**Serum acute phase proteins as biomarkers of pleuritis and cranio-ventral pulmonary consolidation in slaughter-aged pigs**

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**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**

Potential use of long pentraxin 3 (PTX3) as a biomarker in pigs

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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 determined 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
P.347

Development of lung macrophage subpopulations in potbellied minipigs after weaning

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Introduction
Respiratory diseases are one of the main problems in human and animal health. Several models to study the respiratory immune system has been used, but humans and pigs share the unique feature of a non-constitutive bronchus-associated lymphoid tissue (BALT) (1-2). In spite of its importance, no studies on lung macrophage (Mf) development have been carried out. We examined the development of Mf in blood, bronchoalveolar lavage (BAL) and lung parenchyma (LP) of pigs after weaning by flow cytometry.

Material and Methods
Five 5 weeks-old weaned pigs (WP), five 3 months-old (3m) and four 5 years-old (adults) SPF potbellied mini pigs from the animal unit of CINVESTAV (UPEAL), compiling with local and federal regulations of animal care and with no vaccination protocols running, were used. PBMC were obtained by Ficoll/Isopaque gradient, BAL cells were obtained by tracheobroncheal lavage with PBS and lung parenchyma cells (LP) were obtained after enzymatic digestion of fresh tissue. Mouse monoclonal antibodies to pig CD172a (IgG1 -MIL-2-), CD14 (IgG2b -74-22-15-) and MHC-II (IgG2a -MSA-3-) were used with fluorochrome conjugated goat anti-mouse secondary antibodies (IgG1-PE, IgG2b-APC, IgG2a-FITC). The cells were run in a FACScalibur (B-D) flow cytometer. Relative and absolute numbers of positive cells were calculated and the results were analyzed by ANOVA.

Results
The proportion (%) of CD14+MHC-II+ Mf was higher in all tissues of WP (WP>adults=3m) (Fig 1A). CD14+CD172a+cells were higher in 3m PBMC (3m>WP), and LP of adults (Adults>WP=3m) (Fig 1B). CD14+ double positive (DP) cells were higher in all tissues of 3m and adults (3m=adults>WP) (Fig 1C) and CD14+ double negative (DN) cells were higher in WP PBMC (WP>3m) and adult LP (Adults>3m) (Fig 1D). Absolute numbers of CD14+MHC-II+ were higher in PBMC (WP>3m), BAL and LP of WP (WP>3m=adults, Fig 1E). CD14+CD172a+ were higher in PBMC of 3m pigs (3m>WP, Fig 1F) only. CD14+DP cells were higher in 3m BAL (3m>WP) and LP (3m>WP=adults, Fig 1G) and CD14+DN cells were higher in WP PBMC (WP>3m=adults) and LP (WP>3m, Fig 1H).

Discussion
Mf subpopulations in the lung undergone important changes in proportion and number with age. At least four different CD14+ subpopulations were identified. MHC-II+ and CD172a+ cells may have different roles since they tend to inversely change with age (Fig 1A, E & B, F). DP cells tend to increase with age (Fig 1C, G) in contrast with DN cells (Fig 1 D, H). MHC-II+ may correspond to activated Mf, CD172a+ is a SIRP molecule, related to activation control (3-5). DP cells may be related to fully differentiated Mf and DN to scavenger non-activated cells. Their role in lung protection remain to be elucidated. These data also show evidence of important differences among tissues.

Figure 1. Relative (A-D) and absolute (E-H) numbers of CD14+ macrophage subpopulations in PBMC, bronchoalveolar lavage (BAL) and lung parenchyma (LP) of weaned (WP), 3 months-old (3m) and 5 years-old (adults) SPF potbellied mini pigs (n=5/group). Bars show the mean plus SEM. ANOVA test. *P<0.05, **P<0.01, ***P<0.001.

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References
Interaction between Mycoplasma hyopneumoniae and fumonisin B1 toxin in the porcine respiratory tract

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Introduction
Respiratory disease is one of the most important health concerns for swine production. The term porcine respiratory disease complex (PRDC) refers to a condition in which an interaction between various pathogens and inappropriate environmental conditions lead to severe respiratory disorders (1). In this study we examined the possible synergy between M. hyopneumoniae (Mh) and fumonisin B1 (FB1) toxin in the lung of pigs. Computed tomography (CT) proved useful for imaging pneumonia (3). CT was applied to follow up the pathological events in the lung.

