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Interleukin 6, serum amyloid A and haptoglobin as markers of treatment efficacy in pigs experimentally infected with *Actinobacillus pleuropneumoniae*

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Abstract

The possibility to use acute phase proteins to monitor the elimination of a bacterial infection in pigs would facilitate an objective assessment of treatment with various antimicrobial substances. To examine this possibility, the acute phase response (IL-6, serum amyloid A (SAA), and haptoglobin) elicited by *Actinobacillus pleuropneumoniae* and its reduction on treatment with various antibiotics was studied in serum from specific pathogen free (SPF) pigs. Pigs were infected intranasally with *A. pleuropneumoniae* serotype 2, and either left as non-treated control pigs or treated with different antibiotics intramuscularly at onset of respiratory disease (20 h post-infection). Pigs responded to the infection with prominent increases in activity and concentrations of IL-6, SAA, and haptoglobin. These responses were to a certain extent overlapping and covered the time span from a few hours after infection until development of detectable levels of specific antibodies (7–10 days post-infection in untreated pigs). The haptoglobin response lasted until the end of the study on day 17 and thereby partly coincided with the antibody response. Treatment with antimicrobials that effectively reduced establishment of the infection with *A. pleuropneumoniae* also reduced the duration of all three acute phase responses, and reduced the concentration of serum haptoglobin. In contrast, less efficacious treatments did not reduce these acute phase responses. Thus, acute phase reactants can be applied to monitor therapeutic effects of antimicrobial drugs in the pig and measurements of IL-6,

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SAA and haptoglobin could add valuable information about the stage of infection during a disease outbreak.

Keywords: Pig; Bacteria; *Actinobacillus pleuropneumoniae*; Antibiotics; IL-6; Serum amyloid A; Haptoglobin

1. Introduction

Diseases of the respiratory tract constitute a major health disturbance within pig production (Christensen et al., 1999) and one of the dominating causative agents within this disease complex is *Actinobacillus pleuropneumoniae*. The bacteria may, alone or in combination with other microorganisms, cause considerable harm to affected pigs. The acute form of actinobacillosis, that may be life threatening, is manifested by a sudden onset of severe respiratory signs that coincide with increased body temperature and anorexia. However, a chronic onset of disease is more common and the infection can even proceed subclinically (Taylor, 1999). Being a Gram-negative rod, *A. pleuropneumoniae* contains lipopolysaccharides (LPS), which are potent inducers of the acute phase response in several species (Boosman et al., 1989; Rygg et al., 1993a; Syversen et al., 1994) but also other bacterial compounds such as fimbriae, muramyl dipeptide and peptidoglycan can induce the production of pro-inflammatory cytokines (Henderson and Wilson, 1996). Furthermore, *A. pleuropneumoniae* invasion of lungs causes tissue damage (Madsen et al., 1995; Woo et al., 1996) that may contribute to the acute phase response.

The prompt elevation of cytokine and acute phase protein concentrations in serum following microbial invasion makes these proteins useful as inflammatory markers in veterinary medicine (Gruys et al., 1994; Murtaugh et al., 1996; Baarsch et al., 2000; Van Reeth et al., 2002). In the pig, several acute phase proteins, including haptoglobin (Hall et al., 1992; Heegaard et al., 1998), C-reactive protein (CRP), major acute phase protein (pig-MAP) and serum amyloid A (SAA) (Heegaard et al., 1998) have been proven potentially useful as inflammatory markers of *A. pleuropneumoniae* infections. In addition, production of cytokines, such as TNF-α, IL-1 (Huang et al., 1999) and IL-6 (Fossum et al., 1998; Johansson et al., 2001) has been demonstrated in serum following challenge with *A. pleuropneumoniae*.

