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# Porcine Acute Phase Protein Concentrations in Different Diseases in Field Conditions

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With 5 figures and 2 tables

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## Summary

Five acute phase proteins (APPs) [C-reactive protein (CRP), serum amyloid A (SAA), haptoglobin (Hp), pig-MAP and albumin] were measured in pigs with naturally occurring infections by porcine reproductive and respiratory syndrome virus (PRRSV), Aujeszky's disease virus (ADV), porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae*, and in animals with tail and ear bites, arthritis and other acute inflammatory processes. Healthy specific pathogen-free (SPF) pigs were used as controls. In PRRSV-infected pigs, all APPs with the exception of pig-MAP exhibited significant changes compared with controls. In animals affected with ADV only Hp presented changes of statistical significance, whereas pigs with PCV2 showed marked modifications in all APPs tested. Animals affected with Mycoplasmosis showed concentrations of all positive APPs significantly above levels obtained in SPF pigs, though albumin concentrations did not differ from controls. Finally, all APPs studied showed substantial changes in pigs with acute inflammation. The results indicated that an acute phase response was developed in the different diseases studied, this response being higher in animals with clinical signs and concurrent bacterial processes. Haptoglobin would be the APP that better reflects pathological states; however, to get more complete and valuable information it might be advisable to perform APPs profiles including another APP, such as CRP or SAA.

## Introduction

Testing for serum acute phase proteins (APPs) seems to be a tool for the monitoring of the state of health and welfare in pigs (Petersen et al., 2004). Specifically, APPs have been used in pig production to distinguish healthy, subclinically diseased and clinically diseased pigs (Petersen et al., 2001), to evaluate antibiotic treatments (Lauritzen et al., 2003a,b; Carroll et al., 2004), to detect the effect of medication on growth and to assess the effects of production stressors (weaning age, pen density, feeder space limitation) (Francisco et al., 1996a).

Porcine APPs have been mainly evaluated in experimental situations, such as infections with *Actinobacillus pleuropneumoniae* (Hall et al., 1992; Heegaard et al., 1998; Hulten et al., 2003), *Bordetella bronchiseptica* and *Pasterella multocida* type D (Francisco et al., 1996b), porcine reproductive and respiratory syndrome virus (PRRSV) (Asai et al., 1999), *Mycoplasma hyorhinis* (Magnusson et al., 1999), *Toxoplasma gondii* (Jungersen et al., 1999), or *Streptococcus suis* (Knura-Deszczka et al., 2002); or inflammation induced by injection of turpentine (Lampreave et al., 1994; Eckersall et al., 1996) or lipopolysaccharide (Dritz et al., 1996). In contrast, little is known about porcine APPs expression occurring in field conditions. Thus far clinical signs have been correlated with increases in haptoglobin (Hp) or C-reactive protein (CRP) concentrations (Bürger et al., 1992; Petersen et al., 2002a,b). Furthermore, most APPs studies in pigs include only the analysis of selected APPs, although the use of APPs profiles, combining positive and negative APPs determinations, has been highly recommended (Toussaint et al., 1995).

The objective of the present study was to assess the acute phase response in different diseases in field conditions by using APPs profiles. Thus CRP, serum amyloid A (SAA), Hp, pig-MAP and albumin were quantified in pigs with different natural occurring infections [PRRSV, Aujeszky's disease virus (ADV), porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae*], and in pigs with acute inflammation (tail and ear bites, arthritis and ulcerated umbilical hernia).

