Veterinary Microbiology 159 (2012) 499–503

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

Acute phase protein response during subclinical infection of pigs with H1N1 swine influenza virus

Małgorzata Pomorska-Mól *, Iwona Markowska-Daniel, Zygmunt Pejsak

Department of Swine Diseases, National Veterinary Research Institute, 24-100 Pulawy, Poland

ARTICLE INFO

Article history:
Received 23 January 2012
Received in revised form 19 April 2012
Accepted 20 April 2012

Keywords:
Pigs
Acute phase protein
Swine influenza
Subclinical infection
H1N1

ABSTRACT

In the present study acute phase proteins (APPs) responses in pigs after subclinical infection with H1N1 swine influenza virus (SwH1N1) were evaluated. Fourteen 5 weeks old, seronegative piglets, both sexes were used. Ten of them were infected intranasally with SwH1N1. C-reactive protein (CRP), haptoglobin (Hp), serum amyloid A (SAA) and pig major acute phase protein (Pig-MAP) concentrations in serum were measured using commercial ELISAs. No significant clinical signs were observed in any of the infected pigs, however, all infected animals developed specific antibodies against SwH1N1 and viral shedding was observed from 2 to 5 dpi. Only concentrations of Hp and SAA were significantly induced after infection, with mean maximum levels from days 1 to 2 post infection (dpi). The concentrations of CRP and Pig-MAP remained generally unchanged, however in half of infected pigs the concentration of CRP tended to increase at 1 dpi (but without statistical significance). The results of our study confirmed that monitoring of APPs may be useful for detection of subclinically infected pigs. The use of SAA or Hp and Pig-MAP may be a valuable in combination [i.e. Hp (increased concentration) and Pig-MAP (unchanged concentration)] to detect subclinically SIV infected pigs, or to identify pigs actually producing a large amount of virus. Additional studies need to be done in order to confirm these findings.

1. Introduction

The acute phase response is a nonspecific early response of the organism caused by various stimuli (Heegaard et al., 1998; Grau-Roma et al., 2009; Pomorska-Mól et al., 2011a, b). This reaction is mediated by pro-inflammatory cytokines and involves local and systemic reactions, including changes in the concentrations of acute phase proteins (APPs) (Murata et al., 2004; Grau-Roma et al., 2009).

Whilst APP responses have been observed for a large range of infections (Grau-Roma et al., 2009; Pomorska-Mól et al., 2011a, b) whether sub-clinical swine influenza (subSI) also induces an APP response is not known. Swine influenza (SI) is an acute respiratory disease caused by swine influenza virus (SIV) (Olsen et al., 2006; Markowska-Daniel et al., 2011). Typical SI outbreaks are characterized by a rapid onset of high fever, loss of appetite, labored abdominal breathing and coughing. Mortality is low and recovery occurs within 7–10 days (Olsen et al., 2006). However, the infection is much more frequent than the disease. Infection with swine influenza H1N1 virus is frequently subclinical. Typical signs are often demonstrated in only 25–30% of a herd (Brown, 2000; Busquets et al., 2010).

The objective of this study was to assess whether experimentally induced subSI, caused by H1N1 subtype, evokes C-reactive protein (CRP), haptoglobin (Hp), serum amyloid A (SAA) or/and pig major acute phase protein (Pig-MAP) responses in pigs. Additionally, the usefulness of CRP,

* Corresponding author at: Department of Swine Diseases, National Veterinary Research Institute, Al. Partyzantów 57, 24-100 Puławy, Poland. Tel.: +48 81889 32 85.
E-mail address: mpomorska@piwet.pulawy.pl (M. Pomorska-Mól).

0378-1135/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetmic.2012.04.030

Hp, SAA and/or Pig-MAP measurements in identification of subclinically infected animals was investigated.

2. Materials and methods

2.1. Animals

Fourteen 5-week-old piglets of the France Hybrides FH900 line were sourced from high health status herd. Prior to the start of the study all of the piglets were shown to be both influenza A virus and antibody (subtypes H1N1, H1N2, H3N2) negative by Matrix (M) gene real time RT-PCR and hemagglutination inhibition assay (HI), respectively. The herd was seronegative to porcine reproductive and respiratory syndrome virus and pseudorabies virus. No evidence of pleuropneumonia, streptococcosis and atrophic rhinitis was recorded based on clinical, serological and pathological examinations.