A. pleuropneumoniae is generally susceptible to several antimicrobial agents as determined in vitro, and consequently different treatment regimens of diseased pigs have been applied. Substances reported to be effective include penicillin G (Willson and Osborne, 1985), enrofloxacin (Stephano et al., 1988), cephalosporins (Hsu et al., 1990) and tiamulin (Anderson and Williams, 1990). Evaluation of treatment with these antimicrobials using recordings of clinical signs of disease, development of antibodies and pathological lesions at autopsy have, however, indicated different efficacy (Wallgren et al., 1999). Consequently, a relationship between the efficacy of therapy and the magnitude of acute phase protein responses mounted following an infection with *A. pleuropneumoniae* would be expected.

In the present study, three acute phase markers (IL-6, SAA and haptoglobin) were determined in serum from pigs experimentally infected with *A. pleuropneumoniae* and

subsequently treated with different antibiotic substances. The magnitude and duration of the acute phase responses were compared and related to clinical observations in order to evaluate their usefulness in the assessment of treatment efficacy.

2. Materials and methods

2.1. Animals, experimental infection and antibiotic treatment

Specific pathogen free (SPF) Swedish Landrace × Swedish Yorkshire pigs (Serogrisen, Ransta, Sweden; Wallgren et al., 1999) were purchased at the age of 9 weeks. On arrival at the National Veterinary Institute (NVI) the pigs were allotted into groups, taking into account litter origin, weight and sex. The pigs, which were allowed to acclimatise for 1 week before start of the experiment, were fed a commercial dry feed diet (Slaktfor 290, Lantmännen, Svalöv, Sweden) and had free access to water.

The experiment (see Fig. 1) comprised three groups with 10 pigs each which were housed in separate rooms. Eight days after arrival (day 0) all pigs in two of the groups were infected intranasally with 10^8 colony forming units (CFU) of *A. pleuropneumoniae* serotype 2 (strain 700/89) grown overnight on PPLO agar at 37 °C in a humid atmosphere with 5% CO_2. After development of clinical signs, at 20 h post-infection (pi), pigs in one of the infected groups were given intramuscular injections with 2.5 mg enrofloxacin per kg body weight once daily for 3 days (Baytril® 25 mg/ml, Bayer, Leverkusen, Germany), a treatment known to be effective in curing clinical *A. pleuropneumoniae* infection (Wallgren et al., 1999). Pigs in the other infected group were left untreated whereas pigs in the third group served as uninfected, untreated control pigs. The experiment was ended 17 days post-infection.

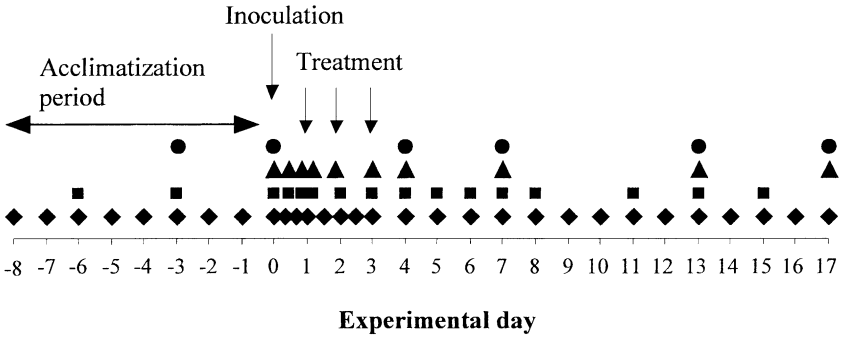

Fig. 1. Schematic description of the *A. pleuropneumoniae* infection model used for assessment of antibiotic therapy in pigs, for details see Wallgren et al., 1999; Fossum et al., 1998. Symbols indicate: clinical examination (rhombs), measurement of rectal temperature (squares), blood sampling for acute phase reactants (triangles) and serum antibodies to *A. pleuropneumoniae* (circles), respectively. The recordings of respiratory symptoms, appetite and rectal temperature were carried out twice daily during the first 2 days of infection. During that period, blood samples were collected at 0 h; 10, 20, 28 and 44 h pi.