## Materials and Methods

### Animals and samples

All pigs used in this study came from farms located in southern Spain. Seventeen clinically healthy 10-week-old pigs from a specific pathogen-free (SPF) farm were used as control animals. The farm was free from PRRSV, ADV, pathogen serotypes of *A. pleuropneumoniae* and *Sarcoptes scabiei var. suis*. For the clinical study, the following animals were used for APPs analysis:

1. Ten 5-week-old pigs with slow growth, respiratory symptoms and positive to PRRSV by PCR from a PRRSV-affected farm and declared ADV free. These pigs were also bled 5 weeks later to assess seroconversion.
2. Seven 17-week-old pigs positive by serology to ADV without clinical signs from a farm included in the Aujeszky eradication program of Murcia region. The farm suffered an acute outbreak of the disease as approximately 50% of the herd was seronegative in that moment but seroconverted 1 week later. All seven pigs had been serologically negative to ADV at 10 weeks and subsequently vaccinated at ages of 10 and 12 weeks with a deleted Aujeszky's disease vaccine (Auskhipra cepa Bartha; Hipra, Gerona, Spain).

3 Ten 16-week-old animals with clinical signs (growth retardation) and positive to specific immunoglobulin (Ig)M and IgG of PCV2 from a post-weaning multisystemic wasting syndrome affected farm. The disease was also confirmed histopathologically; thus pigs showed lymphoid depletion, interstitial pneumonia and granulomatous inflammation in lymphoid tissues.
4 Ten 21-week-old pigs with respiratory symptoms caused by *M. hyopneumoniae* infection coming from a declared free-Aujeszky farm. Animals included in this group were serologically tested at 21, 26 and 29 weeks to assess antibody production, as time-span between infection and seroconversion can take 3–8 weeks (Morris et al., 1995).
5 Sixteen 15-week-old pigs with acute inflammation (nine with tail and ear bites, five with arthritis, one with a rectal prolapse and one with an ulcerated umbilical hernia).

All animals were bled from a jugular vein and blood samples were placed into 7-ml sterile vacutainers (Vacutainers™ Z, Plymouth, UK). Serum was obtained by centrifugation at 3000 ***g*** for 10 min, pooled and stored at −20°C until analysed.

### Determination of serum acute phase proteins

Porcine CRP was determined by using a commercial ELISA kit (Phase™ Range; Tridelta Development Ltd, Maynooth, Ireland).

Serum concentrations of SAA were measured by using a commercially available ELISA kit (Phase™; Tridelta Development Ltd).

Serum concentrations of Hp were quantified by using a spectrophotometric method with commercial kit (Phase™ Range Haptoglobin Assay; Tridelta Development Ltd). The assay was performed according to the manufacturer's instructions on an automated analyser (Cobas Mira Plus; ABX Diagnostics, Montpellier, France). This equipment was also used for albumin determination with a colorimetric assay (Spinreact, Gerona, Spain).

Finally, serum Pig-MAP levels were assessed with an ELISA kit (PigCHAMP Pro Europa S.A., Segovia, Spain).

All samples were analysed in the same analytical run to avoid the imprecision previously reported for the analysis of CRP and SAA (Cerón et al., 2004).

### Serum virus and antibodies detection

The detection of PRRSV was carried out with PCR by using a high pure viral nucleic acid kit (Roche, Penzberg, Germany).

The presence or absence of antibodies to PRRSV, ADV, Mycoplasma and PCV2 was measured by using different ELISA techniques.

Antibodies to PRRSV were tested with the HerdChek* PRRS Virus antibody test kit 2XR (IDEXX Laboratories, Westbrook, ME, USA).

Detection of specific antibodies against glycoprotein E of ADV was carried out by using a blocking ELISA (CIVTEST SUIS ADVgE; Hipra laboratories, Gerona, Spain), which allows differentiation between the vaccinated pigs and those infected with a field strain of ADV.

Mycoplasma antibodies were detected with the CIVTEST™ Suis *Mycoplasma hyopneumoniae* (Hipra Laboratories, Gerona, Spain).

Finally, PCV2 diagnosis was performed by using a capture immunoenzymatic assay for specific IgG and IgM of circovirus (Ingenasa, Madrid, Spain).

The assays were performed according to the manufacturer's instructions and known positive and negative sera were included as controls in each plate.

### Statistical analysis

Data analysis was performed by using a statistical software program (Version 11.5.1, SPSS Inc., Chicago, IL, USA). The significance level was settled at 0.05. After a Kolmogorov–Smirnov test demonstrated a non-parametric distribution of the data, a Mann–Whitney *U*-test was used to compare APPs concentrations in controls and clinical cases.

## Results

A summary of the median and 25th and 75th percentiles values for each protein in the different groups of animals figures in Table 1.

### C-reactive protein

Results of measuring CRP levels in sera from healthy and diseased pigs are shown in Fig. 1. It can be appreciated that SPF pigs exhibited the lowest CRP concentrations, pigs infected with ADV showed small but non-significant CRP increases (2.3-fold), and finally pigs with PRRSV, PCV2, *M. hyopneumoniae* infection and acute inflammation had a significant increase (9-fold, 26-fold, 71-fold and 38-fold respectively) in their CRP levels.

Table 1. Median, 25th and 75th percentiles of CRP, SAA, Hp, Pig-MAP and albumin concentrations for the controls and cases

| | CRP | SAA | Hp | Pig-MAP | Albumin |
|---|---|---|---|---|---|
| SPF | | | | | |
| Median | 5.32 | 3.10 | 0.21 | 0.76 | 3.21 |
| 25th percentile | 0 | 0 | 0.03 | 0.60 | 2.83 |
| 75th percentile | 24.57 | 6.32 | 0.48 | 1.18 | 3.38 |
| PRRSV | | | | | |
| Median | 47.96** | 7.36* | 1.41** | 1.05 | 2.79* |
| 25th percentile | 10.65 | 4.01 | 0.6 | 0.73 | 2.68 |
| 75th percentile | 89.33 | 9.59 | 2.24 | 1.52 | 2.84 |
| Aujeszky | | | | | |
| Median | 12.19 | 0.27 | 1.81** | 1.08 | 3.35 |
| 25th percentile | 10.14 | 0 | 1.36 | 0.89 | 2.66 |
| 75th percentile | 175.53 | 7.79 | 2.44 | 1.14 | 3.83 |
| PCV2 | | | | | |
| Median | 139.04*** | 72.4*** | 5.03*** | 3.32*** | 2.48** |
| 25th percentile | 110.62 | 61.96 | 4.09 | 2.54 | 2.22 |
| 75th percentile | 228.86 | 97 | 5.78 | 3.73 | 3.03 |
| *Mycoplasma hyopneumoniae* | | | | | |
| Median | 380.95*** | 16.24*** | 3.5*** | 2.16** | 3.24 |
| 25th percentile | 233.09 | 7.49 | 3.27 | 1.25 | 3.13 |
| 75th percentile | 415.39 | 43.26 | 4.45 | 3.29 | 3.76 |
| Inflammation | | | | | |
| Median | 203.15*** | 26.05** | 4.06*** | 2.55*** | 2.82* |
| 25th percentile | 174.49 | 13.26 | 2.65 | 1.67 | 2.58 |
| 75th percentile | 206.65 | 184.62 | 5.75 | 3.55 | 3.11 |

CRP, C-reactive protein; SAA, serum amyloid A; Hp, haptoglobin; SPF, specific pathogen free; PRRSV, porcine reproductive and respiratory syndrome virus; PCV2, porcine circovirus type 2.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

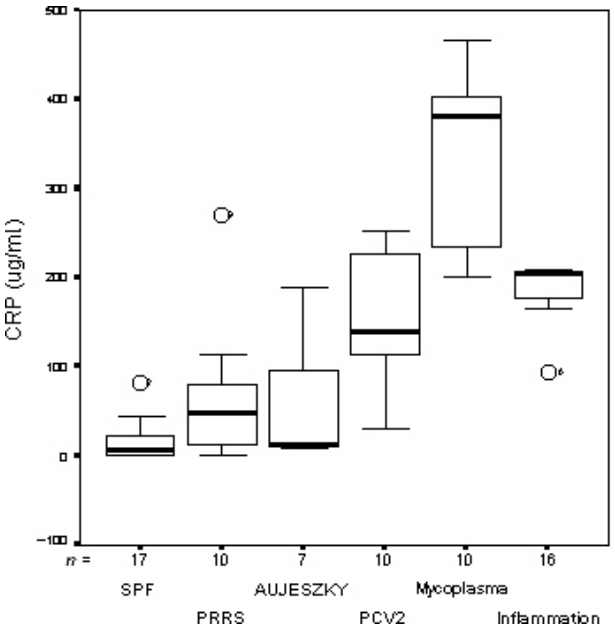


Fig. 1. Porcine c-reactive protein concentrations in different groups of animals. The median is marked with a line, the box shows the 25th to 75th percentile, the whiskers show maximum and minimum values, and the outliers are marked with circles.

