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The porcine acute phase protein response to acute clinical and subclinical experimental infection with *Streptococcus suis*

N.S. Sorensen [a,*], C. Tegtmeier [a], L.O. Andresen [a], M. Piñeiro [b], M.J.M. Toussaint [c], F.M. Campbell [d], F. Lampreave [b], P.M.H. Heegaard [a]

[a] *Department of Veterinary Diagnostics and Research, Danish Institute for Food and Veterinary Research, Bülowsvej 27, DK-1790 Copenhagen V, Denmark*
[b] *Department of Biochemistry and Molecular Cell Biology, Faculty of Sciences, University of Zaragoza, Pedro Cerbuna 12, 50009 Zaragoza, Spain*
[c] *Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL Utrecht, The Netherlands*
[d] *Institute of Comparative Medicine, Division of Animal Production and Public Health, Faculty of Veterinary Medicine, University of Glasgow, Bearsden Rd, G61 1QH Glasgow, UK*

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Abstract

The pig acute phase protein (APP) response to experimental *Streptococcus suis* (*S. suis*) infection was mapped by the measurement of the positive APPs C-reactive protein (CRP), serum amyloid A (SAA), haptoglobin (Hp) and major acute phase protein (pig-MAP) and the negative APPs albumin and apolipoprotein (Apo) A-I. The aim was to elucidate the differences in the acute phase behaviour of the individual APPs during a typical bacterial septicaemic infection. Pigs were inoculated subcutaneously with live *S. suis* serotype 2 and blood was sampled before and on various days *post inoculation* (p.i.), until the pigs were killed and autopsied on day 14 p.i. Clinical signs (fever and lameness) were observed in four of the five inoculated pigs from day 2 p.i., and these pigs also had arthritic lesions at autopsy. CRP and SAA showed fast increases in serum concentrations, CRP being elevated from days 1 to 12 p.i. and peaking at 10 times the day 0-levels on day 1 p.i. SAA rose quickly to peak levels of 30–40 times the day 0-level on days 1–2 and returned to pre-inoculation level on day 5 p.i. Hp and pig-MAP showed slightly slower responses, both peaking around 5 days p.i. Hp was increased throughout the experiment with maximum levels around 10 times the day 0-levels, and pig-MAP was elevated on days 1–12 p.i. with peak levels of around seven times the day 0-levels. Apo A-I was decreased from days 1 to 8 and showed minimum levels of about 40% of day 0-levels around 1–2 days p.i. No clear pattern of changes in albumin levels could be identified. One pig, showing clinical signs on day 2 only, also showed an APP response, although of a relatively short duration, whereas three pigs presenting clinical signs for several days had a more protracted acute phase response. Remarkably, the one pig showing no clinical signs and no arthritic lesions showed an APP response comparable to that of the other, clinically affected pigs. Thus, both acute clinical and subclinical *S. suis* infection could be revealed by the measurement of one or more of the APPs CRP, SAA, Hp, pig-MAP and Apo A-I. The combined measurement

Abbreviations: p.i., *post* inoculation; *S. suis*, *Streptococcus suis*; APP, acute phase protein; CRP, C-reactive protein; SAA, serum amyloid A; Hp, haptoglobin; MAP, major acute phase protein; Apo, apolipoprotein

* Corresponding author. Tel.: +45 72346335/292; fax: +45 72346001.
E-mail address: nss@dfvf.dk (N.S. Sorensen).

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of two or three APPs, including proteins with slow and fast kinetics, should be used to achieve the highest sensitivity for the detection of ongoing *S. suis* infection during a prolonged time period. A diagnostic tool based on such APP-measurements could considerably improve strategic control procedures for this important infection.

Keywords: Pig; *Streptococcus suis*; Acute phase response; C-reactive protein; Haptoglobin; Pig major acute phase protein; Serum amyloid A; Albumin; Apolipoprotein A-I

1. Introduction

The acute phase response is the early, unspecific, systemic reaction of the organism to attacks against its integrity, including infection and tissue damage. It is constituted by various mechanisms of defence and tissue repair, all aiming at counteracting invasion, containing damage and restoring homeostasis. Usually initiated by a local inflammatory reaction at the site of injury, the systemic acute phase response includes neurological, endocrine and metabolic changes giving rise to leucocytosis, the development of fever and changes in hepatic gene expression. This last phenomenon, known as the hepatic acute phase protein (APP) response, involves the up-regulation of some serum proteins, whereas other physiologically expressed proteins present in blood are down-regulated (Ceciliani et al., 2002). The acute phase proteins have been empirically defined as proteins whose plasma concentration increase (the positive APPs) or decrease (the negative APPs) by 25% or more following an inflammatory stimulus (Kushner and Mackiewicz, 1993). APPs are robust indicators of inflammation and are induced by pro-inflammatory cytokines such as interleukin 1 (IL-1), IL-6 and tumor necrosis factor α (TNF-α). Cytokines are released from the inflammatory site into the circulation in waves of relatively short duration and are supplemented by a paracrine production of the same cytokines by the hepatic Kupffer cells (Ramadori and Christ, 1999). Thus, the levels of cytokines in serum may only vaguely mirror the APP response which is the collective result of the actions of these and other factors.

Positive APPs include proteins involved in chemotaxis and opsonization, coagulation, wound healing and angiogenesis, and proteins with anti-inflammatory, antioxidant and protease inhibitory activities (Ceciliani et al., 2002). In the pig, major positive acute phase proteins include C-reactive protein (CRP), serum amyloid A (SAA), haptoglobin (Hp) and major acute phase protein (pig-MAP) (reviewed in Petersen et al., 2004). Hp has been widely studied and is seen to increase following a range of different experimental infections and inflammatory lesions in the pig (Petersen et al., 2004). Likewise, SAA and Hp have been shown to increase following experimental induction of swine dysentery by *Brachyspira hyodysenteriae* (Jacobson et al., 2004). In the few porcine studies in which several different proteins were measured, CRP and Hp (Eckersall et al., 1996) and CRP, Hp and pig-MAP (Lampreave et al., 1994) were shown to be sensitive indicators of inflammation induced by turpentine injection, and, together with SAA, to behave as major acute phase reactants after experimental infection with *Actinobacillus pleuropneumoniae* (Heegaard et al., 1998).