2.2. Clinical recordings and sampling procedures

Clinical recordings and blood sampling were performed as outlined in Fig. 1. Respiratory signs were scored grading severity of symptoms as 0 (no respiratory signs), 1 (moderately forced breathing and hoarseness), 2 (moderately forced breathing and sporadic cough or severely forced breathing) and 3 (severely forced breathing and intermittent cough). The feed intake was graded on group basis from zero (refusal) to five (all consumed). Pigs were restrained by snaring when rectal body temperatures were measured. Blood was collected by jugular venipuncture using tubes without additive (Becton-Dickinson, Meylan Cedex, France). After centrifugation and collection of serum, samples were stored at −20 °C.

2.3. Necropsies and reisolation of A. pleuropneumoniae

All pigs were euthanized at the end of the experiment (on day 17) and the lungs were inspected for signs of pneumonia and pleuritis. Samples for isolation of *A. pleuropneumoniae* were collected with sterile cotton swabs from affected parts of the lung, bronchus, bronchial lymph node, pericardium and, when present, from abscesses. In lungs without lesions the sample was collected from the dorsal part of the diaphragmal lobe. The samples were cultured on blood agar plates and cross-inoculated with a single streak of a nurse strain of *E. coli* at 37 °C (Biberstein et al., 1977). Isolates of *A. pleuropneumoniae* were serotyped according to Gunnarsson et al. (1977).

2.4. Detection of antibodies to A. pleuropneumoniae

Serum samples were analysed for presence of antibodies to *A. pleuropneumoniae* using an ELISA technique as previously described (Wallgren and Persson, 2000). The samples were diluted 1:1000 and absorbance values exceeding 0.3 OD units when read at 450 nm were considered as positive reactions.

2.5. Detection of interleukin-6, SAA and haptoglobin

IL-6 activity in serum was measured using a bioassay based on proliferation of cell line B9 as previously described (Aarden et al., 1987; Fossum et al., 1998). Levels of IL-6 activity were calculated using a serially diluted murine recombinant IL-6 preparation (Genzyme Diagnostics, Cambridge, MA) as a laboratory standard. The detection limit of the assay was 0.3 U/ml and the standard curve range was 0.03–5 U/ml.

Serum concentrations of SAA were measured using a commercially available kit (Phase^{TM} Range SAA Assay, Tridelta Development Ltd., Greystones, Wicklow, Ireland). The assay was performed as recommended by the manufacturer, except for the standard curve range that was extended to a working range comprising sample concentrations from 19.5 to 1250 mg/l (sample dilution 1:500). Within this interval the intra- and interassay coefficients of variation were <10%. Concentrations twice as high as the detection level were considered as positive reactions.

Serum concentrations of haptoglobin were measured using a commercially available kit (Phase^{TM} Range Haptoglobin Assay, Tridelta Development Ltd.). The haptoglobin

assay was performed according to the manufacturers instructions on an automated analyser (Coba's Mira, Hoffmann-La Roche, Basel, Switzerland). The assay is based on a colorimetric reaction and the working range of the assay is 0.05–6 g/l. Intra- and interassay coefficients of variation were <1.5 and <4%, respectively. The haptoglobin concentrations above 1.0 g/l were considered as positive reactions.

2.6. Archived serum samples from a previous experimental infection of SPF-pigs with A. pleuropneumoniae

After evaluation of the initial experiment, the study was expanded by analysing archived serum samples collected during a previous experimental infection with *A. pleuropneumoniae*, using the same experimental model. The serum samples originated from six experimental groups with eight pigs in each. Pigs in one group were infected but not treated and one control group included non-infected, non-treated pigs. Pigs in the other four groups were treated with different antibiotic substances (enrofloxacin, 2.5 mg/kg body weight Baytril®, Bayer, Leverkusen, Germany; ceftiofur, 3.0 mg/kg body weight, Exenel®, Pharmacia & Upjohn Animal Health, Kalamazoo, USA; penicillin G, 2.0 mg/kg body weight Penovet®, Boeringer-Ingelheim Vetmedica, Germany; tiamulin, 15.0 mg/kg body weight, Tiamutin®, Leo, Copenhagen, Denmark). All treatments were initiated at 20 h pi and all substances were administered once a day for 3 days. For a more detailed description see Fossum et al., 1998 and Wallgren et al., 1999.

2.7. