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# Acute phase response in porcine reproductive and respiratory syndrome virus infection

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ABSTRACT

This study was focused on the changes observed in the serum concentration of haptoglobin (Hp), C-reactive protein (CRP), serum amyloid A (SAA) and Pig-major acute protein (Pig-MAP), during experimental porcine reproductive and respiratory syndrome virus (PRRSV) infection and in their relationship with the expression of interleukin 1β (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α). Hp and Pig-MAP serum levels were increased at 10 dpi, but CRP and SAA showed a delayed and highly variable increase. All three proinflammatory cytokines were poorly expressed, and only a mild increase in IL-1β was observed at 7 dpi. The increased expression of Hp coincided with the light enhancement observed in both IL-6 and TNF-α, and might be related with an increased expression of IL-10. The low expression of TNF-α might point to a possible mechanism of viral evasion of host-immune response. This issue and the delayed expression of CRP and SAA should be taken into account in future studies about modulation of the immune response by PRRSV infection.

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## 1. Introduction

The acute phase response (APR) is characterised by the disturbance of the normal homeostasis by several stimuli like infection, inflammation, stress, trauma or tissue damage [1–3]. This APR is triggered by the synthesis of proinflammatory cytokines, namely interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α), at the local site of the injury. These cytokines are released into the bloodstream, reach the liver and induce the production of acute phase proteins (APPs) by hepatocytes [1,4]. APPs have been classified as "positive" or "negative" depending on the increase or decrease of their serum concentration, respectively [2,4]. Haptoglobin (Hp), C-reactive protein (CRP) and serum amyloid A (SAA) are considered as main APPs in pigs [4]. The classification of the pig-major acute protein (Pig-MAP) is controversial, it is considered a major or moderate APP depending on the study [5]. It is generally accepted that APPs are inductors of a proinflammatory reaction and fever, but their over-expression can lead to an anti-inflammatory response [2,4]. Thus, APPs are used today as potential biological markers for monitoring animal welfare and the health status of swine herds and of individual pigs at slaughter

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[1,3,4,6]. Moreover, APPs may be used to determine the virulence of different isolates of the same bacteria or virus, or the efficacy of vaccines [7].

APPs have been tested in pigs after exposure to stress [8] and after natural [5,9,10] or experimental [11–16] infections. Increased levels of Hp, CRP and/or Pig-MAP have been reported in porcine viral and bacterial respiratory infections, like porcine circovirus type 2 (PCV2) [5,10,16], swine influenza virus (SIV) [17], Aujeszky's disease virus [5], *Actinobacillus pleuropneumoniae* [7], *Mycoplasma hyopneumoniae* [5], *Bordetella bronchiseptica* and toxigenic *Pasteurella multocida* type D [11].

Porcine Reproductive and Respiratory Syndrome (PRRS) is a swine disease caused by PRRS virus (PRRSV), which belongs to the *Arteriviridae* family [18]. There are two genotypes of PRRSV, European (EU) and North American (US), which present significant antigenic and pathogenic differences [19–22]. PRRS is characterised by respiratory problems in growing and finishing pigs and reproductive failure in sows, with late term abortion, stillbirth and increased preweaning mortality [23]. The main gross lesions caused by PRRSV consist of a multifocal interstitial pneumonia with tan-mottled areas, hyperplasia of lymph nodes [24] as well as hydrothorax and ascites in weak born and stillborn piglets [25].

To our knowledge, there are few studies on the expression of APPs during PRRS. Moreover, these studies are usually limited to only one APP or to a single time-point or short timeframe of the infection. Enhanced serum Hp concentration has been reported after experimental infection from 7 to 21 dpi [12,26], and this was associated with an elevated expression of IL-6 but not TNF-$\alpha$ [12]. Elevated serum concentrations of Hp, CRP and SAA have also been reported in pigs naturally infected with PRRSV, sometimes before the development of specific PRRSV antibodies [5].

