td>
</tr>
<tr>
<td>Catarrhal bronchopneumonia</td>
<td>0/8</td>
<td>4/8</td>
<td>0/5</td>
</tr>
<tr>
<td>Interstitial nephritis</td>
<td>1/8</td>
<td>5/8</td>
<td>0/5</td>
</tr>
<tr>
<td>Gall bladder oedema</td>
<td>0/8</td>
<td>3/8</td>
<td>0/5</td>
</tr>
<tr>
<td>Fibrinous polyserositis</td>
<td>2/8</td>
<td>5/8</td>
<td>0/5</td>
</tr>
<tr>
<td>Arthritis</td>
<td>0/8</td>
<td>3/8</td>
<td>0/5</td>
</tr>
</tbody>
</table>

Figure 1. Clinical signs (shadowed areas) and body temperatures (lines) in groups G1-G3.
Introduction

Throughout the second half of 2006, large-scale, devastating outbreaks of porcine reproductive and respiratory syndrome (PRRS) affected several provinces of China\(^1\). That epidemic was linked to a hypervirulent genotype-II PRRS virus (PRRSV) harboring a distinctive deletion\(^1\). Economic losses from those outbreaks resulted from deaths but also from loss weight and impaired performance. Control of such outbreaks proved difficult and those hypervirulent strains spread in Asia. At present, genotype-I strains are also present in different countries of Asia. AMERVAC\(^®\)-PRRS (a genotype-I vaccine) is being marketed in Asia for prevention of these genotype-I infections. Is feasible that some herds vaccinated with AMERVAC\(^®\)-PRRS could confront the infection with a hypervirulent Asian strain. The present study was intended to determine if vaccination with AMERVAC\(^®\)-PRRS could produce some beneficial effect on performance of pigs infected with the hypervirulent strain.

Material and methods

Twenty-one 4-week-old, PRRSV-naïve pigs were randomly divided into three groups (G1 n=8, G2 n=8 and G3 n=5) and housed separately in isolation facilities (BSL3 at CReSA, Barcelona, Spain). Animals in G1 received intramuscularly (day 0) 2 ml of a European-type modified live PRRS vaccine (AMERVAC\(^®\)-PRRS containing 10\(^{4.6}\) TCID\(_50\)/ml). G2 and G3 pigs remained unvaccinated. At day +32, pigs in G1 and G2 were intranasally inoculated with 2 ml of 1x10\(^5\) TCID\(_50\)/ml of the strain PRRSV21 that was isolated from a highly virulent case of PRRS in Asia and share 99% similarity in ORF5 with other highly virulent genotype-II Chinese PRRSV strains. Pigs in G3 remained uninfected along the trial. Weekly body weight gain (BWG), average daily feed intake (ADFI) and clinical signs were assessed during the 21 days of the challenge phase of the study. Statistical analyses were done using a non-parametric Mann-Whitney test.

Results

After challenge, pigs in G2 suffered an evident respiratory distress starting the 5\(^{th}\) day post-inoculation (dpi) and lasting in most animals until the end of the trial. In G2, two animals died before the end of the study (25%). In G1 pigs showed milder respiratory signs and suffered no losses. Regarding BWG, groups were equal before the challenge but by 7 dpi animals in G2 showed a lower BWG compared to G1 and G3 (p<0.05) and these differences remained statistically significant along the trial. Significant reduction in ADFI was recorded in G2 pigs from 7 dpi to the end of the study. ADFI differences were remarkably high at the end of the trial (G2=0.44; G1= 1.57 and G3= 1.97 kg/day/animal). Thus, on average, comparing G1 pigs with unvaccinated-challenged pigs, vaccine prevented mortality (2/8 vs. 0/8) and allowed 8.9 kg more of weight gain for the 21 days period examined.

Discussion

PRRSV21 infection severely affected the growth performance of non-vaccinated pigs (G2), as expected from the virulent nature of that strain. In contrast, vaccinated pigs (G1) showed a better performance and lesser mortality indicating that vaccination with AMERVAC\(^®\)-PRRS was able to keep productive performance in the challenge model. The present results suggest that under field conditions, this vaccination may provide beneficial effects in economic terms if a vaccinated herd should confront an infection with a hypervirulent genotype-II strain.

References

**Introduction**

Successful control of PRRS often involves strategies for immunizing gilts and maintaining immunity in sows. The choice of a given strategy depends on the safety/efficacy balance. Objections about using a live PRRSV strain in gestating sows—except in the case of a severe PRRS outbreak—may coexist in the same farm with the use of live PRRSV. In Spain, another approach was tested in which gilts are firstly immunized by using live virus and then booster vaccination of sows is performed using PROGRESSIS®. This may avoid the use of live PRRSV in gestating sows. The present report examines the efficacy of such approach.

**Material and methods**

Thirty-two high health pigs free from all major pig diseases were selected at weaning in a PRRSV-free farm. Animals were confirmed to be free of PRRSV by ELISA (HerdChek 2XR, Idexx Laboratories) ear-tagged and let to acclimatize for two weeks. At six weeks of age, piglets were randomly allocated in four separated groups of eight animals: A, B, C and D and vaccinated with an adjuvanted modified-live PRRSV vaccine or a placebo. Three months later, piglets were revaccinated with PROGRESSIS (once or twice) or a modified-live vaccine (MLV) as shown in Table 1. At 6.5 months of age, all pigs were intranasally challenged with $1 \times 10^6$ TCID$_{50}$ (2 ml) of a Lelystad-like PRRSV strain and were followed for 21 days. Humoral response was evaluated by ELISA (Idexx) and the frequency of PRRSV-specific IFN-$\gamma$ cells in blood was measured by ELISPOT as reported before (1). After challenge viremia was examined by RT-PCR (2) at days 0, +3, +7, +10, +14, +21.

**Results**

No reaction after vaccination were observed showing safety of these treatments. Initial vaccination with the MLV vaccine produced seroconversion and raised the PRRSV-specific cell-mediated response (Table 2 and Figure 1). No differences were seen in the humoral response but the use of PROGRESSIS as a booster antigen was equal or better than the use of a repeated MLV administration (group B) in terms of maintaining the PRRSV-specific IFN-$\gamma$ response. Thus, at the time of challenge, the IFN-$\gamma$ response of animals of group C was higher ($p<0.05$) than the IFN-$\gamma$ frequencies of pigs in groups B and D. After challenge, the higher booster in IFN-$\gamma$ was seen in group C as well. By the end of the experiment, group A showed the higher IFN-$\gamma$ frequencies compared to other groups ($p<0.05$). RT-PCR results showed that all unvaccinated became viremic after challenge while in groups A, B and C only one or two pigs became infected by day 3 PI (data not shown).

**Discussion**

The present results show that the use of PROGRESSIS or MLV vaccine or combination of both gave similar results.

**References**


*PROGRESSIS is a registered trademark of Merial S.A.S., Lyon, France; HERDCHEK is a registered trademark of IDEXX Laboratories, Inc. in the United Kingdom and elsewhere.

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**Table 1.** Design of the experiment.

<table>
<thead>
<tr>
<th>Months of age</th>
<th>Group</th>
<th>1.5</th>
<th>4.5</th>
<th>5.5</th>
<th>6.5</th>
<th>7.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>MLV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>MLV</td>
<td>MLV</td>
<td>Pl.</td>
<td>Ch.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>MLV</td>
<td></td>
<td>Pl.</td>
<td>Ch.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Pl.</td>
<td>Pl.</td>
<td>Pl.</td>
<td>Pl.</td>
<td>Ch.</td>
<td>End</td>
</tr>
</tbody>
</table>

MLV = modified live vaccine; KV= PROGRESSIS, Pl.=Placebo; Ch. = Challenge
PERFORMANCE OF AN IMMUNOENZYMATIC ASSAY BASED ON THE DOUBLE RECOGNITION ELISA FOR EARLY DETECTION OF PRRS VIRUS INFECTION

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Introduction
Routine diagnosis of PRRS is generally done by means of antibodies detection against the virus, most often using indirect or blocking ELISA. With those tests seroconversions are usually detected at +14 post-inoculation (1,2). In other instances, RT-PCR is chosen as diagnostic test for PRRS. RT-PCR usually allows and earlier detection of infected animals compared to serology. Early diagnosis of PRRS is particularly crucial in two circumstances: entry of gilts in PRRS-free farms and testing of boars. INGENASA has recently developed a new ELISA based on the concept of double recognition (DR) of the antigen (3). In the Ingezim PRRS DR (11.PRS.K0) (Ingenasa) for PRRS, plates are coated with the viral protein N. Serum and protein N conjugated to horseradish peroxidase are added to the coated plates in one-step incubation. If PRRS virus (PRRSV)-specific antibodies were bound to the plate, the free binding sites of those antibodies will be able to bind the conjugated N-protein and thus, an increased sensitivity will be obtained after revealing the reaction with a colorimetric substrate. The aim of the present study was to evaluate the performance of the DR for early detection of anti-PRRSV antibodies raised against different genotype-I strains in experimental or field conditions. Comparison with HerdChek 2XR\(^{\circledR}\) (Idexx Laboratories) was also performed.