Negative APPs in the pig include albumin (Lampreave et al., 1994; Gruys et al., 1994), apolipoprotein (Apo) A-I (Carpintero et al., 2005) and transthyretin (Campbell et al., 2005).

The individual acute phase proteins are differentially regulated by different combinations of cytokines resulting in individual APPs having different characteristics of induction and resolution (Kushner and Mackiewicz, 1993; Baumann and Gauldie, 1994). The positive APPs can be classified according to the magnitude of their increase, with class I proteins increasing around 50%, class II proteins increasing 2–10-fold (e.g. haptoglobin) and class III proteins increasing up to 1000-fold (e.g. CRP and SAA). Although species differences exist, in most species studied, serum levels of CRP and SAA increase early (e.g. 4 h) after an inflammatory stimulus, reach maximum levels within 24–72 h followed by a rapid decline, and CRP and SAA may thus be characterized as rapidly reacting, first line APPs (Kushner and Mackiewicz, 1993; Petersen et al., 2004). In contrast,

most class II APPs, including Hp in some species, increase slightly later, reach peak levels within 5 days, and remain elevated for up to 2 weeks, i.e. the second line APPs.

Whereas the acute phase protein response has been documented for a range of clinical infections in the pig, the potential uses of acute phase proteins for the detection of subclinical infection with various pathogens remain to be established. Subclinically infected animals often play an important role in pig-to-pig disease transmission and subclinical infections may reduce growth of the animal. Furthermore, subclinically infected animals may represent a health hazard for humans, and thus the detection of ongoing subclinical infections would be beneficial.

Streptococcus suis (*S. suis*) is an important zoonotic pathogen of pigs causing septicaemia with localizations in synovial structures and meninges. Clinical presentation of *S. suis* infection varies from asymptomatic bacteraemia to fulminant systemic sepsis, and common pathological features include meningitis, arthritis and septicaemia with sudden death (Staats et al., 1997; Higgins and Gottschalk, 1999). *S. suis* causes major economic losses in countries with intensively managed swine production, and occasionally causes serious disease in humans handling infected pigs or pork, most recently in China (Anonymous, 2005).

S. suis is a Gram-positive, facultatively anaerobic coccus with 35 serotypes of which serotype 2 is the one most frequently isolated from diseased pigs and humans (Gottschalk and Segura, 2000; Wisselink et al., 2000). The infection mainly affects weaned pigs housed intensively, and multiple factors are involved in the development of disease, including the herd health status, the virulence of the *S. suis* strain, and environmental and management factors (Higgins and Gottschalk, 1999). Without treatment, mortality in swine herds due to *S. suis* may be high, but prompt antibiotic therapy significantly reduces mortality (Staats et al., 1997). Therefore, the early detection of infection in the individual pig or at herd level may significantly improve animal welfare and reduce the economic losses.

Diagnosis of *S. suis* infection is currently based on the observation of clinical signs and macroscopic lesions, confirmed by the isolation of the infectious agent and the detection of microscopic lesions in tissues. However, acute *S. suis* infection may occur without clinical signs, and even clinical cases of septicaemia and arthritis may go unrecognised (Clifton-Hadley, 1983; Clifton-Hadley, 1984). Subclinical carriers of *S. suis* are infectious to other pigs and play a significant role in transmission of the disease, and such animals represent an unrecognised hazard to uninfected pen-mates, farmers and abattoir workers (Clifton-Hadley, 1983; Clifton-Hadley et al., 1984).

The aim of this study was a detailed mapping of the acute phase response induced by experimental *S. suis* infection with regard to a range of porcine APPs, i.e. the positive APPs CRP, SAA, Hp and pig-MAP and the negative APPs albumin and Apo A-I, in order to characterise overall response patterns and identify differences in response characteristics with regard to these six APPs. The goals were to identify the most appropriate proteins for the monitoring of *S. suis* infection in the pig and to elucidate the differences in the acute phase behaviour of the individual APPs during a typical bacterial septicaemic acute phase reaction.

2. Materials and methods

2.1. Animals and experimental design

The eight pigs initially included in this study came from a specific pathogen free (SPF) herd free from all serotypes of *Actinobacillus pleuropneumoniae*, as well as *Mycoplasma hyopneumoniae*, toxigenic *Pasteurella multocida*, *Brachyspira hyodysenteriae*, *Sarcoptes scabiei* var. *suis* and with no history of *S. suis* serotype 2 infection. The pigs were crossbred Danish Yorkshire/Danish Landrace, 5 weeks of age and with a mean body weight of 8.6 kg at arrival. They were housed in two isolation units (four pigs in each) and fed antibiotic free feed and offered water ad libitum. The pigs were numbered by ear-tagging and acclimatized for 1 week before infection. The experiment was conducted in accordance with a licence from the Danish Animal Experiments Inspectorate.

On day 0, the pigs were inoculated subcutaneously in the back of the neck with 1 ml of live *S. suis* serotype 2 strain SS02-0119 containing 10^{10} CFU and prepared as described before (Andresen and Tegtmeier, 2001).

Blood samples were collected before infection (on day −8 and day 0) and on days 1, 2, 5, 8, 12 and 14 after infection, when the pigs were euthanized.

2.2. Clinical observations

Rectal temperatures were recorded before and every day throughout the experiment, except on days 10 and 11 *post* inoculation (p.i.), and fever was defined as a rise in body temperature to 40 °C or above. The pigs were observed clinically several times a day from the day of infection and throughout the experiment and signs of disease were recorded twice a day. Three of the eight pigs showed a very acute course of infection with the development of severe lameness and were euthanized on day 2 p.i. These animals were excluded from the study.