During the experiment, piglets were housed at the BSL3 animal facility in two independent units: one for the control and the other for the infected pigs. Animal use and handling protocols were approved by Local Ethical Commission.

2.2. Preparation of virus inoculum

Swine influenza virus A/sw/Poland/KPR9/2004 (subtype H1N1) (hereafter referred to as SwH1N1), which had been isolated from a pig with swine influenza, was used for the experimental infection. The stock used for inoculation represented the third passage in eggs. The virus concentration was evaluated in Madin–Darby canine kidney (MDCK) cells.

2.3. Experimental design

On day 0, ten piglets were inoculated with SwH1N1. Inoculations of $10^{7.3}$ $TCID_{50}$ of virus in 3 ml of phosphate-buffered saline (PBS) were given intranasally. Four mock-inoculated pigs (with PBS) served as control pigs.

In order to examine the events taking place at the early stages of infection with SwH1N1 two infected and one control piglet were euthanized on days 2 and 4 after infection. The remaining pigs were euthanized and necropsied at 10 dpi.

2.4. Clinical and pathological examination

Rectal temperatures and clinical signs were recorded daily. Blood samples were collected on −7, 0 (inoculation), 1, 2, 3, 5, 7 and 10 dpi. Nasal swabs were taken at 2, 3, 4, 5 and 10 dpi. Complete necropsy was done on each animal, with special emphasis on the respiratory tract. Samples from lung (all lobes separately) and tracheas were collected for viral RNA extraction.

Lung lesions were scored using the method developed by Madec and Kobisch (1982), according to the following scheme: point 0, no lesion; point 1, lesions affecting <25% of the lobe surface; points 2, lesions affecting 25–49% of the lobe surface; points 3, lesions affecting 50–74% of the lobe surface and points 4, lesions affecting >75% of the lobe surface.

2.5. Laboratory examination

2.5.1. Swabs and tissue samples

The general swine influenza A real time RT-PCR method was used for detection of SIV in swabs and tissues, as described previously (Slomka et al., 2010). Samples with C_t value <30 were considered to be M gene positive, samples having C_t value 30–35 with sigmoidal/logarithmic appearance were considered to be weak positive, samples with C_t value >35 were considered to be negative.

2.5.2. Hemaglutinin inhibition assay (HI)

Antibodies against SIVs were measured using a HI assay, performed according to the standard procedure, using 0.5% chicken erythrocytes and 4HA units of strains SwH1N1 virus. Before inoculation, to check the immune status of the piglets, the HI assay were also performed with H3N2 and H1N2 subtypes. All sera were tested in serial twofold dilutions, starting at 1:20. For estimates of the prevalence of antibodies, titres equal or higher than 20 were considered positive.

2.5.3. APP determination in serum samples

For determination of APP commercial ELISAs were used according to the manufacturer's recommendation (Pig C-reactive protein ELISA and Pig haptoglobin ELISA from Life Diagnostics, Inc., USA; PigMAP KIT ELISA from PigCHAMP Pro Europa S.A, Spain; Phase Serum Amyloid A Assay from Tridelta Development Ltd., County Kildare, Ireland). Serum samples were tested in duplicate. Prior to analyses samples were diluted as follows: 1:1000 for CRP, 1:35,000 for Hp, 1:500 for SAA and 1:1000 for Pig-MAP.

2.6. Statistical analysis

The obtained data were subjected to the W. Shapiro–Wilk test for normality and the Levene's test for equality of variances. The nonparametric Friedman test was used to compare observations repeated on the same subjects. Comparisons between infected and control groups at each time point were assessed using the Mann–Whitney *U*-test. Differences were considered as significant with a $\alpha < 0.05$. All calculations were performed with Statistica 8.0 (Statsoft).

3. Results

3.1. Clinical signs

No relevant clinical sings were observed in pigs either group. However, 5 infected pigs showed transient abnormal rectal temperatures (from 40.1 °C to 40.3 °C) 1–3 dpi. In the control pigs no clinical signs of any disease were seen. The general condition of the piglets and food intake were unchanged in both groups.

3.2. Antibody response against SwH1N1

All infected pigs exhibited specific antibodies against hemagglutinin 7–10 dpi; the HI titre ranged from 20 to 160. Sera from control pigs had no antibody titres (<20 HI titre).