Material and methods
ELISAs. Two different ELISAs were used in the present study. In all cases, sera were examined by Ingezim PRRS DR (11.PRS.K0) (INGENASA) and for particular comparisons HerdChek 2XR\(^{\circledR}\) (Idexx Laboratories) was used. All tests were used as recommended by the manufacturer. Results were expressed as a ratio of the optical density (OD) of a given sample over the OD of the positive control provided by the test (S/P ratio).

Sera from experimental infections. Sera (n=38) obtained from five experimental infections (days 0 to 28 post-inoculation) of piglets (4 week old) with different PRRSV genotype I strains (S1-S5) were used. Selected strains shared from 90.6% to 96.0% of similarity in protein N. In all cases, animals were intranasally inoculated with ≥1x10\(^{5.0}\) TCID\(_{50}\)/ml. All sera were analyzed using DR. Samples for strains S4 and S5 were also examined by the HerdChek ELISA\(^{\circledR}\).

Cross-sectional profiling of an endemic farm. A cross-sectional serological profiling was comparatively performed in an endemic farm (pigs of 3, 6, 9, 12, 15 18 and 21 weeks of age) using both ELISAs.

Results
Sera from experimental infections. At day +7 PI, DR detected 29/38 sera as positive. Interestingly, for strains S4 and S5 HerdChek\(^{\circledR}\) did not detect any positive pig by day +7 PI while DR recognized all animals infected with S4 (8/8) and one pig in the group infected with S5 (1/8). By day +14 PI, all sera were positive in both HerdChek\(^{\circledR}\) and DR tests.

Cross-sectional profiling. Both DR and HerdChek\(^{\circledR}\) showed that PRRSV did spread explosively around 9-12 weeks of age as indicated by the seroprofile but DR had an increased sensitivity for detecting low levels of maternal antibodies or early infections as seen in 3-week-old piglets and in the higher proportion of seropositive pigs from week 15 afterwards (Figure 1).

Discussion
DR-ELISA allowed an earlier detection of experimentally infected pigs (76% at 7 PI). On the basis of the results in field conditions, DR also showed some enhanced sensitivity related to HerdChek\(^{\circledR}\). Present results indicate the suitability of the DR-ELISA for early detection of PRRSV infection.

References
Introduction
Spray-dried porcine plasma (SDPP) is used as a feed ingredient in diets of weanling pigs to provide beneficial effects on productive parameters. Some epidemiological studies have described a significant association of dietary SDPP with lower post weaning mortality and improved productive records in sows from a PRRS unstable farm. According to these studies the positive effect of SDPP could be related to a modulation of the inflammatory immune responses. The aim of the present study was to determine the effect of SDPP as a supplement in the diet on the outcome of experimental PRRS virus (PRRSV) infection.

Material and methods
Experimental design. 55 three-weeks-old piglets obtained from a PRRSV-free farm were randomly distributed in three groups: pigs in I and II were intranasally inoculated with PRRSV (10^6 TCID50/ml) and group III was kept as a control. Group I received a supplemented diet (8% SDPP, AP-820P from APC Europe, SA) from four days before challenge to 28 days post-inoculation (PI) and drinking water supplemented with 2% spray-dried porcine serum until 7 days PI.

Clinical follow-up and sample taking. Individual body temperatures were measured daily (from 0 to 14 PI). Sera samples were taken from 0 to 28 days PI and analyzed for PRRSV viremia by nested RT-PCR. The presence of antibodies to PRRS was checked by a commercial ELISA kit (HerdChek® PRRS 2XR, Idexx).

Histopathology. Serial examination of tissues was performed after humane killing of pigs at days 0, 3, 7, 14 and 28 PI. Severity of microscopic interstitial pneumonic lesions was semi-quantified following a previously reported scoring system.

Immunohistochemistry (IHC). IFN-γ and IL-1 were measured from apical and intermediate lobes of lungs by IHC as described before.

Statistics. Statistical analysis was performed with StatsDirect 2.7.5.

Results
Clinical follow-up. Temperatures of both infected groups showed a biphasic pattern of two peaks (day 4 PI and day 8-12 PI) of fever (≥40ºC). All infected groups seroconverted at day 14 PI and were positive by nPCR until 21 PI. Five of six pigs in group II and only two of five in group I were viraemic at day 28 PI (p=0.07).

Histopathology. Sum of interstitial pneumonia scores from day 3 to 28 PI showed significant differences among groups (II = 129 > I = 94 > III = 25; p<0.05). Interestingly, higher frequency of cells expressing IFN-γ and IL-1 (day 3 PI and days 3, 7 and 14 PI, respectively) were detected in apical and intermediate lobes for group I (p<0.05) (Figure 1). No significant differences were observed for IL-10.

Discussion
After the PRRSV inoculation, a significant lower interstitial pneumonia was found in the group receiving SDPP (I) (p<0.05). Besides, this group also showed less nPCR positive pigs at day 28 PI (p=0.07). Both facts suggest that pigs fed SDPP may have an earlier virus clearance compared to non-SDPP challenged group (II). The increase of cytokine expression (IFN-γ and IL-1) in lungs of group I points to a Th1 enhancement that might be related with the data discussed above. Results of the present report indicate that SDPP used in the diet may have a potential positive effect during a PRRS challenge.

Figure 1. Mean of cells by mm² expressing IFN-γ and IL-1 in apical and intermediate lobes.

References
**GENETIC VARIABILITY AND PHYLOGENY OF SWINE TORQUE TENO VIRUS 1 (TTV1) AND 2 (TTV2) BASED ON COMPLETE GENOMES**

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**Introduction**

Torque teno viruses (TTVs) are circular, single-stranded DNA viruses classified in the family Anelloviridae. They are currently considered non-pathogenic although they have been lately linked to several diseases in human and swine (1-3). The aim of this study was to determine the level of genetic variability in full-length genomes of swine TTVs, study the types and effects of mutations and to determine phylogenetic relationships with all known swine TTV genomes. For these reason 13 novel full-length genomes of swine TTVs were obtained from Europe and analysed together with 10 published genomes.

**Material and methods**

Sera samples used in the study were collected during 2005-2007 and stored in the CReSA sera collection. Full-length genomes of TTV1 and TTV2 were amplified with a proofreading DNA polymerase and sequenced by DNA walking.

**Results**

TTV genomes presented an untranslated region and three potential ORFs. Conserved regions like Arginin rich N-terminus and rolling-circle replication domains were found in all swine TTV genomes. TTV1 genomes showed a high amount of variable positions compared with TTV2 (46.3% vs 23.9%), that were differently distributed along the genome: lower in the UTR (13.2% for TTV1 and 11.4% for TTV2), and higher in the translated region (59.8% for TTV1 and 29.7% for TTV2). Low variability in the UTR region is most likely due to its importance in replication and transcription (4). The pattern of substitutions among ORFs was also different, more skewed for ORF1 and more evenly distributed for ORF2 and ORF3. TTV ORF1 seems to be affected by stronger levels of purifying selection compared with ORF2 and ORF3, most likely caused by the function of the protein products encoded by every ORF (5). TTV1 and TTV2 should be considered as species confirming the classification proposed (6). The phylogenetic reconstructions indicate that TTV1 is divided into three types. One of them (1c) is being reported here for the first time (Fig.1). Also, a certain kind of geographic structure among phylogenetic types may exist. Quite the opposite, the proposed subtypes for TTV2 clustered mixed and its definition was unclear.

**Figure 1.** Neighbor-Joining tree based on the percentage identity among 23 swine TTV genomes.

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**References**

**Introduction**

Torque teno virus (TTV) is a small, non-enveloped single stranded circular DNA virus classified in the floating genus Anellovirus, which infects several vertebrates including human and swine. Two genetically distinct genogroups have been described in swine, genogroup 1 (TTV1) and genogroup 2 (TTV2). TTV is ubiquitous and considered non-pathogenic but it has been linked to several diseases or pathological conditions in humans and, lately, in swine (1). To date, information related with the immunological events behind TTV infection is not available. Several viruses are known to modulate dendritic cell (DC) function. One example is porcine circovirus type 2 (PCV2), related genetically and structurally to TTV. It has been reported that PCV2 DNA is a potent modulator of DC and mediated inhibition of IFN-α production by plasmacytoid DC (pDC) (2). In the present work, the role of viral DNA from two genetically distinct swine TTV genogroups was analyzed for its potential interaction and modulation of DCs activity.

**Material and methods**

PCV2 and TTV genomes were isolated by digestion from plasmids containing double-stranded DNA forms of the complete viral genomes. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque differential centrifugation. pDCs were then enriched by magnetic antibody cell sorting (MACS). Enriched pDCs were incubated with decreasing amounts of PCV2, TTV1 or TTV2 DNA prior to stimulation with CpG-ODN. Supernatants from activated cultures were tested for IFN-α production by ELISA.

**Results**

Preliminary results showed that TTV1 and TTV2 viral DNA mediated inhibition of IFN-α production by CpG-ODN induced pDCs. The effect was dose dependent (Figure 1). TTV DNA was compared with DNA from PCV2, and it was observed that the inhibitory capacity of TTV1 was similar than the one observed for PCV2 and different from that of TTV2 (Figure 2).