Materials and Methods
Twenty-eight 3-day-old pigs, obtained from a high health-status herd, were used in the study. The arrival of the pigs was day 0 of the study. After an early weaning, the piglets were artificially reared receiving milk-replacer until day 16 and then appropriate solid feed until the termination of the experiment (day 58). Four groups of 7 pigs were formed:

Group 1: uninfected untreated controls
Group 2: uninfected - treated with FB1-toxin
Group 3: infected with Mh - untreated
Group 4: infected with Mh - treated with FB1-toxin

Group 3 and 4 were infected with Mh (strain Mp 496) on day 30. The inoculum was prepared by suspending 1.5 g Mh containing lung tissue in 13.5 ml phosphate buffered saline (pH 7.2). Pigs were inoculated intra-tracheally with 0.5 ml of the suspension containing 109 colour-changing units per ml. Groups 2 and 4 were fed with FB1-contaminated feed (10 mg/kg) from day 16 until termination. CT images of the whole lung were acquired at days 16, 30, 44 and 58 using a SIEMENS Somatom Emotion 6 multislice CT scanner. At termination, the pigs were humanly killed and lung lesions were evaluated post mortem (4). CT images were analysed by Medical Image Processing V1.0 software. Affected were compared with and non-affected areas.

Results
From day 37 the infected animals showed clinical signs (coughing, laboured breathing and huskiness). CT examination did not reveal lesions in the negative control pigs (group 1) and the FB1-treated group (group 2) suggesting that the toxin did not induce macroscopic lesion at this dose level. In the Mh infected groups (groups 3 and 4) lung lesions were found in all animals on day 44. The most pronounced lesions were observed in the cranial, middle, and the accessorial lung lobes and in the front of the caudal lobe. The lung lesions appeared first around the small respiratory airway passages, later whole lobules and lobes became affected. Necropsy did not reveal lung lesion in the negative control pigs (group 1). In group 2, mild interstitial oedema was observed in four animals. In the Mh infected groups (group 3 and 4) we found acute catarrhal pneumonia in all animals. The macroscopic lung lesions were more pronounced in group 4 than in group 3. Significant differences were detected between non-infected and infected groups by CT evaluation.

Discussion
The Mh infection produced lung lesions in young piglets that were increased by treatment with FB1 toxin. The only lethal case and the highest rate of lung lesions were found in group 4 that received FB1-toxin treatment beside the bacterial infection indicating that the FB1-toxin increased the severity of pneumonia caused by the bacterial pathogen. These findings confirm the idea of PRDC that various factors may act in harmony to produce pneumonia. Our results also indicate that CT can be applied for studying the pathological conditions in the lower respiratory tract of swine.

References
Virulence genes and PFGE types of Pasteurella multocida field isolates from pigs in South Korea

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Introduction
Pasteurella multocida is widely recognized as the causative agent of progressive atrophic rhinitis and hemorrhagic septicemia in swine and is of considerable economic importance to the pig rearing industry throughout the world. The present study reports the frequency, features and molecular epidemiology of P. multocida isolates from diseased and finished pigs.

Materials and Methods
A total of 140 P. multocida isolates was collected from 751 cases of diseased and finished pigs during 2007-2009 in South Korea. The isolates were analyzed by PCR for the capsular type (A, B, D, E, F) and the presence of virulence genes. The 4 virulence-associated genes were detected with varying frequency. Genotyping of the isolates were performed by Pulsed field gel electrophoresis (PFGE)

Results
The most prevalent capsular type among P. multocida isolates was capA (54.1%), followed by capD (45.9%) from diseased pigs. However, from finished pigs, the prevalent of capA (97.5%) was higher than capD (2.5%). A higher percentage of toxA was detected among diseased pigs (12.5%) compared with strains isolated from finished pigs (3.8%). Among clinical isolates hgbB and tpbA was more frequently identified in strains isolated from diseased pig (95.8%, 54.2%) whereas 78.8% and 18.8% of the finished pig isolates harboured this gene. However, pfh A gene detected more frequently from finished pigs (50.0%) than diseased pigs (16.7%). The PFGE patterns of the isolates revealed five different genotypes.

Discussion
These data enforce further virulence and epidemiological studies, examining the properties of porcine P. multocida strains as well as factors of the porcine hosts themselves, which might be involved in disease susceptibility.

References

Figure 1. Detection rates of virulence-associated genes.