2.3. Pathological and microbiological examination

Necropsy was performed after euthanasia and macroscopically visible lesions were recorded. Macroscopic examination included all joints of both left and right extremities. Microbiological examination was performed by cultivation from swabs, tissue samples and samples of cerebrospinal and synovial fluid essentially as described before (Andresen and Tegtmeier, 2001). Samples from the nasal cavity and tonsils were grown on a selective medium (Columbia agar base supplemented with 5% bovine blood, 30 mg/l nalidixinacid, 1.2 mg/l crystal violet and 1.2 mg/ml gentamycin). Microbiological examination was also performed on blood samples from days 1 and 8 p.i. as described by Houe et al. (1993). Samples from cerebrum, cerebellum, medulla oblongata, plexus choroideus, nasal cavity, tonsils, lung, heart, heart valve, liver, spleen, kidney, gut, joints of the right elbow and knee and joints with macroscopically visible lesions were investigated by histology.

2.4. Acute phase protein assays

2.4.1. Pig major acute phase protein, apolipoprotein A-I and albumin

The concentrations of pig-MAP, Apo A-I and albumin were determined by radial immunodiffusion (Mancini et al., 1965) in 1% agarose gels containing specific rabbit polyclonal antiserum prepared as previously described (Lampreave et al., 1994; Gonzalez-Ramon et al., 1995; Carpintero et al., 2005) and using a porcine serum as a secondary standard. The concentration of pig-MAP, Apo A-I and albumin in the secondary standard was previously determined by radial immunodiffusion using the purified proteins as standard (Lampreave et al., 1994; Gonzalez-Ramon et al., 1995; Carpintero et al., 2005). The determinations were done by single measurements, since intra- and inter-assay coefficients of variation with these experimental conditions are below 5%.

2.4.2. C-reactive protein, haptoglobin and serum amyloid A

The concentrations of CRP, Hp and SAA were measured by ELISA.

With regard to CRP, microtitre plates were coated with phosphoryl choline coupled BSA (5 μg/ml) and blocked with 5% milk powder in saline. Samples and standards were diluted in 50 mM Tris, 0.9% NaCl, 10mM $CaCl_2$, 0.1% Tween 20 (TBS-CT buffer), and bound CRP was detected using an in-house anti-pig CRP monoclonal antibody, followed by a peroxidase-labelled goat anti-mouse Ig antiserum (Jackson Immunoresearch Laboratories, West Grove, PA, USA). All washings and additions of secondary reagents were done in TBS-CT buffer. The ELISA was developed using 50 mM citric acid, pH 4.0, 0.1 mM 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 0.01% H_2O_2 as a color substrate and the absorbance read at 405 nm. A porcine serum calibrated against a homemade purified porcine CRP preparation as well as the Porcine Acute Phase test kit from Tridelta (Tridelta Development Ltd, Bray Co. Wicklow, Ireland) was used as an in-plate standard. The determinations were done by triplicate measurements.

Hp was analysed by a sandwich ELISA, using an in-house monoclonal antibody directed against porcine haptoglobin (3.8/D7) as catching antibody, and biotinylated rabbit anti-human haptoglobin antiserum (DAKO, Glostrup, Denmark) as detection antibody, followed by peroxidase-conjugated streptavidin (DAKO). A pool of pig serum calibrated against a porcine haptoglobin standard from Saikin Kagaku Co. Ltd. (Japan) was used as in-plate

standard. Samples and standard were run in duplicate and the absorbance was read at 490 nm with subtraction of 650 nm.

The concentration of SAA in the samples was assessed by a sandwich ELISA from Tridelta in accordance with the manufacturer's instructions. The determinations were run in duplicate. The lowest SAA concentration detected with the assay as determined by the lowest standard concentration used and the lowest dilution of samples tested was 6 μg/ml. The SAA data have been published in a paper describing the negative acute phase response of serum transthyretin during experimental *S. suis* infection (Campbell et al., 2005). In that paper only the group mean SAA-values were shown, whereas here data from each individual pig are presented.

2.5. Statistical analysis

The temperature and APP data were analysed using the statistical software SigmaStat, version 2.0 for Windows. Comparisons of the measurements from before and from various time points p.i. were performed by initially running a one way repeated measures analysis of variance (ANOVA) for data passing the normality and variance homogeneity tests. For data failing either of these tests, a Friedman repeated measures ANOVA on ranks was performed. In both cases, if the between group variability was found to be significant using a threshold *P*-value of 0.05, a multiple comparisons versus control group test (Dunnett's method) was performed to isolate the days p.i. showing APP levels significantly different from levels before inoculation. The overall level of significance for conclusions on Dunnett's method is 0.05. However, exact *P*-values are not produced by the software for this method.

3. Results

3.1. Microbiology, pathology and clinical course of infection

Five pigs inoculated on day 0 with *S. suis* serotype 2 strain SS02-0119 developed relatively mild or no clinical signs and lived until the end of the experiment. In all of these, the establishment of the infection was confirmed by the re-isolation of *S. suis* serotype 2 from blood cultures on days 1 and 8 p.i. (Table 1). At autopsy, *S. suis* serotype 2 was re-isolated from synovial fluid of one of the five pigs (not shown). In addition, *S. suis* serotype 8 was isolated from the tonsil of one pig, whereas *S. suis* serotype 26 was isolated from the heart valve of another. Apart from this, all other *post mortem* samples from the five pigs were sterile.

Clinical signs of arthritis were observed in four of the five animals during the experiment. Thus, mild to moderate lameness was observed on days 2–4 in pig no. 1, on days 2–7 in pig no. 2, on day 2 in pig no. 3 and on days 2–6 in pig no. 4, whereas one pig (no. 5) showed no visible clinical signs of *S. suis* infection at any time point (Table 1). In all animals, the body temperature was seen to rise on day 1 p.i. (Fig. 1). However, only four of the animals developed fever (40 °C or above). Pig no. 5 did not develop fever. In the group as a whole, the observed increase in body temperature induced by infection was statistically significant on days 1 and 2 p.i.