**Discussion**

TTV viral DNA was found to interfere in the ability of swine pDC to produce IFN-α in response to known CpG-ODN stimuli (2). This study also reconfirms the effect or PCV2 DNA (2). The immunomodulatory effect observed for these viruses could potentially interfere in the development of efficient host innate and adaptative immune responses.

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**References**


ROLLING CIRCLE AMPLIFICATION FOR DETECTING ANELLOVIRUSES FROM PIG SERUM SAMPLES

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Introduction
Anelloviruses are vertebrate infecting single-stranded circular DNA viruses which have been recently linked to different swine diseases (1). In swine two genetically distinct Anellovirus genogroups are described, namely Torque teno virus 1 (TTV1) and Torque teno virus 2 (TTV2) (2). It is probable that novel swine genogroups exist as shown in human TTV counterpart. Rolling-circle amplification (RCA) is a novel technique used to amplify circular DNA templates using random hexamers, yielding 10^9 or more copies of a circular sequence in about an hour (3). The potential of this technique has been shown to be increased when combining it with Sequence-Independent Single Primer Amplification (SISPA) (4). The aim of our study was to apply the combined RCA-SISPA approach and optimize a novel RCA using Anellovirus-specific primers (Anello-RCA) to discover new members of the genus Anellovirus. Furthermore, the efficiencies of these two techniques were compared.

Material and methods
A swine sera sample resulted TTV2 positive by the common nested PCR was selected to test RCA-SISPA technique. Standard RCA involves the prolonged elongation of random primers bound to a circular DNA template by Phi29 DNA polymerase. The RCA products, that are tandem repeat copies of the viral complete genome, were used for the SISPA method, based on the use of endonuclease restriction of target sequences. These products were separated on agarose gel and different length products were ligated to specific linkers followed by PCR amplification and sequencing. Short Anellovirus-specific primers were corresponding to highly conserved region among all full-length Anellovirus genomes from several species available in the GenBank. Anello-RCA was optimized using short Anello-specific primers (6 nt) using swine sera known to contain TTV. Furthermore, Anello-RCA was applied using swine sera and plasma samples negative for TTV. The resulting products from Anello-RCA were used as template in inverse-PCR with Anello-specific PCR primers (12 and 15 nt in length). PCR products purified from agarose gel were sequenced.

Results
Typically the combined RCA-SISPA method resulted in several fragments of various lengths (Figure 1A). After sequencing 66 fragments 4.5% resulted positive for TTV demonstrating that the RCA-SISPA is applicable to detect TTV in swine sera. Anello-RCA approach resulted in fragments between 2 and 3 kb of length (Figure 1B). Sequencing of 12 fragments 50% resulted positive for TTV.

Discussion
Both methods described in this study are applicable for Anellovirus detection in swine samples. However, the RCA-SISPA is more tedious than Anello-RCA due to the high number of different size fragments to be analysed. Anello-RCA technique resulted in larger and fewer fragments for subsequent analysis and is therefore more straightforward and less laborious. However, Anello-RCA is only detecting Anelloviruses while RCA-SISPA could detect any circular DNA virus present in a given sample. Considering that the primers used in Anello-RCA are located in the highly conserved region, this technique could be applicable for Anellovirus detection in other species as well.

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References

Figure 1. Digestion products obtained using RCA-SISPA approach (A). Amplification patterns from inverse-PCR on Anello-RCA product (B).
Materials and methods

Cells and tissues. PK-15 cells were transfected with 0.5 µg/well (six-well plate) of cloned TTV2 genome and fixed on cover slips. Formalin-fixed, paraffin-embedded tissues (lymph node, lung, kidney and liver) from healthy and PMWS pigs were tested with a TTV semi-quantitative PCR (data not shown) and tissues with the highest amounts of virus were selected for ISH and FISH.

ISH and FISH. To prepare TTV2 specific probe for hybridizations, total TTV2 viral DNA was labelled with digoxigenin (DIG), previous digestion with XhoII and SmaI. Negative controls and specificity test of the TTV probe were: non transfected, pGFP (plasmid-green fluorescent protein) transfected, and PCV2 transfected PK-15 cells. The detection was made by an anti-DIG antibody and developed with NBT/BCIP as chromogen. The FISH method was performed using Cy3 labelled anti-DIG-antibody. In tissues, to enhance the signal, TSA (tyramine signal amplification) system was used. ISH and FISH methods were based on previously described protocols (5,6) respectively.

Discussion

ISH and FISH were successfully developed to detect TTV2 nucleic acid in PK-15 transfected cells. In contrast, no specific ISH and FISH positive signal was found in formalin-fixed, paraffin embedded tissues from TTV2 infected pigs. These tissues were positive by PCR; considering the lower sensitivity of ISH techniques versus PCR, it is likely that the TTV2 DNA amounts in the tissues were below detection limit of ISH. However, we can still not rule out if formalin fixation and paraffin embedding alters the detection by ISH or FISH. Nowadays, experiments are being done to determine the detection limits of the technique.

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References

Introduction

Torque teno virus (TTV) is a small, non-enveloped virus with a circular single-stranded DNA genome, belonging to the family Anelloviridae, genus Iotatorquevirus and widely distributed in different animal species and humans. In swine, two genetically distinct genogroups have been identified, Torque teno sus virus 1 (TTV1) and 2 (TTV2). No clear-cut pathogenic role of TTVs has been demonstrated so far. However, it has been shown that TTV2 prevalence is higher in postweaning multisystemic wasting syndrome (PMWS) affected pigs, and TTV1 has been linked to PMWS and a porcine dermatitis and nephropathy syndrome (PDNS)-like condition (1,2,3). Nevertheless, the TTV viral load in diseased animals has not been investigated. Therefore, the aim of the present study was to quantify TTV1 and TTV2 viral load in serum of PMWS-affected and healthy animals using a newly developed quantitative PCR (qPCR) method.

Material and methods

Serum samples of sixty animals ranging from 11 to 21 weeks of age included in a previous study on Porcine circovirus type 2 (PCV2) (5) were analyzed by TTV1 and TTV2 qPCRs. From them, 29 pigs were diagnosed as PMWS by histopathology and PCV2 in situ hybridization (ISH) (4), and 31 were healthy, age-matched animals.

A qPCR assay was developed using TTV1 and TTV2 D-Lux primers technology (Invitrogen). Standard curves were generated using ten-fold serial dilution (10^8 - 10^0 genomic equivalents) of purified TTV1 and TTV2 whole genome plasmids. Serum samples as well as standard dilutions were run in triplicates. The average log10 copies of TTV1, TTV2 and PCV2 per ml of serum were compared globally, and between healthy and PMWS pigs, using Mann-Whitney’s U and Kruskal-Wallis test. Significance was set at P<0.05. PCV2 viral load was obtained from data generated in the abovementioned study (5).

Results

PCV2 log10 copies/ml from the previous published work (5) as well as TTV1 and TTV2 log10 DNA copies/ml of serum in PMWS and healthy pigs are represented in figure 1. TTV2 DNA was detected and quantified in all the PMWS affected animals but not in all healthy animals. TTV1 was detected in most but not all the animals of both groups. In general, TTV1 viral loads in serum were similar in healthy and PMWS animals, with values comparables to TTV2 viral loads in healthy animals. On the contrary, PMWS animals had significantly higher (p<0.05) TTV2 viral load than healthy animals. PCV2 viral load in healthy animals was significantly lower than TTV2 loads. No significant differences were found between PCV2 and TTV2 loads in PMWS animals.

Discussion

Results from this study showed that TTV2 and TTV1 were highly prevalent among studied pigs. Moreover, the fact that TTV2 loads in serum were higher in PMWS affected than in healthy pigs would support the previously suggested association between TTV2 and PMWS (1). On the contrary, this relation was not observed for TTV1. However, results obtained in this study encourage further studies on the role of TTVs in pig diseases or disease triggering.

Figure 1. TTV1 and TTV2 log10 copies/ml of serum in PMWS and healthy pigs. PCV2 viral loads of studied animals are from Grau-Roma et al.

Acknowledgements

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References

Introduction

Torque teno viruses (TTVs) are small, non-enveloped viruses with a circular single-stranded DNA genome, belonging to the family Anelloviridae, genus Iotatorquevirus. TTVs are widely distributed and may infect human, non-primate and domestic species including pigs. In swine, two genetically distinct genogroups have been identified so far, TTV1 and TTV2 (1). The aim of the present study was to study the distribution and age-relation of swine TTV1 and TTV2 in tissues. Viral DNA obtained from tissue extractions were tested by a semi-quantitative PCR for the presence of both TTVs.

Material and methods

Thirty healthy animals corresponding to six different age-groups (second and last third of gestation, 5 days and 5, 15 and 24 weeks of age), 5 pigs per group, were sampled for 9 different tissues: brain, lung, mediastinal lymph nodes, heart, liver, spleen, kidney and bone marrow. Presence of TTV1 and TTV2 was tested with a semi-quantitative PCR, amplifying the virus in three different dilutions of extracted DNA (90 ng, 9 ng and 0.9 ng) at 43 cycles (optimal cycling for detecting the lowest standard concentration of 10^1 molecules). Negative samples at 43 cycles were tested at 50 cycles to enhance the detection sensitivity and ensure the negativity of the sample. The degree of TTV1 or TTV2 positivity was graded into highly positive (4), medium (3), low (2), very low (1), and negative (0). Contingency tables (Chi-square) were used to compare prevalence and grades of TTV1 and TTV2 in different samples at the different studied ages and in the different tissues. Statistical significance level was set at p<0.05.

