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Short communication

C-reactive protein, haptoglobin and Pig-Major acute phase protein profiles of pigs infected experimentally by different isolates of porcine reproductive and respiratory syndrome virus

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ABSTRACT

Porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) is the etiologic agent of PRRS, one of the most important diseases in swine worldwide. In the present work, the effects of different PRRSV strains were tested on a piglet experimental model to study the induced acute phase response. For this purpose, pigs ($n = 15$ for each group) were intranasally inoculated with one of five PRRSV strains (isolates EU10, 12, 17, 18 from genotype 1 and isolate JA-142 from genotype 2). The acute phase response was monitored by measuring acute phase proteins (APPs). Specifically, the serum concentration of haptoglobin (Hp), C-reactive protein (CRP) and Pig-Major Acute Protein (Pig-MAP) was determined at 0, 3, 6, 9, 12, 15, 18 and 21 days p.i. Clinical signs and growth performance were also monitored during the experiment. All animals became viremic after inoculation during the study period. The APP response was heterogeneous and dependent on the strain, being strains EU10, EU 18 and JA-142 those that induced the highest response and the strongest clinical signs. In general, Hp was the most sensitive biomarker for PRRSV infection, CRP behaved as moderate and Pig-MAP was the less responsive during the course of PRRSV experimental infection. Hp and CRP were significantly discriminatory between infected and control pigs, but not Pig-MAP.

1. Introduction

Acute phase proteins (APPs) are part of the systemic acute phase response and components of the innate immune system. The concentration of APP in the plasma is altered in animals subjected to challenges such as infection, inflammation, trauma or stress (Murata et al., 2004). The synthesis of APP takes place mainly in the liver under the stimulus of the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α. So far, many advances of APPs monitoring in animals for clinical and experimental purposes have been described since they can easily be measured in plasma whereas quantification of cytokines displays several problems, mainly associated to their short plasma half-lives (Eckersall and Bell, 2010; Heegaard et al., 2011; Murata et al., 2004; Petersen et al., 2004).

In swine, haptoglobin (Hp), C-reactive protein (CRP) and pig major acute phase protein (Pig-MAP) are the main positive APPs (Heegaard et al., 1998; Lampreave et al., 1994). Plasma Hp is a major antioxidant protective agent against haemoglobin-driven free radical accumulation (Alayash et al., 2013). CRP plays a significant role in the clearance of infectious agents and damaged cells, through its ability to bind phosphocholine (Black et al., 2004). Pig-MAP is the homolog to human ITIH4, a member of the Inter-α-Trypsin Inhibitor Heavy Chain family (Gonzalez-Ramon et al., 2000; Zhuo and Kimata, 2008), but its biological functions are still unknown.

Porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) is a major swine pathogen. The syndrome in growing pigs is characterized by systemic signs, such as fever and anorexia, and respiratory disease including dyspnoea and tachypnoea. One of the

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hallmarks of PRRSV is the significant variability between isolates. Thus, the high genomic variability observed among PRRSV strains has led to the classification of PRRSV in two different genotypes: type 1, which comprises isolates similar to the European prototype strain Lelystad virus and type 2, which includes isolates similar to the American prototype strain VR-2332 (Meng, 2000). Even more, several clades have been described within each genotype (Murtaugh et al., 2010; Shi et al., 2010; Stadejek et al., 2013). Accordingly, high antigenic variation has been proven between PRRSV isolates, which cross-reactivity is limited between genotypes and even within a given genotype (Martinez-Lobo et al., 2011b). Besides, although in general the immune response against PRRSV is weak and inefficient, due to the ability of the virus to suppress the production of type I IFNs (Darwich et al., 2010), production of other cytokines such as TNF-α, IL-1β, IL-10 and IL-6 is dependent on the strain considered and sometimes conflicting (Baumann et al., 2013; Darwich et al., 2010; Mateu and Diaz, 2008; Van Reeth and Nauwynck, 2000). Finally, the severity of clinical signs is also variable and influenced by the virus strain and also be host immune status, host susceptibility and management factors (Opriessnig et al., 2011; Zimmerman et al., 2012). In general, it is accepted that type 2 isolates induce more severe respiratory disease than type 1 strains although no clear differences exist between genotypes in the occurrence of systemic clinical signs (Martinez-Lobo et al., 2011a).

APP serum concentrations have been previously evaluated in experimental and field PRRSV infections (Diaz et al., 2005; Gomez-Laguna et al., 2010; Gutierrez et al., 2009; Heegaard et al., 2011; Parra et al., 2006). However, to date, no systematic comparative studies using different PRRSV strains have been performed to determine the usefulness of APPs as markers of pathogenicity or infection. Thus, the objective of this study was to determine serum profiles of CRP, Hp and Pig-MAP during experimental infections with five different PRRSV isolates.