The main aim of this study was to analyze the kinetics of APPs and proinflammatory cytokines in the blood stream during the APR in pigs infected with a European PRRSV field isolate.

## 2. Materials and methods

### 2.1. Animals and experimental design

Thirty-two, five-week-old, PRRSV-free pigs were housed in the biocontainment level III facility at the Centro de Investigación en Sanidad Animal (CISA-INIA, Valdeolmos, Madrid, Spain) for 10 days prior to challenge. Twenty-eight pigs were randomly located in batches of four each, inoculated by intramuscular route with 1 ml of $10^{3.0}$ $TCID_{50}$ of the PRRSV field isolate 2982 (kindly provided by Dr. E. Mateu) and humanely killed at 3, 7, 10, 14, 17, 21 and 24 days post-inoculation (dpi) [27]. The four remaining pigs, used as control pigs, were injected intramuscularly with 1 ml of sterile RPMI 1640 medium and humanely killed at the end of the study (24 dpi). All animals were sedated with tiletamine-zolazepam (Zoletil, Virbac) followed by intravenous injection of a lethal dose of 5% sodium thiopental (Thiovet, Vet Limited). Tissue samples were subjected to *in situ* hybridization and were shown to be negative for PCV2. This experiment was carried out according to the guidelines of the European Union (Directive 86/609/EEC) and was approved by the local ethical committee of "Centro de Investigación en Sanidad Animal" (CISA-INIA, Valdeolmos, Madrid, Spain).

### 2.2. Clinical signs, gross pathology and histopathology of the lungs

The pigs were monitored daily for clinical signs, i.e. rectal temperature and a clinical respiratory score, as described previously [24]. Post mortem examination was carried out following standard operational procedures and any observed lesion was recorded. Macroscopic lung lesions were evaluated by visual inspection following the scoring system described by Halbur et al. [24]. Samples from the right lung (cranial lobe, medial lobe, accessory lobe, caudal dorsal lobe and caudal ventral lobe) were fixed in 10% buffered formaldehyde and embedded in paraffin-wax. Four-micrometer sections were stained with haematoxylin and eosin (HE) for histopathological examination. Microscopic lesion scores from 0 to 4 were assigned as previously described [24].

### 2.3. Serum samples and virus titre

Blood samples were taken prior the inoculation, considered as day 0, and at 3, 7, 10, 14, 17, 21 and 24 dpi. Samples were collected into evacuated tubes, allowed to clot at room temperature and centrifuged, and then the serum was harvested and frozen at $-80\,^{\circ}C$ until testing.

Viraemia in serum samples was determined as described previously [28]. Briefly, the virus titration was carried out cultivating 50 $\mu$l of tenfold serial dilutions of serum samples on PAMs, from PRRSV-negative pigs, and incubating at 37 °C for 1 h. The samples were replaced by medium, and the PAMs were incubated for 72 h at 37 °C for developing cytopathic effect, washed once with PBS and further stained using an immunoperoxidase monolayer assay (IPMA) [29].

### 2.4. Detection of APPs in serum

Serum samples were analyzed for APPs by means of commercial kits, previously validated in our laboratory [30]. Porcine serum Hp concentrations were quantified by using a non-species-specific spectrophotometric method with a commercial kit (Phase$^{TM}$ Range Haptoglobin Assay; Tridelta Development Ltd). The assay presented a detection limit of 0.02 mg/ml and was performed according to the manufacturer's instructions on an automated analyzer (Cobas Mira Plus; ABX Diagnostics, Montpellier, France). Serum CRP and Pig-MAP levels were assessed with porcine specific ELISA kits based on monoclonal antibodies (Phase$^{TM}$ Range; Tridelta Development Ltd, Maynooth, Ireland; PigCHAMP Pro Europa S.A., Segovia, Spain). Their detection limits were determined as 2.00 and 0.18 mg/ml, respectively. SAA concentration was determined by using a commercial non-species-specific ELISA kit based on a

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