Results

No significant differences were found between genogroup prevalence in the same tissue at any age, while the prevalence increased with the age reaching 100% in all the organs by 5 weeks of age onwards. The first three age groups had negative to low levels of virus, and the last three groups, medium to high levels. Negative tissues were only found in foetuses and 5 day-old pigs. The only TTV1 PCR negative organs in some pigs were kidney, mediastinal lymph node, brain and heart. For TTV2, those negative tissues were brain and heart, mediastinal lymph node in some pigs.

Discussion

Both TTV genogroups followed similar infection dynamics in all the tested tissues, increasing progressively in prevalence and virus load over the time. The highest prevalence (100%) was reached at 5 weeks of age, and the highest loads of virus in the different tissues were seen in the oldest animals (15 to 24 weeks of age). Those results are in agreement with an earlier study reporting increasing prevalence in sera in a longitudinal analysis (4). No animals were found to be negative to TTV, even foetuses, supporting the role of vertical transmission of TTV (2,3). In conclusion, the present study indicates that swine TTV1 and TTV2 can be found in tissues like brain, lung, mediastinal lymph nodes, heart, liver, spleen, kidney, mesenteric lymph nodes and bone marrow. Both swine TTV genogroups were present in high levels in most if not all adult animals while viral negative tissues were only found in 5 week-old pigs or foetuses.

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References

Introduction

Torque teno virus (TTV) is a non-enveloped virus with circular single-stranded DNA genome belonging to the floating genus Anellovirus. TTV was discovered in humans and later identified in other species including pigs. In pigs, two different genogroups have been described, genogroup 1 (TTV1) and genogroup 2 (TTV2). Swine TTV infection is ubiquitous and species-specific (1). However, its potential role on disease occurrence is yet to be defined. TTV infection has been associated with pigs affected by postweaning multisystemic wasting syndrome (PMWS) (1,2) and linked with development of a porcine dermatitis and nephropathy syndrome (PDNS)-like condition in pigs co-infected with TTV1 and porcine reproductive and respiratory syndrome virus (PRRSV) (3). Faecal-oral transmission route is considered the most common route of TTV dissemination but vertical transmission route may play an important role (4).

In the present work, a prospective study was designed to assess the prevalence of both swine TTVs in cases of abortions. Healthy foetuses collected from pregnant sows at slaughterhouse were used as age-matched controls.

Material and methods

A total of 98 abortions cases from 22 different Spanish farms and 55 foetuses collected from pregnant sows (from 14 farms) at slaughterhouse were studied. Foetal age was estimated and foetuses collected were classified according first (A) or second half (B) of gestation.

All foetuses were necropsied and relevant tissues were collected, pooled and submitted to DNA extraction. In order to evaluate the presence of TTV1 and TTV2, samples were analyzed by a specific polymerase chain reaction (PCR). Contingency tables were used to compare prevalence of TTV1 and TTV2 in different samples and association between gestation stage and TTV infection.

Results

Results on TTV prevalence are summarized in table 1. There were no statistically significant differences when comparing prevalence of swine TTVs by type of sample or by gestation stage.

Table 1. Prevalence of swine TTV1 and TTV2 in foetuses coming from abortions and from slaughtered sows with no reproductive problems. Foetuses were classified as from first (A) and second half (B) of gestation.

<table>
<thead>
<tr>
<th>Gestation</th>
<th>TTV1</th>
<th>TTV2</th>
<th>TTV1/TTV2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n=10)</td>
<td>2 (20%)</td>
<td>2 (20%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>B (n=88)</td>
<td>15 (17%)</td>
<td>27 (30.7%)</td>
<td>5 (5.7%)</td>
</tr>
<tr>
<td>A (n=22)</td>
<td>2 (9.0%)</td>
<td>8 (36.4%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>B (n=33)</td>
<td>4 (12.0%)</td>
<td>14 (42.4%)</td>
<td>3 (9.0%)</td>
</tr>
</tbody>
</table>

Discussion

The present work represents the first description of swine TTV infection in pig foetuses at different stages of gestation. Results obtained confirm that vertical transmission is an important route of TTVs dissemination in swine. In addition, data obtained suggest that swine TTV infection is commonly present in aborted foetuses and foetuses collected from slaughtered sows. However, TTVs should not apparently be considered as infectious agents responsible for abortion occurrence.

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References

A CASE ON CHRONIC SALT INTOXICATION IN GROWING PIGS

Núria Llanesa, Carmen Alonso, Joaquim Segalés

Introduction
Sodium (Na+) is necessary for the electrolytic balance in animals. Most ion sodium in the diet of different livestock species, including swine, is given by means of salt (sodium chloride). The standard recommendation of 0.2-0.25% meets the dietary Na+ and Cl- requirements of growing and finishing pigs for most meal diets. Sodium ion toxicosis is also called water deprivation or salt poisoning, and it is rather well documented in swine (1). The occurrence of this toxicosis is inversely related to water intake, usually related to water deprivation due to inadequate water supply or changes in husbandry. Based on available data, salt intoxication results in an acute or sub-acute clinical problem. Chronic salt toxicity has not been reported so far in pigs. Therefore, the objective of the present study is to report the clinical, pathological and laboratorial findings of a chronic salt intoxication case in growing pigs.

Materials and Methods
Pigs from a single-origin fattening farm located in North Eastern Spain displayed increased mortality. Historical mortality records in the finishers ranged between 5-7%, but in May 2008, mortality percentage increased dramatically to 49%. Approximately, 30% of the pigs developed central nervous disorders and 70% of them respiratory problems. Affected pigs displayed hyperesthesia, paddling and, usually, lack of palpebral reflex, with no fever. Animals with respiratory clinical signs (dyspnea) adopted dog-sitting position. Also, affected pigs showed paleness of the skin, and diarrhoea was identified occurring in all pens of diseased animals. A number of pigs were on-farm necropsied. Based on gross findings, oedema disease, postweaning multisystemic wasting syndrome (PMWS) and porcine reproductive and respiratory syndrome (PRRS) were suspected, besides the existence of concomitant bacterial-like pneumonia. Based on suspected etiologies, antibiotic was prescribed in water (neomycin) and several drugs were used parenterally (including enrofloxacyn, florfenicol and apramycin) in the different groups of affected animals. No significant response to antibiotic treatment was noted after 5 days of application. A number of laboratorial studies were implemented, including necropsy and histopathology of two pigs with central nervous clinical signs, antibody profile against PRRS virus, and urea and creatinine levels in serum. Further studies included determination in feed of heavy metals (cadmium and mercury), vitamin E, vitamin D, selenium, ochratoxine, oxalates and sodium chloride content were measured.

Results
The most significant pathological findings in both animals were a severe, bilateral, diffuse interstitial fibrosis in the middle and deep area of the renal cortex, with loss of glomeruli and glomerulosclerosis and a mild mononuclear multifocal meningo-encephalitis with very occasional presence of eosinophils. Both pigs were also positive to PRRS virus detection in lungs. Both pigs had very high levels of both urea and creatinine. Obtained results so far, mainly the diffuse renal fibrosis, pointed out to a potential chronic toxicity scenario. Among the feed analyses performed, all determinations were within the normal ranges, but the sodium content of the ration, which was 2.21% on dry matter (those pigs ingested 30 times more sodium than that recommended for dry feeding with free access to drinking water (2). To determine the origin of these abnormally high salt levels, feed mix compounds were analysed for sodium chloride. Among 8 sources of whey, 2 of them had values of 17.2 and 21.3% of salt on dry matter; the remaining whey samples had values from 3.5 to 10.6% on dry matter.

Discussion
The present case report describes a complex situation in which clinical signs pointed towards an acute process, while kidney pathological features suggested a potential chronic toxicity process. Therefore, once the clinico-pathological findings were obtained, laboratorial investigations were directed towards a potential intoxication process. The confirmation of extremely high levels of salt in the diet, not previously reported in the literature as far as the authors’ know, allowed confirming a diagnosis of salt toxicosis in the affected herd. Moreover, the presence of an apparent novel kidney lesion (characterized by diffuse cortical fibrosis and glomeruloscrosis) associated to this intoxication let us classify the clinical problem as chronic.

References
Introduction

Streptococcus suis is a major swine pathogen and a zoonotic agent causing mainly meningitis and septicemia. Serotype 2 is considered the most virulent and the most frequently type isolated from both in pigs and humans\(^1\). Mechanisms involved in the host innate and adaptive immune responses toward S. suis as well as the mechanisms used by S. suis to subvert these responses are unknown. The capsular polysaccharide (CPS) is the only proven critical virulence factor\(^1\). Dendritic cells are powerful antigen presenting cells that initiate the immune response against pathogens, and interactions between DCs and pathogens can strongly influence the outcome of a disease. DCs capture and process the antigens, and then migrate to the adjacent lymphoid organs where they activate T cells. In the present study, we used porcine bone marrow-derived DCs (bmDCs) to investigate the capacity of S. suis to interact with DCs and to induce their maturation and activation.