Table 1
Summary of blood cultures, clinical observations and pathological findings of individual pigs inoculated s.c. with 10^{10} CFU of *S. suis* serotype 2 strain SS02-0119 on day 0

Pig no.	Recovery of *S. suis* serotype 2 by blood culture		Lameness (day p.i.)	Arthritis by macroscopic examination at necropsy (day 14 p.i.)	Arthritis[a] by histopathology at necropsy (day 14 p.i.)
	Day 1 p.i.	Day 8 p.i.			
1	+	+	2–4	None	+
2	+	+	2–7	+	+
3	+	+	2	None	+
4	+	+	2–6	+	+
5	+	+	None	None	None

[a] Lesions included chronic proliferative (pig nos. 1–3), subacute suppurative (pig. no. 4) and fibrinous suppurative (pig no. 2) arthritis.

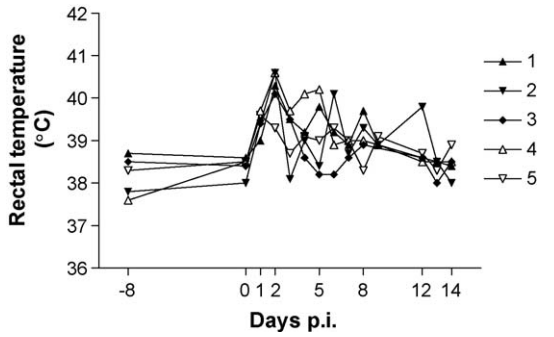

Fig. 1. Rectal temperatures of individual pigs before and on various time-points after s.c. inoculation with 10^{10} CFU of *S. suis* serotype 2 strain SS02-0119.

There was good accordance between the clinical signs of arthritis in the individual pigs and the lesions observed at necropsy. Two of the five pigs (nos. 2 and 4) showed macroscopic signs of arthritis at necropsy, and both of these also showed lameness for a protracted time-period (Table 1). Furthermore, all four pigs developing lameness during infection also showed lesions associative with arthritis by histology (Table 1). The microscopic lesions included chronic proliferative arthritis (pigs nos. 1–3), subacute suppurative arthritis (pig no. 4) and fibrinous suppurative arthritis (pig no. 2). Within the joints investigated, no arthritic lesions were observed in pig no. 5, which also showed no clinical signs of infection. A large number of different organs and tissues from the five animals were subjected to histological examination, but apart from the arthritic lesions detected, no other lesions were revealed which could be associated with *S. suis* infection or any other infectious agent.

3.2. Acute phase protein responses

Fig. 2 shows the individual CRP-, SAA-, Hp-, pig-MAP- and Apo A-I responses of the five pigs after inoculation with *S. suis*. *CRP* (Fig. 2A) had a mean pre-challenge level on day 0 of 5.13 μg/ml (mean + 2S.D.: 14.25 μg/ml, range: 0.55–12.33 μg/ml). Already on day 1 p.i., CRP-levels increased sharply in all animals and in general reached maximum between days 1 and 5 p.i., showing an approximately 10-fold increase as compared to day 0. From around day 8 p.i. the response started to decrease, but was still elevated in pig nos. 1 and 2 on day 14 p.i. By statistical analysis, the changes in CRP level induced by the infection were significantly different from the day 0-level on days 1, 2, 5, 8 and 12, and thus covered a large part of the experimental period.

SAA (Fig. 2B) showed pre-challenge levels of 6 μg/ml or below in all animals (no variation, samples below detection limit of the assay). After challenge, SAA levels rose sharply in all pigs on day 1 p.i., and peaked already days 1 and 2, reaching peak levels of at least 30–40 times the levels of before infection. On day 5 p.i. the SAA-levels had decreased and generally reached baseline around day 8 p.i. The increases in SAA levels induced by infection were statistically significant on days 1 and 2 p.i. only.

Hp (Fig. 2C) showed a mean pre-challenge level on day 0 of 0.43 mg/ml (mean + 2S.D.: 1.28 mg/ml, range 0.01–0.94 mg/ml). Levels increased day 1 p.i. in all animals, but the increase was slightly less steep as compared to CRP and SAA, and peak levels of about 10 times the day 0-levels were seen around day 5 p.i. From day 8 the response generally decreased, but levels were still elevated as compared to day 0 on day 14 p.i. In accordance with this, the increases in Hp seen on days 1, 2, 5, 8, 12 and 14 p.i., i.e. throughout the experiment, were all statistically significant.

Regarding *pig-MAP* (Fig. 2D), a mean pre-challenge level of 0.63 mg/ml (mean + 2S.D.: 0.76 mg/ml, range 0.53–0.70 mg/ml) was seen. The level of pig-MAP also increased by day 1 p.i., and as with Hp the increase was less abrupt than that of CRP and SAA, and peak responses of seven times the day 0-levels were seen day 5 p.i. The pig-MAP levels in general decreased from day 8 p.i. but were still elevated as compared to before inoculation on day 14 p.i. in pig nos. 1, 2 and 4. The changes in pig-MAP as compared day 0-levels were statistically significant on days 1, 2, 5, 8 and 12, and thus covered the main part of the experimental period.

Apo A-I (Fig. 2E) showed a mean day 0-level of 2.64 mg/ml (mean − 2S.D.: 2.13 mg/ml, range 2.33–3.01). On day 1 after inoculation with *S. suis* levels of Apo A-I were seen to decrease sharply to minimum levels of about 40% of day 0-levels on day 1–2 p.i. From day 2 p.i. the levels gradually increased and approached day 0-levels days 12–14 p.i., latest in pig nos. 1, 2 and 4. The decreases in Apo A-I as compared

