ACUTE PHASE PROTEIN RESPONSE IN PIGS EXPERIMENTALLY INFECTED WITH *HAEMOPHILUS PARASUIS*

Fablet, C., Marois, C., Eono, F., Kobisch, M. and Madec, F.

French Food Safety Agency, Zoopôle Les Croix, B.P. 53, 22 440 Ploufragan, FRANCE

SUMMARY

The aim of the trial was to look at the course of the serum concentration of the acute phase proteins C-Reactive Protein, Haptoglobine and Serum Amyloïde A after infection of SPF pigs with a strain of *Haemophilus parasuis*. The results indicated that APP response followed up clinical symptoms of the outcome of the disease. Hp, CRP and SAA seems to be sensitive and non specific bio marker of clinically and subclinically infected pigs with Glässer's disease. The determination of these protein concentrations may be a useful tool to distinguish acute from chronic phases.

Keywords: pigs, *Haemophilus parasuis*, acute phase response, C-Reactive Protein, haptoglobine, serum Amyloïd A, experimental trial

INTRODUCTION

The acute phase response is an unspecific systemic reaction of the organism that occurs shortly after infection, inflammation or trauma and includes changes in the concentration of plasma proteins called acute phase proteins (APPs) such as Haptoglobin (Hp), C-Reactive Protein (CRP) and Serum Amyloid A (SAA). Quantification of their concentrations in plasma or serum of pigs could provide valuable diagnostic information for prognosis and monitoring disease (Eckersall, 2000). In recent years, a growing interest in infections caused by Hps in pigs has been identified due to the severity of the disease when the infectious agent is introduced in high health status herds (Rapp-Gabrielson et al., 2006). Even if the evolution of the acute phase response of pigs induced by experimental infections has been documented for some bacteria or virus, to the best of our knowledge, no trial related to the APPs behaviour following Hps infection has been reported. The aim of the trial was to look at the course of the serum concentration of the acute phase proteins C-Reactive Protein (CRP), Haptoglobine (Hp) and Serum Amyloïd A (SAA) after infection of pigs with a strain of *Haemophilus parasuis* (Hps).

MATERIALS AND METHODS

A total of 16 specific pathogen free pigs (SPF) was used for the assay. They were divided into 4 groups that differed in the age and route of infection:

- **Group 1**: two 10-week-old pigs were not inoculated and constituted the control group,
- **Group 2**: six 10-week-old pigs intranasally infected with $2x10^8$ colony-forming units (CFU) of Hps,

–**Group 3**: four 7-week-old pigs intranasally infected with $2x10^8$ CFU of Hps,
–**Group 4**: four 7-week-old pigs infected by intratracheal injection with $2x10^8$ CFU of Hps.

On day 0, the pigs were inoculated with a strain of Hps isolated from septicaemia. The pigs were monitored daily before and after challenge by measuring rectal temperature and recording specific clinical symptoms such as lameness, swollen joints, dyspnoea and nervous signs (tremor). Blood samples were obtained at days 0, 7 and 14 for determination of CRP, Hp and SAA concentrations. On day 14 after challenge, survival pigs were euthanized and necropsied. Macroscopic lesions were recorded. At necropsy, nasal swabs, tonsillar, lung and arthritic joints samples of every pig were taken and submitted to PCR analysis for Hps detection (Olivieira et al., 2001). Serum levels of CRP, Hp and SAA were measured with commercial assay kits (Tridelta Development, Greystones, Ireland).

RESULTS

Fever was observed in all infected groups within day 01 and 09 p.i. In group 2, 2 pigs showed a rectal temperature above 40.5°C. One pig had elevated temperature on days 01 and 02 p.i. and died soon after. The second pig developed fever on day 04 p.i. and was euthanized on day 05 p.i. In group 3, 2 pigs had elevated body temperature on days 01–05 p.i. and were euthanized on days 02 and 05. Fever concerned 2 pigs in group 4. One pig died on day 02. The other pig had a body temperature exceeding 40°C during 7 days after the challenge (days 03–09). Mortality was observed during the first week following the challenge (day 02 to day 07). Lameness was identified after infection whatever the age and route of infection. The number of affected pigs was highest for the group intranasally infected at 7 weeks-old (3/4 pigs, group 4). In this group, nervous signs were recorded in one pig (Table 1).