2. Materials and methods

2.1. Animals and viral infection

The animals used for the present study corresponded to a subset of a larger study carried out at the Universidad Complutense de Madrid (Martinez-Lobo et al., 2011a and unpublished data). A total of ninety 3-week-old, cross-bred piglets from a PRRSV and *Mycoplasma hyopneumoniae*-seronegative herd were randomly divided into six groups (15 piglets/group) and housed in pens with a concrete floor and an automatic watering system. For this study, thirty pigs, five per group, were randomly selected and monitored during the whole experimental period. All experimental procedures were approved by the Animal Ethics Committee of Universidad Complutense de Madrid. After an acclimation period of 6 days, pigs of groups 1–5 were challenged by the intranasal route with 5×10^5 tissue culture infectious doses 50 ($TCID_{50}$) of one of the selected PRRSV isolates (Table 1). Pigs of the sixth group were sham-inoculated using an uninfected PAM culture lysate and were used as negative controls.

Table 1
Summary of the PRRSV isolates used in the present study together with their genotype and origin.

Strain #	PRRSV isolate	PRRSV genotype	ORF5 % nucleotide homology	
			Lelystad	VR-2332
1	EU-17	1 (subtype 1)	86	59
2	EU-18	1 (subtype 1)	87	61
3	EU-12	1 (subtype 1)	91	61
4	EU-10	1 (subtype 1)	88	59
5	JA-142	2	61	91

2.2. Experimental design and sampling

Details of the experimental outline have been already published (Martinez-Lobo et al., 2011a). Clinical signs were evaluated daily for each pig following a score system that takes into account systemic signs, including depression, lethargy, anorexia, cyanosis, and respiratory signs, including sneezing, coughing, laboured and abdominal breathing and respiratory rate as described in Martinez-Lobo et al., (2011a). Besides, average daily weight gain (ADWG) was estimated for each pig from day 0 to day 21. Blood samples were collected in serum clot vacuum tubes on days 0, 3, 6, 9, 12, 15, 18 and 21 p.i. (post-infection). Sera obtained on the corresponding day were stored at −80 °C until analyzed. Lungs samples obtained after necropsy at day 21 were analyzed for the presence of virus by immunohistochemistry (Martinez-Lobo et al., 2011a). Viremia was assessed by PRRSV isolation in porcine alveolar macrophages and RT-PCR as described in Martinez-Lobo et al., 2011a.

2.3. Acute phase protein measurement

Haptoglobin was quantified in serum by a spectrophotometric method (haemoglobin binding assay) using a commercial reagent from Tridelta Development Ltd. (Ireland) and performed on an automated analyzer (Olympus AU400, Hamburg, Germany). Pig-MAP concentrations were assessed with an ELISA kit (PigCHAMP ProEuropa, Segovia, Spain). Intra-assay and inter-assay coefficients of variation of both techniques have been reported previously (Saco et al., 2010b). Serum CRP concentration was determined using a commercial immunoturbidimetric method (Olympus System Reagent, OSR 6147) and the assay was performed in the above mentioned Olympus AU400 analyzer following a protocol validated for porcine samples (Saco et al., 2010a).

2.4. Statistical analyses

The normality of the dataset was evaluated with the Kolmogorov-Smirnov and Shapiro-Wilk tests, while the Levene statistic was used to test the homocedasticity (evaluation of variance homogeneity). The *U* Mann-Whitney test was performed to investigate: (a) the differences between PRRSV infected animals (all five strains together) and non-infected controls, and (b) the differences in the observed serum concentrations of APPs between PRRSV infected groups at each time point after inoculation and on day 0 of the experiment. The clinical signs scores recorded during three different periods (from day 1 to 7 p.i., from day 8 to 14 p.i. and from day 15 to the end of the experiment) were converted to an approximate area under the curve (AUC) using the trapezoidal rule (Hennen, 2003) as described previously in Martinez-Lobo et al. (2011a). Pearson correlations were calculated between accumulated clinical signs at 7, 14 and 21 days p.i. and mean APP concentrations at the same days. Statistical analyses regarding comparisons of clinical scores, IHC and viremia were performed as indicated in Martinez-Lobo et al. (2011a).

3. Results

3.1. Acute phase protein concentrations

Pigs inoculated with PRRSV, independently of the isolate, had higher overall serum APP concentrations than control animals. Globally, during the course of the study, serum Hp values ranged from 0.71 ± 0.54 mg/mL to 1.26 ± 0.99 mg/mL; those of Pig-MAP