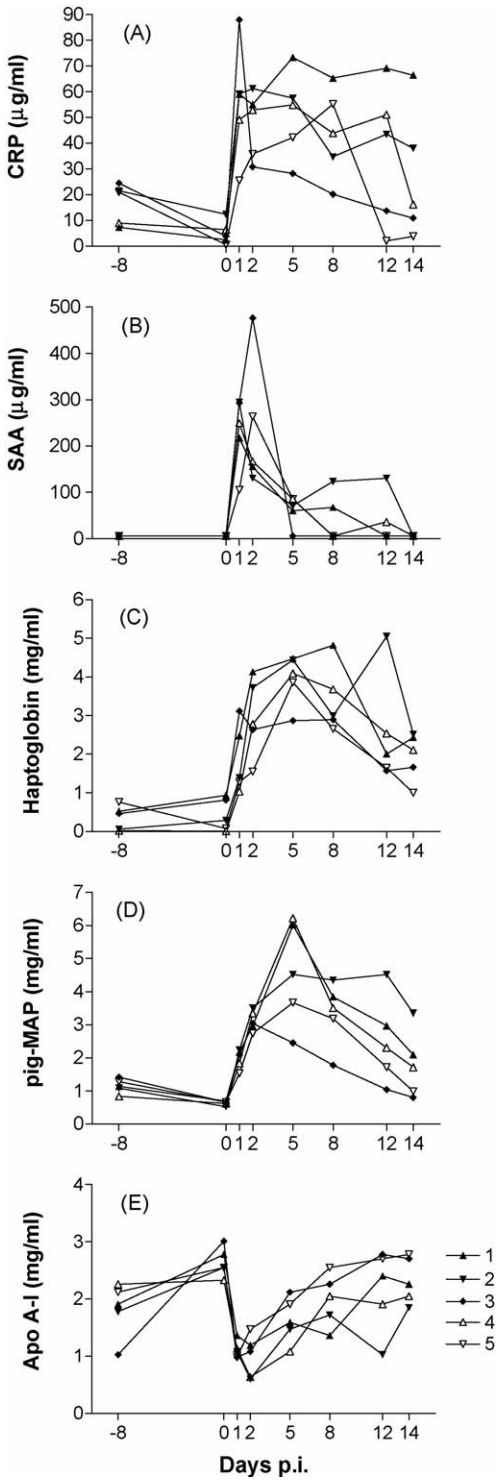

Fig. 2. Concentrations of CRP (A), SAA (B), Hp (C), pig-MAP (D) and Apo A-I (E) in serum of individual pigs before and on various time-points after s.c. inoculation with *S. suis*.

to day 0-levels were statistically significant on days 1, 2, 5 and 8, and thus covered a considerable part of the infection period. Serum levels of *albumin* were also determined, but no clear pattern of changes from before infection-levels could be identified, and no significant difference was found in mean albumin level between the days (results not shown).

In Fig. 3, the CRP-, SAA-, Hp-, pig-MAP- and Apo A-I responses of each of the five individual pigs have been plotted separately in order to enable a direct comparison of the response kinetics of the individual APPs for each animal and to elucidate between-animal differences in overall APP response-kinetics. From this, SAA and CRP are confirmed as early responders in all five animals. However, whereas SAA displays a relatively early decrease, CRP stays elevated for the main part of the experiment in all animals. In comparison, Hp and pig-MAP are seen to increase somewhat slower in all pigs, to generally reach their peak levels a couple of days later than SAA and CRP, and to stay elevated throughout the experiment. Apo A-I is seen to behave as an early, negative responder, reaching its minimum when SAA and CRP peak. When comparing the overall APP response-kinetics of the individual animals, differences can also be noted. Thus, pig no. 3 (Fig. 3C) displays a particularly early APP-response with regard to all five reactants, followed by a relatively quick return to near-normal levels. Pig no. 5 (Fig. 3E) displays an APP-response of intermediate length, which appears slightly delayed with regard to SAA, CRP and Hp, whereas pig no. 1 (Fig. 3A), pig no. 2 (Fig. 3B) and pig no. 4 (Fig. 3D) all show relatively protracted APP-responses, most clearly seen as prolonged responses of SAA, CRP and Apo A-I.

3.3. Acute phase protein responses—correlation with clinical findings

When correlating the responses of the individual, responding APPs to the clinical observations and pathological findings, there was good accordance: Thus, all five APPs showed significant changes as compared to day 0-levels on day 1 p.i., corresponding

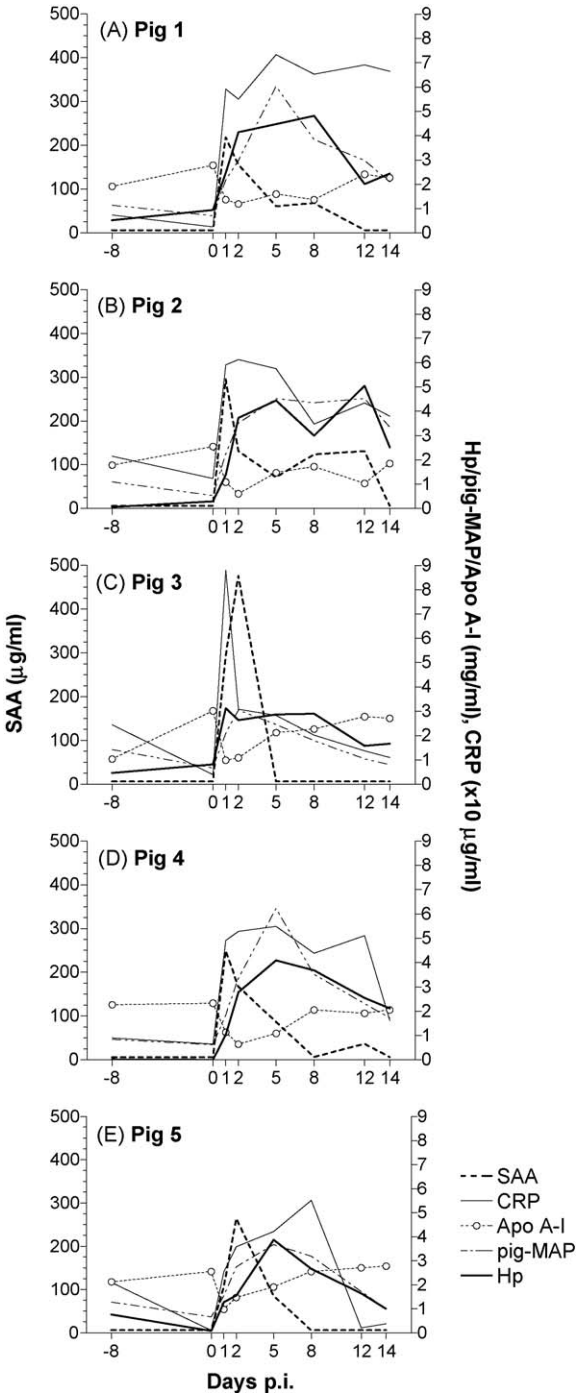

Fig. 3. Concentrations of the acute phase proteins CRP, SAA, Hp, pig-MAP and Apo A-I in pig no. 1 (A), pig no. 2 (B), pig no. 3 (C), pig no. 4 (D) and pig no. 5 (E) before and on various time-points after s.c. inoculation with *S. suis*. The left *Y*-axis corresponds to the SAA-measurements, whereas the right *Y*-axis corresponds to the measurements of CRP, Hp, pig-MAP and Apo A-I. Please note that different scales apply to the different APP-measurements.