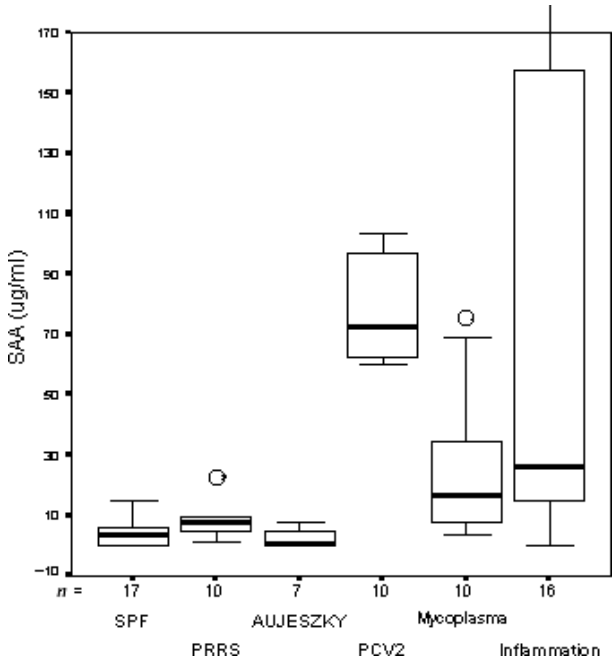


Fig. 2. Serum amyloid A levels in specific pathogen-free pigs and diseased pigs (whiskers explanation in Fig. 1).

## Serum amyloid A

Specific pathogen-free pigs showed SAA levels that did not exceed 15 $\mu$g/ml and similar values were observed in pigs positive to ADV; however, pigs infected with PRRSV and pigs with PCV2, *M. hyopneumoniae* infection and acute inflammation showed SAA concentrations significantly increased (3-fold, 30-fold, 6.8-fold and 10.9-fold respectively) (Fig. 2).

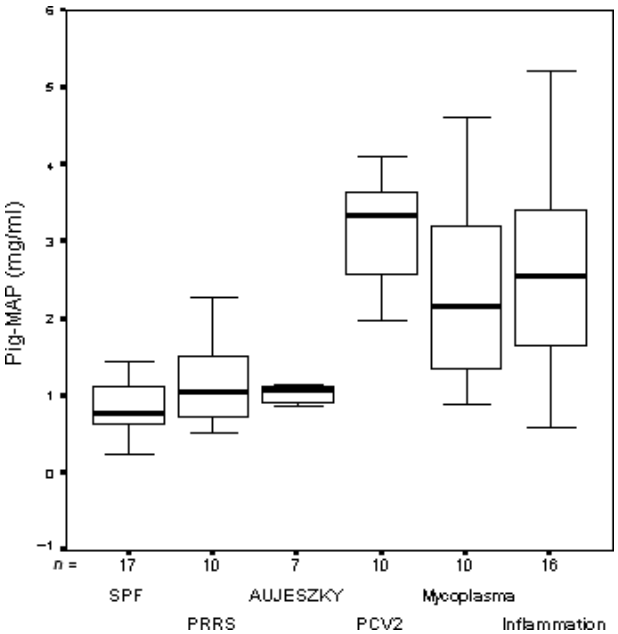


Fig. 3. Pig-MAP levels in pigs under different field conditions (whiskers explanation in Fig. 1).