Material and methods

**Bacterial strains:** S. suis serotype 2 virulent strain 31533, originally isolated from a case of porcine meningitis, and its isogenic CPS-negative mutant B218 were used.

**Generation of bmDCs:** Bone marrow was removed from femurs of 7 week-old SPF piglets. Cells were obtained and characterized by morphology and appropriate receptors by FACS\(^2\).

**Phagocytosis assay and intracellular survival:** Bacteria were either not opsonized or pre-opsonized with complete normal serum. Phagocytosis was left to proceed for different incubation times before antibiotics were added into the wells for 1h to kill extracellular bacteria. Cells were then washed and lysed to determine viable intracellular bacteria. The intracellular survival of S. suis was performed in a similar manner, but antibiotics were added for a period times up to 6h. Cells were processed as described above.

**Microscopy analysis:** The interactions between S. suis and bmDCs were analyzed by scanning electron microscopy (SEM). Internalization of S. suis by bmDCs was confirmed by confocal microscopy.

**Cytokine measurement and expression of co-stimulatory molecules:** Levels of IL-6, IL-8, IL-12 and TNF-\(\alpha\) in cell culture supernatants following S. suis stimulation were measured by ELISA. The expression of MHC-II and CD80/86 by bmDCs were analyzed by FACS.

Results

**In vitro interactions:** S. suis interacts with bmDCs. This interaction is modulated by the CPS, as shown by SEM. Phagocytosis: The CPS interferes with the uptake of S. suis by bmDCs (Fig.1). Once internalized, both strains are equally destroyed.

**Cytokines and Co-stimulatory molecules:** S. suis triggers the release of IL-6, IL-8, IL-12 and TNF-\(\alpha\) as well as the expression of MHC-II and CD80/86 by bmDCs. However, the CPS-negative mutant induced higher levels of cytokine production and co-stimulatory molecule expression by bmDCs than the wild-type strain.

Discussion

- S. suis interacts with bmDCs, which results in an activation of these cells.
- The CPS highly interferes with S. suis internalization by and activation of bmDCs, as previously shown with other professional phagocytes\(^1\). This reduced level of antigen uptake/activation due to CPS might lead to a reduced host immune response.

References

Introduction

Infection and environmental conditions make inflammation a manifestation of respiratory disease in pigs that decrease the ability of the lungs to exchange gases. In fact, the inflammatory response may become so overwhelming as to be life threatening in itself (1, 2).

The benefits of therapeutic intervention with non-steroidal anti-inflammatory drugs (NSAIDs) are because of their properties as inhibitors of the actions, synthesis or release of inflammatory mediators. In addition, one of the effects of NSAIDs, such as acetyl-salicilic acid, is to act as antipyretics, improve the general clinical status of animals and increase food and water intake. The aim of this study was to investigate the clinical efficacy of acetyl-salicilic acid (AAS) as an adjunct to the antibacterial treatment of porcine respiratory disease.

Material and methods

142 piglets of 4 months of age were divided in two groups (experimental and control group). These animals were suffering a respiratory disease. The inclusion criteria was the presence of pyrexia (>39.7 °C) in the animals. Experimental group (A) received Doxycyline hlicate and acetyl-salicilic acid by drinking water at a dose of 10 mg/Kg (doxiporc®, Laboratorios Policherm, Spain) and 100 mg/kg of body weight (fiebrina porcino®, Laboratorios SYVA, Spain) for five consecutive days, respectively whereas control group (B) received only the antibiotic treatment. The animals were clinically examined before treatment (0 h/pretreatment) and at 1, 2, 3, 4, 5 and 6 days after treatment for rectal temperature (°C) and abdominal breathing, cough and depression. Each parameter was scored using a scale of 0 = normal, 1 = slight or moderate and 2 = severe according to Moore et al. (1986). A statistical analysis was performed by paired t-test using the statistical software SPSS System v15. The alpha level used for determination of significance for all analyses was $P < 0.05$.

Results

The experimental group showed always a mean temperature lower than the negative control group (group B) throughout the trial (see figure) and this fever decrease for animals receiving AAS (group A) is statistically significant at days 2, 5 and 6 of the trial ($p<0.05$) in comparison with animals not receiving AAS (group B). However, it was not observed significant differences for respiratory symptoms throughout the trial between both groups.

Discussion

NSAIDs are used to block the production and/or the effects of inflammatory mediators and modulators which may have a deleterious effect on alveolar exchange of gases (3). Results support the idea that AAS is an effective adjunct in the treatment of porcine respiratory disease complex to decrease fever. However, we did not observe a synergic effect to decrease respiratory symptoms in animals receiving antibiotic and AAS. Nevertheless, it was not possible to check the lung inflammation to observe the anti-inflammatory effect at lung level as observed in bovine for other NSAIDs (4).

References

Introduction
Gastric ulcers in pigs have been extensively studied (1). Annual mortality due to gastric ulcers is estimated to be between 0.5 and 0.75%. Therefore, its economical weight on the pork industry is high. The specific causes of gastric ulceration are not well known, but a number of factors have been pointed as risky. A severe case of acute gastric ulcers affecting several farms from an integration company located in a high density pig rearing area in North-Eastern Spain allowed the study of potential risk factors associated with such condition.

Material and methods
An estimated number of around 5000 pigs of a single integration pig company died because of acute gastric ulcers during a 4-month period. It was known that other pig producing companies in the area had also a similar problem. Most of affected pigs weighted between 30 and 40 kg and the gastric ulcer appeared when the adaptation diet was shifted to the growing diet at the fattening units. No previous clinical signs were found prior the gastric ulceration outbreaks. Most affected farms were in a good health conditions prior the appearance of the problem.

Acute gastric ulceration with massive haemorrhage appeared in 90% of the dead animals in affected farms. Mesenteric oedema, hepatomegaly and soft, orange-yellowish livers were found also at necropsy in 80% of the deaths. Mortality rate rapidly increased from 0.5-5% (normal mortality values in fatteners) to 30-40% in most affected farms.

A number of laboratorial analyses were performed, including necropsy and histopathology (with in situ hybridization to detect Porcine circovirus type 2 (PCV2)), haemogram, hepatic enzyme determination, measurement of vitamin E and selenium in blood of affected animals and feed, mycotoxins in pig livers and raw feed material, heavy metals in liver and perirenal fat, and a general toxicological analysis on feed, some raw feed material and pig livers. In the meantime, in order to counteract the problem, the feed particle size was increased in size (from 700 to 800 µm). Concomitantly, a dramatic change in the diet formulation was performed: reduction of wheat content from 45 to 25%, a new source of fibre was added (soybean hulls at 3%), sodium bicarbonate was added at a dose of 2 kg/ton and levels of vitamin E and selenium were increased from 10 and 0.2 to 40 and 0.5 ppm, respectively. Feed were served in mash form in most affected farms. Moreover, vitamin E and selenium were also given in pigs as injection. Finally, ranitidine (inhibitor of gastric acid production) was added in the water of some farms.

Results
Clinical response to farm actions: Injection of vitamin E and selenium had a positive response in some of the farms.
Pathology: Most consistent lesion, besides gastric ulceration, was massive hepatic centrolobular necrosis and or liver plate disruption with massive loss of hepatocytes, suggesting either a chronic intoxication or hepatosis dietetica. PCV2 was not detected in tissues of affected animals.
Haemogram and biochemical determinations: Most significant results were severe anaemia and increased levels of hepatic enzymes in serum.
Vitamin E and selenium determination: Low levels of vitamin E (<0.5µg/ml) and selenium (0.78µg/ml) were found (2).
Other analyses: No significant mycotoxins levels (but traces of aflatoxins) or heavy metals were detected in analysed samples. Toxicological analyses were indicative of peroxidation.

Discussion
Clinical response and performed analyses pointed to a deficiency in vitamin E and selenium. However, it is very likely that other factors should be involved in the triggering of the problem, since these vitamin E and selenium levels had been used in the company for more than 5 years. Since raw feed material prices increased dramatically by the end of 2007, it is speculated that the use of materials of lower price with less quality, low vitamin E bioavailability and higher levels of potential contaminants (mycotoxins, peroxides or others) may have accounted as major predisposing factors for the clinical situation observed.

References
Introduction

African swine fever (ASF) is a highly contagious hemorrhagic disease of domestic pigs. The disease was reported for the first time in Kenya in the 1920s, and actually remains endemic in Sardinia and in East and Southern Africa, producing important economical losses in the pig industry (1). The recent outbreak in Georgia last 2007, and its extension to some Russian counties, has increased the chances for the ASFV to be extended to European and Asian countries. Due to the absence of vaccines available against this disease, ASF control is based on an efficient diagnostic and the slaughter of infected animals.

The aim of the present work was to develop an optimized in situ hybridization (ISH) protocol to detect ASF virus genome in formalin-fixed paraffin-embedded tissues.

Material and methods

Animal samples

Tissue samples including liver, spleen, kidney, lung, and gastro-hepatic lymph node were obtained from experimentally infected animals using Ba71L and E75L virulent ASFV isolates.

