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Infection dynamics and acute phase response of an *Actinobacillus pleuropneumoniae* field isolate of moderate virulence in pigs

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ABSTRACT

Actinobacillus pleuropneumoniae, the causative agent of porcine contagious pleuropneumonia (PCP), causes significant economic losses associated mainly with growth stunting of animals. Although serotypes can be distinguished according to their virulence, most of the studies are focused in *A. pleuropneumoniae* infections with virulent serotypes. There is little information regarding the role of acute phase proteins (APPs) and proinflammatory cytokines in infections with isolates of mild or moderate virulence. Thus, the present study aims to evaluate the kinetics of infection with an *A. pleuropneumoniae* serotype 6 (*Ap6*) field isolate of moderate virulence and the changes in the serum concentration of specific antibodies and different APPs and proinflammatory cytokines. Control animals showed no clinical signs or lesions throughout the study. Infected animals showed increased rectal temperature, respiratory distress and depression from 24 hpi, and typical gross and microscopic lesions of PCP from 6 hpi onwards. *Ap6* was isolated from nasal swabs of four out of five inoculated animals at 24 hpi, and from nasal swabs, tonsil and lung samples from all inoculated animals at 72 hpi. Specific antibodies against *Ap6* or changes in the serum concentration of IL-1β, IL-10 and TNF-α were not detected throughout the study. The serum concentration of IL-6 increased from 6 hpi as well as serum A amyloid, C-reactive protein and haptoglobin from 24 hpi onwards. Our results highlight the onset of the acute phase response after the infection with a field isolate of *A. pleuropneumoniae* of moderate virulence from 24 hpi onwards which may be of interest in the study of the pathogenesis of this disease.

1. Introduction

Porcine contagious pleuropneumonia (PCP) causes marked economic losses associated to the retardation in the growth of infected pigs (Losinger, 2005). Different clinical forms may be observed, from peracute to subacute or chronic. Clinical signs are characterized by respiratory dyspnoea with nasal and oral bloody discharges, affecting pigs from any age but mostly 12–16 week old pigs (Gottschalk and Taylor, 2006; Quinn et al., 2011). The characteristic lesion consists of a mostly bilateral necrotic haemorrhagic pneumonia of the caudal lung lobes (Gottschalk and Taylor, 2006).

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PCP is caused by *Actinobacillus pleuropneumoniae*, an encapsulated, non-sporulated, immobile, facultative aerobic, haemolytic and fermentative, Gram-negative coccobacillary bacteria (Dubreuil et al., 2000; Vadillo et al., 2002). Fifteen different serotypes are recognized based on the capsular antigens (K antigen) (Angen and Jessing, 2004; Ciprián et al., 2004). Subtypes or sub-serotypes may be distinguished within serotypes 1 and 5 (Haesebrouck et al., 1997; Gottschalk and Taylor, 2006). Serotypes 1, 2, 5, 9 and 11 are considered as virulent isolates, being responsible for either acute or chronic outbreaks of PCP (Gottschalk and Taylor, 2006). In central Chile (from Metropolitan to Biobío regions), serotypes 4, 6 and 7 are the most frequently identified isolates, having mild to moderate virulence compared with serotypes 1 and 5 (Gottschalk and Taylor, 2006; Neira-Ramírez et al., 2012).

Different virulence factors associated to *A. pleuropneumoniae*, such as APX toxins, which belong to the repeats-in-toxin (RTX) toxin family, capsular or membrane lypopolyssacharides (LPS), and adhesins (Paradis et al., 1994; Ward and Inzana, 1998; Bossé et al., 2002; Velthuis et al., 2002; Bandara et al., 2003; Shin et al., 2003), induce the activation of the inflammatory response through the secretion of interleukin (IL)-1, IL-6, chemokine-8 or tumour necrosis factor-alfa (TNF-α) (Chen et al., 2011). However, regulatory cytokines, such as IL-10, have also been reported to reduce infiltration and proliferation of inflammatory cells in other swine respiratory diseases (Gómez-Laguna et al., 2013). Likewise, the synthesis of proinflammatory cytokines may trigger off the onset of an acute phase response with the secretion of acute phase proteins (APPs) mainly by the hepatocytes (Eckersall, 2000; Petersen et al., 2004).