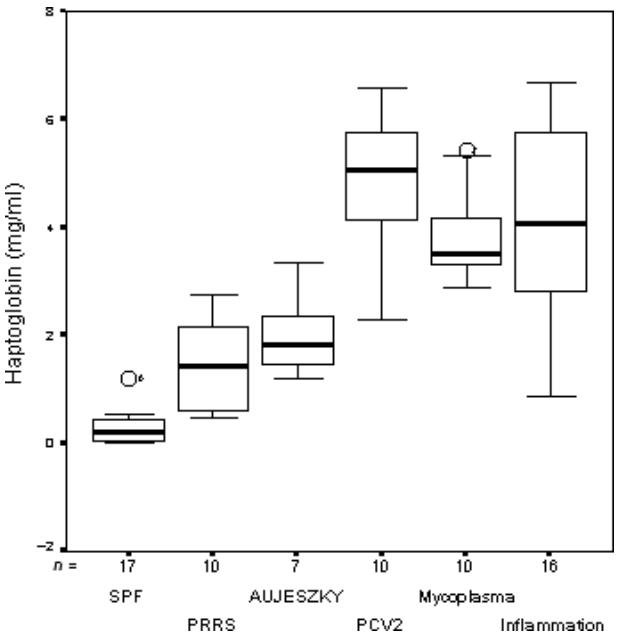


Fig. 4. Haptoglobin concentrations in healthy pigs and pigs with different pathological processes (whiskers explanation in Fig. 1).

## Pig-MAP

Pigs infected with PRRSV and ADV presented pig-MAP concentrations that did not differ statistically from controls. Nonetheless, pigs with PCV2 (4.4-fold), with respiratory symptoms caused by *M. hyopneumoniae* (2.8-fold) and acute inflammation (3.3-fold) showed protein levels significantly higher than control pigs (Fig. 3).

## Haptoglobin

This protein showed a marked increase in its concentration in all pathological states evaluated (Fig. 4). The increase was

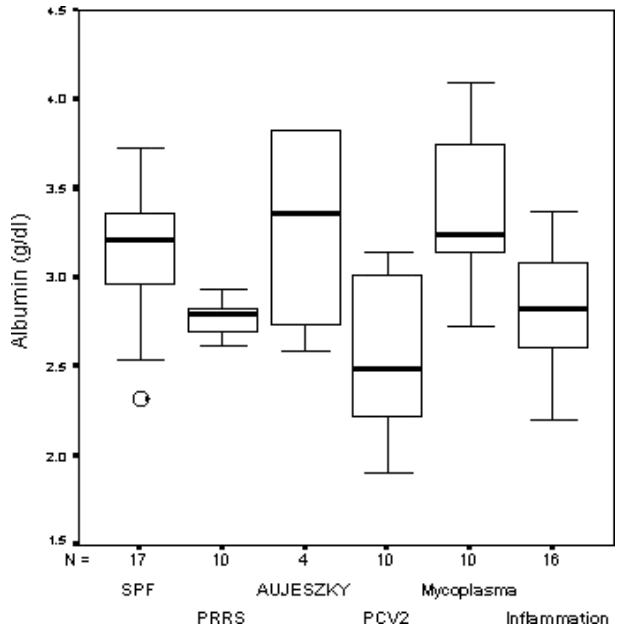


Fig. 5. Albumin concentrations in clinically healthy specific pathogen-free pigs and diseased pigs (whiskers explanation in Fig. 1).

higher for pigs with PCV2 signs, mycoplasmosis and inflammation (23.9-fold, 16.6 fold and 19.3-fold increases) than in viral infections by PRRS or Aujeszky (6.7-fold and 8.6-fold).

### Albumin

Albumin showed reduced concentrations compared with controls in pigs with PRRSV (0.87-fold), PCV2 (0.77-fold) and different traumas (0.88-fold) (Fig. 5) that were statistically significant. However, the protein kept similar values to SPF pigs, in animals infected with ADV and *M. hyopneumoniae*.