ISH method

Three different digoxigenin (DIG) labeled probe approaches were used: a pool of DIG-oligonucleotide probes complementary to ASFV sequences, a 18.5kb SalI fragment (SD probe) of the genomic Ba71V DNA, and the total E75 viral DNA (CG probe). Two different proteolytic treatments were also tested: proteinase K and pepsin digestion.

Combined ISH and immunohistochemistry (IHC)

In order to verify the presence of ASFV DNA in the nucleus of lymphocytic-like cells, a double ISH-IHC protocol was performed in different tissues. T-lymphocytes were first identified by IHC using a polyclonal anti-human CD3 antibody (Dako). ISH was next performed using the CG probe after proteinase K digestion.

Results

Positive specific ASFV signal was obtained in most of ASFV-infected tissues tested either using SD or CG probes. Nonetheless, no signal was detected using the DIG-oligonucleotide probes. While the SD probe was able to detect the presence of ASFV DNA only in Ba71L infected tissues, the CG probe hybridized with both E75L and Ba71L infected tissues. Proteinase K treatment resulted more efficient than pepsin digestion, yielding a better tissue integrity and a lower background.

Regarding ISH staining distribution, intranuclear and perinuclear positive signal was mainly found in cells of the monocyte-macrophage cell lineage. Positive staining was also found in other cell types, including endothelial cells, hepatocytes, neutrophils, and T-lymphocytes (double staining performed).

Discussion

In the present study, a new optimised protocol to detect ASFV genome in formalin-fixed paraffin-embedded tissues has been developed. The viral distribution described in this study is in accordance with that previously described (2). However, this is the first time that intranuclear location of the ASFV DNA has been described in different cell types, such as macrophages and T-lymphocytes, from ASFV-infected tissues. This surprising result might be the in vivo reflection of an early step of intranuclear ASFV replication, as it has been previously described after the in-vitro infection of cell lines with the virus (3,4).

Presented results indicated the utility of this ISH protocol to study ASFV pathogenesis and open new avenues to be used in the future as a diagnostic tool.

Aknowledgements

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References

EFFECTS OF CARBOHYDRALASE SUPPLEMENTATION ON GUT MICROBIOTA AND IMMUNOLOGY OF GROWING PIGS

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Introduction
Exogenous carbohydrolase enzymes used in feed to improve animal performance, hydrolyse non-starch polysaccharides (PNA) from raw materials. A study conducted in our laboratory has shown that this type of enzymes modify the intestinal microbiota of growing pigs (dos Santos et al. 2009). These changes in microbiota could play a positive effect on intestinal health as suggested by Kim et al. (2003). The aim of our study was to determine the effects of enzyme supplementation on gut microbiota and immunological parameters of growing pigs.

Material and methods
Thirty-six pigs (25 kg BW.) were fed during 28 days with one of 4 different treatments based on the type of diet (WBR or C) and the supplementation or not (+ or -) with enzymes. The pigs were slaughtered to obtain samples of serum, bile and ileal mucosa for immunological studies. In addition, samples of ileal and caecal digesta and ileal mucosa were collected for microbiota studies. The microbiota composition was studied by RFLP-PCR. The serum IgA, IgG and IgM and bile IgA were examined by ELISA. The terminal ileum was used to study some immunological cellular markers by immunofluorescence (Table 1) and some morphological parameters by HE and PAS stains.

Results and discussion
WBR+ diet presented an increase in the mucosal biodiversity and in the villous height: crypt depth ratio compared to WBR- diet (Table 2). These results are in accordance with the gut health concept: a higher microbiota biodiversity promotes a lower aggression on the mucosa. The corn based diet supplemented with enzymes promoted an increase of neutral Goblet cells, a reduction of secretory IgA, intra epithelial lymphocytes (IEL) and CD3+ cells of villi compared to corn based diet without enzymes (Figure 1). An increase of secretory IgA, an increase of IEL and an increase in CD3+ cells can be indicative of a higher aggression of gut mucosa in the corn based diet without enzyme supplementation.

The biodiversity degree of ileal mucosa presented significant correlation with the bile IgA concentration (R=−0.42, p=0.001), the IEL of villi (R=−0.43, p=0.008) and neutral Goblet cells (R=0.53, p=0.03). Our results suggest that a more complex intestinal microbiota could be induced by changes of feed components and by the addition of exogenous enzymes. The beneficial effects of enzymes could be related with an improvement of the adaptive capacity of commensal microbiota as a natural barrier defence against the overgrowth of pathogens.

References
Introduction

Actinobacillus pleuropneumoniae (APP) is the causative agent of porcine pleuropneumonia. Pigs can become asymptomatic carriers of the organism in their tonsils for long periods. Attempts to eradicate APP from pig herds have been made with antibiotics but there is a paucity of information about the penetration of antibiotics in tonsils. Marbofloxacin (MB) is a fluoroquinolone widely used in veterinary medicine to control APP under field conditions. The goal of this study was to quantify the MB penetration in tonsils after applying several MB regimen dosages.

Material and methods

Pigs were randomly divided in three groups (control, P1 and P2) of ten animals each one. The control group received 2 mL of serum saline. On the other hand, P1 group received a dose of 2 mg of MB/Kg every day for three consecutive days and P2 group received a dose of 4 mg of MB/Kg every 48 hours two times. The animals were sacrificed 24 hours after finishing the treatment to obtain tonsils and serum samples. The concentration of marbofloxacin in sera and tonsils was quantified by an HPLC analytical method. Finally, It was also calculated the ratio MB tonsil concentration versus APP MIC90 value (0.03 µg/mL,) determined following the CLSI recommendations (4, 7). This ratio is one of the PK/PD efficacy parameters described for fluoroquinolones (5).

Results

Average marbofloxacin serum concentration (AMSC) was 0.16 and 0.24 µg/mL for the P1 and P2 group, respectively. Average marbofloxacin tonsil concentration (AMTC) was 0.50 and 0.70 µg/gr for the P1 and P2 group, respectively (see figure). Thus, AMSC and AMTC increased in a dose-dependent fashion. However, the tonsil MB versus serum MB concentration ratio was close to 3 independently of the dose administered to animals. Moreover, the MB tonsil concentration: APP MIC90 ratio was 16.6 and 23.3 for P1 and P2 groups, respectively.

Discussion

Marbofloxacin achieves a good penetration in tonsillar tissue, which compares favourably with tonsil/plasma mean concentration ratios reported for other fluoroquinolones (2,3). However, tonsil/plasma ratio observed for MB was very similar to the described by Esposito et al. (2006) for moxifloxacin in humans. A great deal of information is now available on the PK/PD relationships for fluoroquinolones. Ratios of 125 for AUC0--24:MIC and 10 for Cmax:MIC have been recommended to achieve high clinical efficacy for concentration-dependent antimicrobial drugs like MB. The MB tonsil:MIC ratio described is above the threshold value (10) that is associated with clinical efficacy for all the doses studied (6). Thus, all the posology regimens tested might be efficacious to eliminate APP from tonsils. Obviously, the information provided here paves the way to carry out studies whose main goal could be to detect APP in tonsils of animals receiving several posology regimens of marbofloxacin.

References

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Introduction
In Europe, imported human cases of hepatitis E virus (HEV) infection are declining while autochthonous cases although sporadic in nature have become more frequent (1). These autochthonous cases are most often caused by genotypes 3 and 4 of HEV whereas in endemic countries genotypes 1 and 2 are the commonest and cause epidemics transmitted through drinking water (2). In industrialized countries, the ubiquitous nature of the virus in domestic pigs and the genetic similarity between porcine and human HEV isolates support the notion of a potential zoonotic transmission of the infection. Thus, some human cases have been associated with the consumption of inadequately cooked meat products (3). In Spain, >90% of pig farms have HEV-seropositive pigs and the infection is suspected to be present from at least the decade of 1980 (4). The objective of the present study was to determine the percentage of pigs that could reach the abattoir being infected.

Material and methods
A longitudinal survey was conducted in six farrow-to-finish swine herds. Farms have been previously diagnosed as HEV positive. Twenty piglets per farm were serologically monitored from nursery to slaughter age (3, 7, 13, 18 and 25 weeks (wk) of age). Sera were analyzed for anti-HEV IgM and IgG antibodies using an in-house ELISA. At slaughter, liver (n=96) and bile (n=80) were taken and tested by RT-PCR. In order to compare HEV sequences, serum and faeces collected at 13wk of age from positive animals at slaughter, were also analyzed by RT-PCR.

Results
Anti-HEV IgM were firstly detected in pigs of 7wk of age in five farms whereas in the remaining farm, animals did not show anti HEV IgM until 13wk of age (farm 6). At slaughter age, for the first five farms between 50%-100% of pigs had seroconverted for anti-HEV IgG whereas in farm 6 only 5% of pigs were IgG seropositive. Six out of 96 livers (6%) and five out of 80 bile (6%) were HEV positive by RT-PCR. None of the pigs was simultaneously positive in liver and bile and therefore, the total percentage of infected animals was the sum of both cases (12%). Interestingly, positive animals from five farms had already seroconverted at slaughter, whereas positive animals from farm 6 – the one seroconverting later – had not seroconverted yet. All RT-PCR product sequences corresponded to HEV genotype 3 and shared 83.5-98.7% nucleotide similarity among them or with other human or porcine HEV isolates. Three infected pigs (farms 3 & 4) were also positive in faeces at 13wk of age. The sequences from one of them had 85.8% nucleotide identity between 13wk and slaughter sequences, whereas another pig had 93.3% nucleotide identity between them.