Table 1. Clinical signs observed in SPF pigs after experimental infection with a strain of *Haemophilus parasuis* (14 pigs, group 2: 10-weeks-old pigs intranasally infected with $2x10^8$ colony-forming units; group 3: 7-weeks-old pigs intranasally infected with $2x10^8$ colony-forming units; group 4: 7-weeks-old pigs infected by intratracheal injection with $2x10^8$ CFU)

Clinical observation	**Group 2**		**Group 3**		**Group 4**	
	No of affected pigs/No of inoculated pigs	Day p.i.	No of affected pigs/No of inoculated pigs	Day p.i.	No of affected pigs/No of inoculated pigs	Day p.i.
Lameness	1/6	3–4	1/4	3–5	3/4	2–14
Nervous signs	–	–	–	–	1/4	2

At necropsy, polyserositis was observed in all infected groups (Table 2). Macroscopic lesions were more frequent in pigs intranasally infected at 7 weeks of age. In this group, only one pig was free from gross lesions whereas 2/4 pigs and 5/6 pigs had no visible lesions in group 3 and 4 respectively. Hps was detected from nasal and tonsillar samples of all infected pigs, indicating that all pigs have been contaminated.

Table 2. Pathological findings at necropsy in 3 groups of SPF pigs infected with a strain of *Haemophilus parasuis* (14 pigs, group 2: 10-weeks-old pigs intranasally infected with $2x10^8$ colony-forming units; group 3: 7-weeks-old pigs intranasally infected with $2x10^8$ colony-forming units; group 4: 7-weeks-old pigs infected by intratracheal injection with $2x10^8$ CFU)

Lesion	Nbre of affected pigs/Nbre of inoculated pigs		
	Group 2	**Group 3**	**Group 4**
Pleuritis	1/6	2/4	–
Peritonitis	1/6	2/4	2/4
Arthritis	1/6	3/4	2/4
Pneumonia	–	1/4	1/4
Pericarditis	–	2/4	–
Total	**1/6**	**3/4**	**2/4**

Individual CRP, Hp and SAA responses of the pigs are given Figures 1, 2, 3 and 4 for groups 1 to 4 respectively. Since 5 pigs died before day 07 p.i., their acute phase responses were not investigated. Dealing with the remaining pigs, one pig in group 4 (247) developed an acute phase response 7 days p.i. CRP level showed a 25-fold increase as compared to day 0. Hp and SAA concentrations rose respectively from 0.03 to 7.15 mg/ml and 24.41 to 1094.86 µg/ml. High levels of CRP and Hp were still observed 14 days p.i. SAA decreased more rapidly to reach 80.2 µg/ml, 14 days after challenge. To a lesser extent, a slight increase of Hp level was observed in one pig (248) in group 3 between days 7 and 14 p.i. (0.07 mg/ml to 1.39 mg/ml). When correlating responding APPs with clinical signs and pathological findings, the pig 247 showed fever from days 04 to 06 p.i. and lameness until the end of the trial (day 14 p.i.). At necropsy, polyserosits was observed (peritonitis and arthritis). Pig 248 suffered from arthritis. All infected pigs which did not showed severe fever or clinical symptoms between days 0 to 14, or macroscopic lesions at necropsy, had acute phase proteins levels quite similar to those of control pigs.

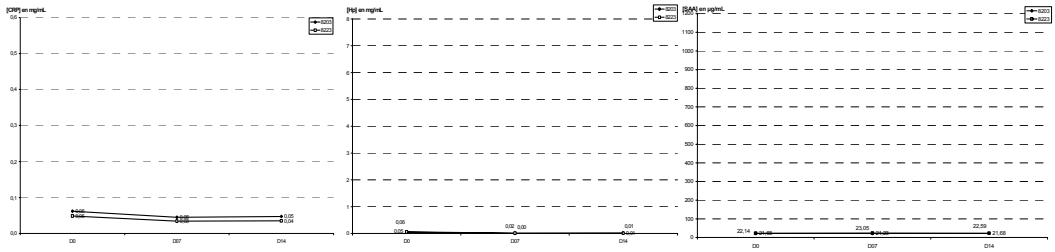

Figure 1. Concentrations of C-Reactive Protein (A), Haptoglobine (B), Serum Amyloid A (C) in serum of individual control pigs before and on various time-point after inoculation with *Haemophilus parasuis*

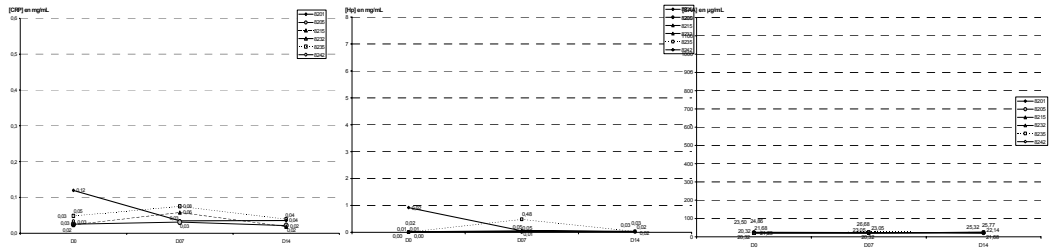

Figure 2. Concentrations of C-Reactive Protein (A), Haptoglobine (B), Serum Amyloid A (C) in serum of individual pigs of group 2 before and on various time-point after inoculation with *Haemophilus parasuis*