### Serodiagnosis of the different infectious diseases evaluated

Results of the serological screenings are presented in Table 2. It can be appreciated that six out of 10 PRRSV-affected pigs showed negative results at 5 weeks; however, all of them had positive results 5 weeks later. All animals tested for ADV were negative at 10 weeks but positive at 17 weeks. Nine pigs with clinical signs of PCV2 were positive to IgM and IgG and one was negative to IgM and positive to IgG. Finally, the 21-week-old pigs with respiratory symptoms were seronegative for *M. hyopneumoniae*, some of these animals seroconverted at 26 weeks and they all were seropositive at 29 weeks.

## Discussion

Use of APPs to assess the hygienic status and welfare of pigs is of great interest. Recently, European projects have been developed to harmonize the methods for APPs measurement (European Concerted Action number QLK5-CT-1999–0153) or for the APPs application in pig production (Shared Cost Project QLK5-2001-02219). Acute phase proteins present several advantages with regard to other inflammatory markers, such as cytokines or total leucocyte numbers. Cytokine expression, such as Interleukin-6, is brief and of less magnitude than APPs (Hulten et al., 2003). Compared with leucocytes, APPs may offer an improved diagnostic sensitivity as pigs with clinical signs and severe pathological lesions did not develop leucocytosis but had elevated APPs concentrations (Baarsch et al., 2000; Lauritzen et al., 2003b). In addition, they are more stable than blood cells and assays can be performed on frozen serum or plasma samples.

In the present study, pigs infected either with virus or bacteria, or those with acute inflammation were evaluated. Pigs naturally infected with PRRSV did not show increased pig-MAP levels compared with controls, however, they exhibited significant changes in the remaining APPs tested. There is a previous report (Asai et al., 1999), in which increases in Hp concentrations (3- to 4-fold) were also detected following experimental PRRSV infection and after exposure to the virus in a farm with chronic PRRS. Concerning pigs infected with ADV, only haptoglobin exhibited changes of statistical significance compared with controls. Carpintero et al. (2005) also detected a minor APP response in pigs experimentally infected with ADV that had been previously immunized.

In both viral diseases (PRRSV and Aujeszky) APPs increase was of less magnitude than in the rest of diseases assessed in the present study. This finding could be attributed to the

Table 2. Detection of antibodies to PRRS virus, ADV, *Mycoplasma hyopneumoniae* and PCV2 by using different ELISA techniques in porcine serum

| PRRSV | | | Aujeszky's virus | | | PCV2 | | | *M. hyopneumoniae* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pig | 5 weeks | 10 weeks | Pig | 10 weeks | 17 weeks | Pig | IgM | IgG | Pig | 21 weeks | 26 weeks | 29 weeks |
| 1 | − | + | 1 | − | + | 1 | + | + | 1 | − | + | + |
| 2 | − | + | 2 | − | + | 2 | + | + | 2 | − | + | + |
| 3 | + | + | 3 | − | + | 3 | + | + | 3 | − | + | + |
| 4 | + | + | 4 | − | + | 4 | + | + | 4 | − | − | + |
| 5 | + | + | 5 | − | + | 5 | + | + | 5 | − | + | + |
| 6 | + | + | 6 | − | + | 6 | + | + | 6 | − | + | + |
| 7 | − | + | 7 | − | + | 7 | + | + | 7 | − | + | + |
| 8 | − | + | | | | 8 | + | + | 8 | − | + | + |
| 9 | − | + | | | | 9 | + | + | 9 | − | + | + |
| 10 | − | + | | | | 10 | − | + | 10 | − | − | + |

(+), positive result; (−), negative result; PRRSV, porcine reproductive and respiratory syndrome virus; ADV, Aujeszky's disease virus; PCV2, porcine circovirus type 2; Ig, immunoglobulin.
Shading columns represent pigs groups in which acute phase proteins were determined.

aetiology of the process as in human medicine, it was demonstrated that bacterial infections cause higher CRP levels compared with viral infections (McCarthy et al., 1978; Peltola, 1982). Furthermore, a subacute state of disease or the previous immunization in the Aujeszky's pigs group could also contribute to the low acute phase response found.