Discussion
The present study demonstrates that HEV could be present in slaughter age pigs (12%). Other papers reported values ranging from 1.3% to 11.5% (5-7). In the present case, three of the infected animals were already RT-PCR positive at 13wk. This finding suggests either that HEV persisted in that pig longer than expected or that pigs might be infected more than once with the same or a different HEV strain during its productive life. Also, as seen in farm 6, if infections take place late in the pig’s life, infected but seronegative pigs could reach the slaughterhouse. In Spain, consumption of viscera is very low (0-2.9 grams/person/day) and thus, the risk of acquiring HEV by consumption of infected pig livers should be extremely low. However, manipulation or cross-contamination of HEV infected livers could be considered a potential route of transmission.

References
Introduction

Long pentraxin 3 (PTX3) is a conserved pattern-recognition molecule and a host-defense-related component of the humoral innate immune system. PTX3 is produced in a variety of cell types and tissues, most notably dendritic cells (DC) and macrophages, in response to Toll-like receptor engagement and inflammatory cytokines. PTX3 regulates the complement cascade, facilitates the pathogen recognition via cellular receptors (opsonisation) and plays a regulatory role during inflammation. Moreover, PTX3 is related to female fertility in human and mice (1, 2, 4).

PTX3 has an antiviral role in early host defence against influenza infections (5) and it might be a useful biomarker of acute lung injury (4) in mice. No information on PTX3 role in pigs is available at the moment.

The aim of our study was to investigate the role of PTX3 as a biomarker in vivo and in vitro in pigs.

Material and methods

For in vitro study, porcine bone marrow-derived DC were generated and infected with a H3N2 swine influenza virus (A/Swine/Spain/80598-LP1/2007).

For in vivo study, sixteen conventional pig farms with history of pleuritis lesions and cranio-ventral lung consolidation were used (3). Blood from 20 randomly selected pigs from each farm was collected and selected acute phase proteins were determined in serum. Hp was quantified by a spectrophotometric method (haemoglobin binding assay). Pig-MAP levels were assessed with an ELISA kit (PigCHAMP ProEuropa, Segovia, Spain). Finally, CRP was determined using a commercial immunoturbidimetric method (Olympus System Reagent, OSR 6147). Moreover, sera from 48 conventional sows from parity one to eight showing high swine influenza virus (SIV) antibody titres were also used.

PTX3 concentration in sera and culture supernatant was determinate by sandwich ELISA (2C3 and 6B11 antibodies) as previously described (8).

Statistical analysis was performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). A linear regression between different variables was carried out and a Kruskal-Wallis test was used to compare PTX3 serum levels between different sow parities. All this statistical analysis was performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA).

Results

The in vitro study showed that porcine myeloid bone marrow-derived DC produced PTX3 after infection with porcine influenza virus H3N2.

The in vivo results have shown that PTX3 serum levels are correlated neither with Hp, CRP or Pig-MAP nor with lung lesions score in conventional swine farms. PTX3 concentration in SIV antibody positive sera of studied sows increased with the parity number showing statistical tendency between the youngest and the oldest sows (p=0.07). Moreover, there was no statistical correlation between SIV antibody titres and PTX3 amount in serum.

Discussion

PTX3 is produced by myeloid DC in human and mice and the present study confirmed in vitro production on porcine myeloid DC. In vivo results have shown that PTX3 is not a good biomarker for chronic lung lesion status in swine farms when compared with Hp and Pig-MAP (7). However, PTX3 concentration in SIV antibody positive sera correlated with a greater exposure to infections, indicating that PTX3 might be used as a biomarker under certain conditions. Further studies should be performed to elucidate its possible practical application. This is the first analysis of PTX3 role in pigs and future studies will determine its contribution in porcine innate immunity.

References

Introduction

Actinobacillus suis (A. suis) is considered an early colonizer of the upper respiratory tract, which may also cause fatal cases of septicaemia in young piglets. A. suis infection has been also described in adult animals with fever, inappetence and erysipeloid skin lesions (1). However, in high-health farms this pathogen has been linked as well to different pathological conditions including arthritis, pneumonia, enteritis, meningitis, abortion and endocarditis in pigs at different ages (2). Besides, A. suis infection can cause very similar lesions to those of Actinobacillus pleuropneumoniae (App). In fact, both pathogens share some virulent factors including Apx toxins, urease, and iron-regulated outer membrane proteins. Moreover, distinction of A. suis isolates from App biotype II ones can be difficult if it is not confirmed biochemically or by molecular biology techniques. This high similarity between both pathogens may sometimes lead to erroneous diagnosis (2).

Little information on the A. suis prevalence in animals of different ages and clinical conditions is nowadays available. Therefore, the goal of the present study was to gain insight into A. suis prevalence in upper and lower respiratory tract samples from pigs with different clinical conditions.

Material and methods

Forty seven pigs were included in this study. From these, 34 pigs (from 6 to 18 weeks of age) were submitted for necropsy to the Pathology Diagnostic Veterinary Service at the Veterinary School of Barcelona (Spain) with different clinical signs (mainly, growth retardation, digestive and respiratory problems). The remaining pigs were 13 6-week-old healthy pigs. The following samples were collected from each animal: nasal swab (NS), tonsillar swab (TS), tonsillar brush (TB), tonsil tissue (T), bronchial swab (BS) and lung (L). TB was not taken in 9 of the 47 animals. Lung was always taken from the accessory lobe, independently on the presence or absence of lesion.

DNA from swabs and tissues was extracted using commercial kits and processed according to the manufacturer’s instructions. In order to differentiate between A. suis and App, samples were tested with two PCRs. One to detect the Apx IV gene (3), present exclusively in all App serotypes, and another to detect the Apx II gene (4), present in A. suis and in most of App serotypes. The Apx IV PCR was done in a singleplex mode in all samples but when ambiguous results were obtained the nested PCR was performed (5).

Results

Apx II and IV toxin genes were detected in a number of animals showing different clinical signs. Positivity was found in samples from the upper (NS, TS, TB and T), but not in those from lower respiratory tract (BS and L). On the other hand, all tested samples from healthy animals were PCR negative to both toxins. Apx II was more frequently detected (15 out of 47 [32%]) than Apx IV (9 out of 47 [19 %]). In both cases, the number of PCR positive samples per animal varied from one (mainly TS) up to 3 (TS, TB and T or NS). From the 47 tested animals, 31 (66%) were negative to both Apx II and IV PCRs in all the tested samples. Nine (19%) out of 47 pigs were PCR positive to both Apx II and IV at least in one sample. Finally, 6 (13%) animals were positive only to Apx II PCR while only 1 (2%) was positive exclusively to Apx IV.

Discussion

Detection of Apx II in absence of Apx IV amplification in some samples would indicate A. suis infection in such animals. These preliminary results suggest that A. suis detection in upper respiratory tract by means of direct PCR is feasible and relatively frequent in animals of different ages and showing different clinical conditions. The best sample to be tested by direct PCR resulted to be the TS or TB.

The concomitant detection of both toxins in some animals together with the existence of an animal in which only Apx IV was found, indicated the presence of different App serotypes in the samples. More studies will be useful to assess the role and prevalence of A. suis in porcine respiratory pathologies.

References

SEROPEVALENCE AND RISK FACTORS OF SWINE INFLUENZA IN SPAIN


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Introduction
Swine influenza is caused by type A influenza viruses. The three commonest swine influenza virus (SIV) subtypes are H1N1, H1N2 and H3N2 although other less frequent or sporadic subtypes can infect pig farms as well H4N6 (2). In Spain, previous serological studies revealed high seroprevalences for H1N1, H1N2 and H3N2 viruses in sows (2) and fattening pigs (3). Nevertheless, available information indicating risk factors for the introduction and spread of the infection in farms is very much scarce. The main objectives of the present study were to estimate the seroprevalence of H1N1, H1N2 and H3N2 subtypes in sows and fattening pigs and to explore the potential risk factors associated with seropositivity to SIV.

Material and methods
Between 2007 and 2009 a cross-sectional survey was carried out in swine farms of pig-producing regions of Spain. Sampling was designed to guarantee a 95% probability of detecting at least one positive animal for an assumed within-herd seroprevalence of 50%. As a result 100 swine farms located all over Spain were examined. Epidemiological data were gathered through on-farm interview of the farmer based on “closed-ended” questions. For each farm, blood samples were collected from 14 sows and 20 finishers, totaling 2,249 pigs for the whole survey.