with a rise in rectal temperature on the same day in all animals (compare Figs. 1 and 2). Likewise, all five APPs showed maximum (minimum with regard to Apo A-I) response levels around or shortly after day 2 p.i., concurrent with the development of fever and lameness in four of the five animals (Fig. 2). In addition, all five APPs were seen to reflect differences in the duration of clinical symptoms. Thus, the three pigs showing lameness for an extended period of time (nos. 1, 2 and 4), also were the ones to show the latest return towards baseline levels of all five APPs (Fig. 3A, B and D). Of these, two pigs showing lameness for a particularly protracted period (pig no. 2: days 2–7 p.i. and pig no. 4: days 2–6 p.i.) displayed arthritic lesions at autopsy both by macroscopic examination and at the microscopic level, whereas one pig (no. 1), showing lameness days 2–4, only showed arthritic lesions at the microscopic level. However, these slight differences in duration of lameness and the extent of the *post mortem*-evidence of arthritis did not result in differences in the duration of APP-responses of these three pigs (compare Fig. 3A, B and D). Remarkably, even subclinical infection with *S. suis* (pig no. 5) resulted in APP-responses of comparable duration and magnitude as did infection associated with clinical manifestations and pathologic lesions at autopsy (nos. 1–4) (compare Fig. 3A–D with Fig. 3E). Thus, from this it appears that both clinical and subclinical *S. suis* infection may be detected by the measurement of one or more of the APPs CRP, SAA, Hp, pig-MAP and Apo A-I.

4. Discussion

In this study, the non-induced concentrations of APPs were clearly defined by the pre-inoculation samples and the APP response following inoculation with *S. suis* was closely correlated with other clinical signs and pathological lesions typical of *S. suis* infections, and no evidence of other infections were found. The one animal showing no clinical signs of lameness or arthritis (no. 5), but showing a full blown acute phase response, might theoretically have been

affected by an undetected inflammation. Although by no means certain, an aberrant infection might have been revealed by a control group receiving sterile saline only. Such a control group was not included in the present work as it is well-known from other studies that subcutaneous injection of sterile saline, the suspension medium used in the present study, does not cause an acute phase reaction (see, e.g. (Eckersall et al., 1996)). Also, it does seem more likely that the acute phase response detected in the subclinical animal was in fact elicited by the experimental *S. suis* infection, which was demonstrated in the blood of this animal on days 1 and 8 p.i.

The haptoglobin acute phase response to experimental infection with a *S. suis* serotype 2 strain was studied recently by Knura-Deszczka et al. (2002). In complete accordance with our findings, a significant increase in the Hp concentration was seen on day 1 p.i., peaking 4 days p.i. with levels of around 3.5 mg/ml, and staying significantly elevated until the end of the experiment (day 10 p.i.). Furthermore, the response of Apo A-I and pig-MAP was studied more recently in the same experimental model (Carpintero et al., 2005). Similarly to our findings, Apo A-I reacted with a strong reduction, reaching minimum values around days 2 and 3 p.i., whereas the positive response of pig-MAP was slightly slower, peaking 4–5 days p.i. However, in that study both proteins showed stronger and more protracted responses compared to our results, corresponding well with the fact that the animals of the Knura-Deszczka/Carpintero studies experienced a more severe course of infection with higher peak temperatures and more prolonged clinical signs. This therefore further supports our findings that the duration of the period of clinical signs is reflected in the duration of the acute phase protein response.

The finding that albumin did not show a clear response pattern is surprising, as albumin has previously been shown to behave as a negative APP in the pig after turpentine-induced inflammation (Lampreave et al., 1994). The response of albumin to *S. suis* infection was characterized by an extreme between-animal variation, and further studies with larger groups of animals are needed in order to firmly establish the behaviour of serum albumin during a bacterial septicaemic acute phase reaction in the pig.

The APP response has been studied during a number of other experimental infections in the pig, but except for one study (Heegaard et al., 1998), only Hp (Hall et al., 1992; Francisco et al., 1996; Jungersen et al., 1999; Magnusson et al., 1999; Knura-Deszczka et al., 2002) or Hp and one additional positive acute phase protein (Asai et al., 1999; Enemark et al., 2003; Jacobson et al., 2004) were measured. In the study by Heegaard et al. (1998), the APP response of Hp, CRP, pig-MAP and SAA to experimental infection with *Actinobacillus pleuropneumoniae* (*A.p.*) was studied. The kinetics of the responses of these APPs to *A.p.*-infection was similar to the responses seen in the present study. Thus, in accordance with our results, Hp was shown to respond rapidly and with protracted response kinetics. This was also seen in other studies covering a sufficient time-period (Jungersen et al., 1999; Asai et al., 1999; Magnusson et al., 1999; Enemark et al., 2003; Jacobson et al., 2004). Also in agreement with the present study, Heegaard et al. (1998) found the CRP response to be very fast, and slightly less protracted than the response of Hp. In contrast, in our study, pig-MAP increased nearly as quickly as Hp in response to *S. suis* infection, whereas after *A.p.*-challenge pig-MAP responded slightly slower than Hp (Heegaard et al., 1998). In both studies the response of pig-MAP was slightly more pointed than that of haptoglobin. Finally, SAA was expressed very transiently and very strongly upon *A.p.*-challenge (Heegaard et al., 1998) in agreement with our results as well as with the transient increase of SAA seen after induction of swine dysentery (Jacobson et al., 2004).