In pigs Haptoglobin (Hp), C-reactive protein (CRP), serum amyloid A (SAA) and Pig-Major Acute Protein (Pig-MAP) are considered as "positive" APPs, showing up to 100- to 1000-fold increase in serum concentrations, whereas albumin, transferrin and fibrinogen are considered as "negative" APPs, with a decrease in their serum concentration after a specific stimulus (Petersen et al., 2004). APPs are used today as potential biological markers for monitoring infectious diseases and detecting subclinical diseases with impact on animal performance (Eckersall, 2000; Petersen et al., 2004; Pallarés et al., 2008). Changes in the concentration of APPs have been associated to different porcine diseases or pathogens (Parra et al., 2006; Grau-Roma et al., 2009; Gómez-Laguna et al., 2010a,b; Barbé et al., 2011; Pomorska-Mól et al., 2013). Several APPs have proven potentially useful inflammatory biomarkers of *A. pleuropneumoniae* infections with virulent isolates (Hall et al., 1992; Heegaard et al., 1998; Hultén et al., 2003; Lauritzen et al., 2003); however, there is scarce information regarding the role of these reactants in *A. pleuropneumoniae* infections with isolates of mild or moderate virulence.

Therefore, the present study analyzes the dynamics of an early infection with an *A. pleuropneumoniae* serotype 6 (*Ap6*) field isolate, evaluating the changes in the serum concentration of pro- (IL-1, IL-6 and TNF-α) and anti-inflammatory (IL-10) cytokines, and their correlation with the expression of Hp, CRP and SAA.

2. Materials and methods

2.1. Animals, inoculum and experimental design

Forty, 8-week-old, Large White × Landrace pigs from a unit of a high health industrial pig farm, which was frequently monitored for *A. pleuropneumoniae*, were used. No clinical signs or *A. pleuropneumoniae*-associated lesions have been reported in the selected farm during the past five years. In addition, the exposure of the animals to *A. pleuropneumoniae* was ruled out by means of microbiological isolation from nasal swabs during the acclimatization period and by a commercially available ELISA kit (IDEXX APP-ApxIV Ab Test, IDEXX, Maine, USA). After the post-mortem examination, all the animals yielded negative results for PCV2 *in situ* hybridization and SIV immunohistochemistry analysis. Chile has been PRRSV-free from November 2012 to October 2013, the period in which this study was conducted.[1] The pigs were clinically healthy and were housed in bio-containment animal facilities at the Agricultural and Animal Laboratories and Quarantine Station of the Agricultural and Livestock Service (SAG, Lo Aguirre, Santiago, Chile). The pigs were housed in the bio-containment facility for two weeks prior to challenge. The animals were randomly assigned into two groups (control and inoculated) of twenty animals each (four subgroups of five animals each). Inoculated animals received intranasally 5 mL of a suspension of 9.3×10^9 CFU/mL of the field isolate 418/07, a *A. pleuropenumoniae* serotype 6 (*Ap6*) of moderate virulence, which was isolated from the lungs of animals with PCP at central region of Chile (VII Biobío Region) during 2007. The purity and viability of the inoculum was determined by means of flow cytometry (Berney et al., 2007) and propagation of the bacteria in PPLO (pleuropneumonia-like organism) agar as described in Section 2.3 (microbiological examination). This inoculation procedure has previously been reported to successfully reproduce the disease (Muñoz et al., 2010). Control animals were intranasally inoculated with 5 mL of a suspension of sterile culture medium without bacteria. The instillation of the inoculums was made under sedation with maleate acepromacine (Acedan, Holliday Scott) in both nostrils (during inspiration) using a fenestrated catheter.

2.2. Clinical signs, gross pathology and histopathology

Pigs were clinically monitored daily with rectal temperatures and evidence of respiratory signs recorded (Morton and Griffiths, 1985). Five animals from control and inoculated groups were euthanised using maleate acepromacine (Acedan, Holliday Scott) followed by an overdose of sodium thiopental (Thiovet, Vet Limited) at 6, 24, 48 and 72 hpi. Post mortem examinations were carried out following standard operational procedures and any observed lesion was recorded. Macroscopic lung lesions were evaluated by visual inspection following the scoring

[1] http://www.sag.cl/noticias/caso-confirmado-prrs-en-plantel-industrial-de-cerdos-en-la-region-metropolitana.