Blood samples were analyzed by means of an indirect ELISA (CIVTEST Influenza, HIPRA) directed to detect antibodies against SIV nucleoprotein that was performed according to the manufacturer’s instructions. A hemagglutination inhibition (HI) test was standardized for H1N1 (A/swine/Neth/Best/96), H1N2 (strain A/swine/Gent/7625/99) and H3N2 (strain A/swine/Neth/St.Oedenrode/96) according to procedures previously described (4). IH titres ≥ 80 were considered as positive. SPSS 14.0 statistical program was used to explore risk factors associated with swine influenza using the method of generalized estimating equations (GEE).

Results
By ELISA, all farms had at least one seropositive animal, and in total 1,435/2,249 animals yielded positive ELISA results (63.8%; IC95%: 61.8-65.8%). Seroprevalence of sows was 77.8% (IC 95%: 75.5-79.9%) significantly higher (p<0.05) than seroprevalence in fattening pigs (40.9%; IC95%: 37.6-44.2%). Average seroprevalence in positive farms was 68.3%. A total of 1,231 animals (54.7%; 95% IC: 52.7-56.8%) were positive in the HI test. Again, sows had a significantly higher (p<0.05) seroprevalence compared to fattening pigs (72.9%; IC9%: 70.6-76.3 against 24.7%; IC95%: 21.8-27.6). Table 1 summarizes the results obtained by individuals and age group. In the epidemiological analysis, four variables (open vs. solid partitions between pens, pig density, natural vs. forced ventilation and the percentage of replacement rate) were identified as risk factors.

Discussion
This results shows that SIV is widespread in Spain. The high seroprevalence observed against H1N1 and H3N2 compared to H1N2 could be explained by a reduced ability to spread the latter. The higher seroprevalence observed in sows could be explained by the fact that these animals have a long lifespan. As a consequence, sows would have more chances to be exposed to SIV. The epidemiological analysis also show results obtained in GEE have shown that major contact between animals favor SIV circulation. Low air renewal enhances a major concentration of SIV in environment, which explains higher seroprevalence in farms without forced ventilation. Finally, an increasing % of replacement, could favor a higher number of susceptible animals to SIV infection.

References

Table 1.

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Number and percentage of positive animals

SEROPREVALENCE AND RISK FACTORS OF SWINE INFLUENZA IN SPAIN...
Introduction

Pleuritis and cranio-ventral pulmonary consolidation (CVPC) are the most frequent pathological findings in pig lungs at slaughter (3). Several environmental factors and infectious agents have been associated with the occurrence of lung lesions in swine. Mycoplasma hyopneumoniae and Actinobacillus pleuropneumoniae are considered to be the most important primary bacterial respiratory pathogens (6). On the other hand, the acute phase protein (APP) response plays an important role in lung infection (5). Therefore, the purpose of this study was to investigate the relationship between the existence of lung lesions in pigs at slaughter and the concentration of three APPs, haptoglobin (Hp), pig-major acute protein (Pig-MAP) and C-reactive protein (CRP).

Material and methods

Twenty-four farms were used for the present study. Pleuritis lesions were assessed based on the Slaughterhouse Pleurisy Evaluation System (SPES score). An A. pleuropneumoniae index (APPI) was defined to provide information on the prevalence and severity of pleuritis lesions as previously described (1). Farms were classified as "pleuritis negative (P-) or positive (P+)" if they had low or high APPI values, respectively. Moreover, farms were classified also as "CVPC negative (M-) or positive (M+)" if the percentage of lungs with these lesions at farm level were lower or higher than 60%, respectively. CVPC lesions were evaluated using a previously described method (2). Selected farms were divided into four combined groups (P-M-, P-M+, P+M-, P+M+), including 6 farms per category. Blood from 20 randomly selected pigs from each farm was collected and selected APPs determined in serum. Hp was quantified by a spectrophotometric method (haemoglobin binding assay). Pig-MAP levels were assessed with an ELISA kit (PigCHAMP ProEuropa, Segovia, Spain). Finally, CRP was determined using a commercial immunoturbidimetric method (Olympus System Reagent, OSR 6147).

A non-parametric test (Mann-Whitney) was used to evaluate the effect of pleuritis or CVPC lesions on the concentration of APPs. Finally, a Receiver Operating Characteristic (ROC) analysis was carried out to determine the capacity of discrimination of APPs in farms classified as P- or P+ or M- or M+ and when the four groups were taken into account (P-M-, P-M+, P+M-, P+M+).

Results

All APPs concentrations were significantly higher for M+ farms than for M- ones. However, only Hp and Pig-MAP showed significantly higher values for P+ farms than for P- ones (p<0.05). Pig-MAP was the most sensitive indicator since it was able to clearly discriminate between P-/P+ and M-/M+ groups (p<0.001 in both cases). Hp was an excellent marker for pleuritis and good for CVPC lesions. CRP was able to discriminate for CVPC lesions but not for pleuritis. ROC analysis showed that Pig-MAP was the best biomarker, with 67.8% sensitivity and 78.8% specificity to discriminate between P-M- and P+M+ farms.

Discussion

There is little information in the literature about the relationship between serum APP and the degree of lung lesions of pigs at slaughter (4). The present study shows that both Pig-MAP and Hp can be used as unspecific markers for the presence of pleuritis and/or CVPC lesions at slaughter. None of the tested APPs was able to discriminate both types of lesions; such result is not surprising, taking into account to their unspecific role in the inflammatory response. It is very important, however, to note that pigs involved in the present study did not show any clinical sign of disease at slaughter, and lesions were identified in a retrospective evaluation by abattoir surveillance.

References

T CELL AND NEUTRALIZING ANTIBODY RESPONSES TO SYNTHETIC DENDRIMERIC PEPTIDES DERIVED FROM E2 AND NS2-3 PROTEINS OF CLASSICAL SWINE FEVER VIRUS


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Introduction

Classical swine fever virus (CSFV) is the causative agent of one of the most devastating porcine viral diseases; classical swine fever (CSF). There is a current need for efficient and safer marker vaccines to assist in the control of future CSF outbreaks (1). Multimerization is a nature-mimicking strategy of antigen presentation. Indeed, pigs vaccinated with a dendrimeric peptide against foot-and-mouth disease virus, did not develop significant clinical signs upon challenge and inhibited replication and excretion of virus (2). Here, we describe the design and construction of three dendrimeric peptides, that integrates B (three different epitopes from E2 protein) and T (from NS2-3 protein) cell epitopes, and their capability to induce T cellular and neutralizing humoral immune responses against CSFV challenge in domestic pigs.

Material and methods

The dendrimeric peptides were synthesized by Stepwise Solid Phase Peptide Synthesis on a Rink Amide ChemMatrix resin using standard Fmoc chemistry. P1: 4B (aa 694-712)_1T (1-446-1460), P2: 4B (aa712-728)_1T and P3: 4B (aa829-842)_1T. To evaluate the immune response induced by the dendrimeric peptides, four groups of six domestic pig were placed in four different sections of the BSL3 animal house from CRESA (24 pigs in total). Three of these groups were inoculated twice, at day 0 and at day 21, by intramuscular injection (i.m.) with 1.4 mg of P1, P2 and P3, respectively. Peptides were emulsified with Montanide V206 adjuvant (Seppic). The fourth group of animals was used as the control (non-vaccinated pigs).

Ten days after the last immunization, all pigs were challenged with 10^6 DICT- of CSFV (strain Margarita) (3). The clinical signs of disease were scored daily and pigs were bled weekly to follow the specific antibody induction by neutralization peroxidase-linked assay. Peptide specific antibodies were detected by indirect ELISA. E2 specific antibodies were detected using ELISA (IDEXX). Evaluation of the interferon gamma (IFN-γ)-producing cells was performed as described (4). The presence of CSFV RNA in serum was analyzed using the Q RT-PCR real time assay (5).

Results

Immunization of pigs with dendrimeric peptides induced dendrimer-specific antibodies in the three groups of immunized pigs, mainly in those that received dendrimer P1. These dendrimer-specific antibodies not recognize the E2 CSFV protein in ELISA, and were not neutralizing. On the other hand, the three dendrimeric constructions were able to induce IFNγ-producing cells in immunized pigs after two doses, being the higher response also observed with dendrimer P1. After viral challenge, non vaccinated pigs developed fever and severe clinical signs of CSF from day 3 pi. Contrary, immunized groups showed lower score values, mainly in pigs immunized with dendrimer P1 in two animals of this group. Induction of ELISA antibodies against E2 protein and neutralizing antibodies, as well as a decrease in viral charge at 13 dpi were observed. On the other hand, the highest values of IFN γ-producing cells in these animals was observed at 13 dpi.

Discussion

Multimerization of peptides, such as that provided by dendrimeric constructs, has been recognized as an effective tool for enhancing peptide immunogenicity (2). In this work, we report that immunization of pigs with dendrimers P1, P2 and P3 induces non-neutralizing peptide-specific antibodies, as well as IFN γ-producing cells, the latter probably due to the NS2-3 T cell epitope included in their formulation). With the three dendrimers, a significant lower score of CSF clinical signs after viral challenge was observed compared to that of naive animals. Interestingly, two of the animals immunized with dendrimer P1 developed neutralizing antibodies and showed the highest values of IFN γ-producing cells after CSFV challenge, suggesting that dendrimer P1 can induce efficient T cell and humoral responses that correlated with protection against CSFV.

References

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