Taken together, different patterns of responses could be identified within the APPs tested. CRP could be characterized as a very fast/protracted responder, SAA as a very fast/transient responder and Apo A-I as a very fast/intermediately protracted responder. Hp could be described as a fast/very protracted responder and pig-MAP as a fast/protracted responder. This corresponds quite well with the general description based on other species of SAA and CRP as rapid reacting first line or ‘‘type 1’’ APPs induced by the cooperative action of IL-6- and IL-1-type cytokines (Ramadori and Christ, 1999) and characterized by an early dramatic increase in serum concentration followed by a rapid normalization (Kushner and Mackiewicz, 1993; Baumann and Gauldie, 1994; Steel and Whitehead, 1994; Ramadori and Christ, 1999). Likewise, Hp and MAP in the pig seem to act as

second line or "type 2" APPs induced by IL-6-type cytokines (Ramadori and Christ, 1999; Gonzalez-Ramon et al., 2000) and characterized by a later increase and a response lasting up to two weeks. In reality, however, CRP in this study behaved like a "type 1/2" protein with an SAA-like kinetics of induction combined with a relatively prolonged response. These results underscore the differential regulation of each individual APP within and between species.

The differences in responsiveness affected the information that can be derived from the proteins. Thus, the very quickly reacting proteins CRP and SAA, and to some degree Apo A-1, were particularly efficient in elucidating differences in response kinetics between the individual pigs. Such differences were less evident with regard to pig-MAP and Hp, which instead were the most robust indicators of infection. The finding that the initial rise in rectal temperature and the initial changes in APP-levels completely coincided is to be expected, since both parameters reflect the onset of the systemic acute phase response to infection. Furthermore, since these initial changes seen on day one p.i. were similar in all five animals, this could indicate that the *initiation* of the acute phase response mainly depended on the bacterial challenge. LPS-induced inflammation is a frequently applied model of the acute phase reaction, and the surface LPS and peptidoglycan of gram-positive bacteria may also induce an acute phase response by the direct activation of mononuclear phagocytes (Ramadori and Christ, 1999) as was recently shown by the induction of inflammatory cytokines (including TNF-α, IL-1 and IL-6) in a human monocytic cell line after exposure to *S. suis* (Segura et al., 2002). In contrast, the *duration* of the acute phase response in the individual animals seemed to reflect the extent of tissue damage, since protracted APP responses in general were associated with a prolonged period of clinical signs.

The observation that subclinical infection with *S. suis* resulted in APP-responses of similar magnitude as did *S. suis* infection associated with clinical manifestations further supports the idea that the acute phase response to this pathogen may appear by its mere presence in blood and internal tissues, rather than by the actual development of pathology. If this is the case, this would enable the early detection of even subclinical *S. suis* infection and thus help limit the (sometimes unrecognized) spreading of the organism at the herd level. However, this single finding needs confirmation by other studies in order to be given significance.

In conclusion, even though all five responding acute phase proteins to some degree reflected differences in the duration of clinical signs there was a tendency for the early responders (SAA, CRP and Apo A-I) to better reflect such differences than the protracted responders pig-MAP and Hp. These, on the other hand, showed strong and protracted responses to infection in all five pigs and could thus be seen as particularly robust indicators of infection at various stages of disease progression, including subclinical infection. The combined measurement of two to three APPs with different response characteristics, e.g. CRP, Apo A-I and Hp, would give a high sensitivity of detection throughout the course of an ongoing *S. suis* infection. The measurement of one or a combination of these acute phase proteins would enable the detection of ongoing *S. suis* infection for a prolonged time-period (all of the APPs tested were significantly elevated from day 1 p.i. and some of them were significantly elevated for up to 2 weeks) as compared to the measurement of rectal temperature (significantly elevated on days 1 and 2, only) and would furthermore enable the detection of ongoing *S. suis* infection even when accompanied by limited or no clinical signs. Though not specific, strategic APP-measurements in the pig herds may reveal ongoing bacterial infection, enable the tracing of infection and pinpointing of infected animals for targeted treatment, and improve the monitoring of treatment efficiency, all of which would improve herd health and human safety and reduce economic losses (Petersen et al., 2004).

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References

Andresen, L.O., Tegtmeier, C., 2001. Passive immunization of pigs against experimental infection with Streptococcus suis serotype 2. Vet. Microbiol. 81, 331–344.

Anonymous, 2005. Outbreak associated with *Streptococcus suis* in pigs, China. Weekly Epidemiol. Rec. 80, 269–270.

Asai, T., Mori, M., Okada, M., Uruno, K., Yazawa, S., Shibata, I., 1999. Elevated serum haptoglobin in pigs infected with porcine reproductive and respiratory syndrome virus. Vet. Immunol. Immunopathol. 70, 143–148.

Baumann, H., Gauldie, J., 1994. The acute phase response. Immunol. Today 15, 74–80.

Campbell, F.M., Waterston, M., Andresen, L.O., Sorensen, N.S., Heegaard, P.M., Eckersall, P.D., 2005. The negative acute phase response of serum transthyretin following *Streptococcus suis* infection in the pig. Vet. Res. 36, 657–664.

Carpintero, R., Pineiro, M., Andres, M., Iturralde, M., Alava, M.A., Heegaard, P.M., Jobert, J.L., Madec, F., Lampreave, F., 2005. The concentration of apolipoprotein A-I decreases during experimentally induced acute-phase processes in pigs. Infect. Immun. 73, 3184–3187.

Ceciliani, F., Giordano, A., Spagnolo, V., 2002. The systemic reaction during inflammation: the acute-phase proteins. Protein Pept. Lett. 9, 211–223.

Clifton-Hadley, F.A., 1983. *Streptococcus suis* type 2 infections. Br. Vet. J. 139, 1–5.

Clifton-Hadley, F.A., 1984. Studies of *Streptococcus suis* type 2 infection in pigs. Vet. Res. Commun. 8, 217–227.

Clifton-Hadley, F.A., Alexander, T.J., Upton, I., Duffus, W.P., 1984. Further studies on the subclinical carrier state of *Streptococcus suis* type 2 in pigs. Vet. Rec. 114, 513–518.