Despite being a viral disease, pigs with PCV2 showed a high and significant acute phase response, which has been previously reported for Hp and pig-MAP (Segalés et al., 2004). It is possible that the robust APPs expression is because of the acute state of the disease, as confirmed by increased levels of IgM and IgG (Martínez et al., 2005). In addition, PCV2 is characterized by a systemic inflammation (Rosell et al., 1999) with frequent co-infection with additional bacterial or mycoplasmal pathogens (Chae, 2005).

The most significant increases in positive APPs in our study were found in animals with respiratory symptoms, infected with *M. hyopneumoniae*. The airway damage caused by mycoplasma often leads to secondary infections by bacterial pathogens, such as *A. pleuropneumoniae* and *P. multocida* (Calsamiglia et al., 2000). It is possible that the high APP levels detected in our study were due in part to concurrent infections. We had not found former studies about APPs measurements in pigs infected with *M. hyopneumoniae*, though it has been reported that *M. hyorhinis* infection caused an increase in serum Hp concentration (Magnusson et al., 1999). Overall, these results would support that APPs increase more significantly in animals at an acute stage of disease, especially in cases of bacterial infections.

The animals with acute inflammation (by tail and ear bites, lameness... etc.) were included as a positive control group with evident inflammation, in which an acute phase reaction would be expected. All APPs studied showed substantial changes; however, the high increases detected for Hp in our study (19-fold) contrast with previous reports (Petersen et al., 2002a,b), in which only increases between 3- to 5-fold were observed in these conditions. The use of a different ELISA kit by these authors (Petersen et al., 2001) might have influenced the results.

Our serological study showed that six out of 10 pigs affected with PRRSV exhibited increased CRP, SAA and Hp levels, and low albumin concentrations before seroconversion could be detected. On the other hand, all positive APPs assessed were increased in all pigs infected with *M. hyopneumoniae* at the time they showed negative serological results. Thus, in some cases, increased APPs were seen before specific antibodies could be found and therefore, although less specific than serology, APPs could be the earlier and better general markers of disease. According to other authors (Carroll et al., 2004), APPs might be even useful to detect subclinical processes as the acute phase response precedes the antibodies synthesis.

In human beings and dogs, APPs can be classified into major (10- to 100-fold increases), moderate (2–10) and negative (decrease) by the magnitude of their response to stimuli (Eckersall, 2000; Cerón et al., 2005). Results obtained in the present study suggest that CRP, SAA and Hp behave as major APPs in pigs, increasing their concentration by more than 10 times, and that pig-MAP could be considered as a moderate APP as its highest increase was of 4.4-fold. Previous studies pointed out that pig-MAP acts as a major APP (Lampreave et al., 1994; Heegaard et al., 1998), whereas others (Carpintero et al., 2005a,b; Sorensen et al., 2005) reported pig-MAP elevations that did not exceed 10-fold. Our results indicate that Hp is the most sensitive APP to detect pathological states in pigs as this protein was increased in acute inflammation, in animals with clinical signs and in pigs without evidence of disease but suffering from ADV infection. However, to get a more complete and valuable information about the general health status of the animal, it might be advisable to perform APPs profiles (Toussaint et al., 1995) including another APP, such as CRP or SAA, which have higher responses than Hp and perhaps a negative acute phase protein different from albumin, as its quantification did not show changes of high magnitude in our study.

In conclusion, an acute phase response was observed in different porcine diseases in field conditions this response being higher in animals with evident clinical signs and concurrent bacterial processes. Consequently, APPs may be useful markers for disease in pig production. To our knowledge, this is the first time that APPs have been evaluated in naturally occurring infections. Therefore, results obtained should be interpreted with caution as the environment could have had a huge impact on the expression of APPs. Ideally, animals with the same pathologies from herds exposed to different environments should be included in further studies to prove a similar trend in APPs behaviour. In addition, it should have been interesting to include control animals for each of our different age groups to rule out possible increases in APPs concentrations due to the age of the animal.

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