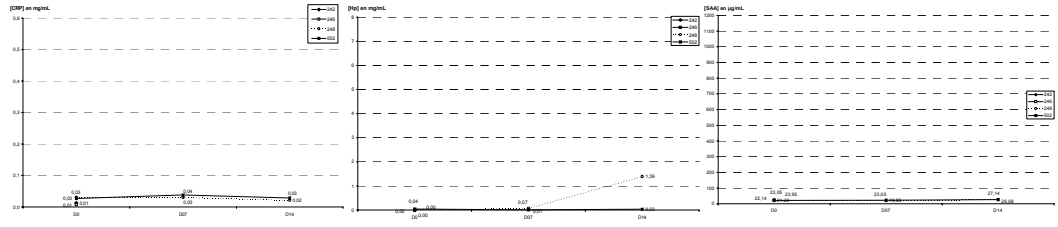

Figure 3. Concentrations of C-Reactive Protein (A), Haptoglobine (B), Serum Amyloid A (C) in serum of individual pigs of group 3 before and on various time-point after inoculation with *Haemophilus parasuis*

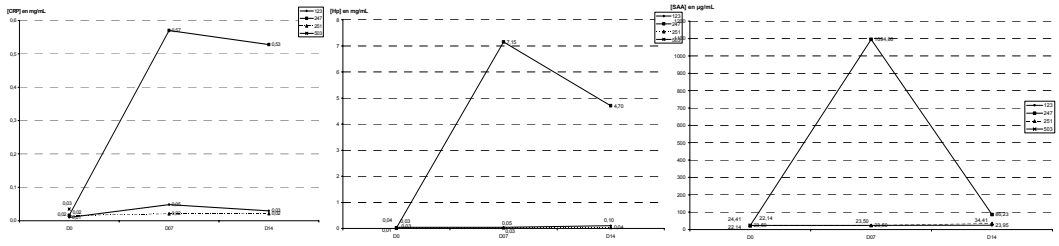

Figure 4. Concentrations of C-Reactive Protein (A), Haptoglobine (B), Serum Amyloid A (C) in serum of individual pigs of group 4 before and on various time-point after inoculation with *Haemophilus parasuis*

DISCUSSION-CONCLUSION

The *Haemophilus parasuis* strain used for this trial was isolated from a pig affected by Glässer's disease. Experimental infection of SPF pigs with this strain by nasal or intratracheal route induced for 7 out of 14 infected pigs, clinical (fever, lameness, nervous signs) and typical macroscopic lesions (polyserositis) of the disease. Furthermore, mortality occurred within the first days after challenge as described in the acute phase of the disease (Oliveira and Pijoan, 2004). Half of the

infected pigs did not developed clinical signs, especially an severe increase of body temperature. Nevertheless, Hps was detected in the upper respiratory tract of this pigs, indicating a colonisation of the mucosa. An acute phase response was observed 07 and 14 days post infection in pigs showing clinical signs of Glässer's disease during the trial. On the other side, no significant changes in the CRP, Hp and SAA concentrations were noticed at these time for pigs carrying *Haemophilus parasuis* in the upper respiratory tract and free from clinical signs and macroscopic lesions. These results indicated that APP response clearly followed the clinical symptoms of the disease. Hp, CRP and SAA seem to be sensitive and react as non specific bio markers of clinically and subclinically infected pigs. The determination of the concentrations in these proteins may be useful to distinguish acute from chronic phases.

REFERENCES

Eckersall, P.D.. 2000. Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. Revue de Médecine Vétérinaire. 151: 577–584.

Oliveira, S., Galina, L., Pijoan, C. 2001. Development of a PCR test to diagnose *Haemophilus parasuis* infections. J. Vet. Diagn. Invest. 13: 495–501.

Oliveira S. and Pijoan, C. 2004. *Haemophilus parasuis*: new trends on diagnosis, epidemiology and control. Veterinary Microbiology, 99,1–12.

Rapp-Gabrielson, V.J., Oliveira, S.R., Pijoan, C. 2006. Haemophilus parasuis. (Eds), In: B.E. Straw, J. Zimmerman, S. D'Allaire, and D.J. Taylor (Eds), Diseases of swine, 9th ed., 681–690. Iowa State University Press, Ames, Iowa.