Eckersall, P.D., Saini, P.K., McComb, C., 1996. The acute phase response of acid soluble glycoprotein, alpha(1)-acid glycoprotein, ceruloplasmin, haptoglobin and C-reactive protein, in the pig. Vet. Immunol. Immunopathol. 51, 377–385.

Enemark, H.L., Bille-Hansen, V., Lind, P., Heegaard, P.M., Vigre, H., Ahrens, P., Thamsborg, S.M., 2003. Pathogenicity of *Cryptosporidium parvum*—evaluation of an animal infection model. Vet. Parasitol. 113, 35–57.

Francisco, C.J., Shryock, T.R., Bane, D.P., Unverzagt, L., 1996. Serum haptoglobin concentration in growing swine after intranasal challenge with *Bordetella bronchiseptica* and toxigenic *Pasteurella multocida* type D. Can. J. Vet. Res. 60, 222–227.

Gonzalez-Ramon, N., Alava, M.A., Sarsa, J.A., Pineiro, M., Escartin, A., Garcia-Gil, A., Lampreave, F., Pineiro, A., 1995. The major acute phase serum protein in pigs is homologous to human plasma kallikrein sensitive PK-120. FEBS Lett. 371, 227–230.

Gonzalez-Ramon, N., Hoebe, K., Alava, M.A., Van Leengoed, L., Pineiro, M., Carmona, S., Iturralde, M., Lampreave, F., Pineiro, A., 2000. Pig MAP/ITIH4 and haptoglobin are interleukin-6-dependent acute-phase plasma proteins in porcine primary cultured hepatocytes. Eur. J. Biochem. 267, 1878–1885.

Gottschalk, M., Segura, M., 2000. The pathogenesis of the meningitis caused by *Streptococcus suis*: the unresolved questions. Vet. Microbiol. 76, 259–272.

Gruys, E., Obwolo, M.J., Toussaint, M.J.M., 1994. Diagnostic significance of the major acute phase proteins in veterinary clinical chemistry: a review. Vet. Bull. 64, 1009–1018.

Hall, W.F., Eurell, T.E., Hansen, R.D., Herr, L.G., 1992. Serum haptoglobin concentration in swine naturally or experimentally infected with *Actinobacillus pleuropneumoniae*. J. Am. Vet. Med. Assoc. 201, 1730–1733.

Heegaard, P.M., Klausen, J., Nielsen, J.P., Gonzalez-Ramon, N., Pineiro, M., Lampreave, F., Alava, M.A., 1998. The porcine acute phase response to infection with *Actinobacillus pleuropneumoniae*. Haptoglobin, C-reactive protein, major acute phase protein and serum amyloid A protein are sensitive indicators of infection. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 119, 365–373.

Higgins, R., Gottschalk, M., 1999. Streptococcal diseases. In: Straw, B.E., Allaire, S., Mengeling, W.L. (Eds.), Diseases of Swine. Iowa University Press, Iowa, pp. 563–578.

Houe, H., Eriksen, L., Jungersen, G., Pedersen, D., Krogh, H.V., 1993. Sensitivity, specificity and predictive value of blood cultures from cattle clinically suspected of bacterial endocarditis. Vet. Rec. 133, 263–266.

Jacobson, M., Fellstrom, C., Lindberg, R., Wallgren, P., Jensen-Waern, M., 2004. Experimental swine dysentery: comparison between infection models. J. Med. Microbiol. 53, 273–280.

Jungersen, G., Jensen, L., Riber, U., Heegaard, P.M., Petersen, E., Poulsen, J.S., Bille-Hansen, V., Lind, P., 1999. Pathogenicity of selected *Toxoplasma gondii* isolates in young pigs. Int. J. Parasitol. 29, 1307–1319.

Knura-Deszczka, S., Lipperheide, C., Petersen, B., Jobert, J.L., Berthelot-Hérault, F., Kobisch, M., Madec, F., 2002. Plasma haptoglobin concentration in swine after challenge with *Streptococcus suis*. J. Vet. Med. B 49, 240–244.

Kushner, I., Mackiewicz, A., 1993. The acute phase response: an overview. In: Mackiewicz, A., Kushner, I., Baumann, H. (Eds.), Acute Phase Proteins: Molecular Biology, Biochemistry and Clinical Applications. CRC Press, Boca Raton, pp. 3–19.

Lampreave, F., Gonzalez-Ramon, N., Martinez-Ayensa, S., Hernandez, M.A., Lorenzo, H.K., Garcia-Gil, A., Pineiro, A., 1994. Characterization of the acute phase serum protein response in pigs. Electrophoresis 15, 672–676.

Magnusson, U., Wilkie, B., Artursson, K., Mallard, B., 1999. Interferon-alpha and haptoglobin in pigs selectively bred for high and low immune response and infected with *Mycoplasma hyorhinis*. Vet. Immunol. Immunopathol. 68, 131–137.

Mancini, G., Carbonara, A.O., Heremans, J.F., 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2, 235–254.

Petersen, H.H., Nielsen, J.P., Heegaard, P.M., 2004. Application of acute phase protein measurements in veterinary clinical chemistry. Vet. Res. 35, 163–187.

Ramadori, G., Christ, B., 1999. Cytokines and the hepatic acute-phase response. Semin. Liver Dis. 19, 141–155.

Segura, M., Vadeboncoeur, N., Gottschalk, M., 2002. CD14-dependent and -independent cytokine and chemokine production by human THP-1 monocytes stimulated by *Streptococcus suis* capsular type 2. Clin. Exp. Immunol. 127, 243–254.

Staats, J.J., Feder, I., Okwumabua, O., Chengappa, M.M., 1997. *Streptococcus suis*: past and present. Vet. Res. Commun. 21, 381–407.

Steel, D.M., Whitehead, A.S., 1994. The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein. Immunol. Today 15, 81–88.

Wisselink, H.J., Smith, H.E., Stockhofe-Zurwieden, N., Peperkamp, K., Vecht, U., 2000. Distribution of capsular types and production of muramidase-released protein (MRP) and extracellular factor (EF) of *Streptococcus suis* strains isolated from diseased pigs in seven European countries. Vet. Microbiol. 74, 237–248.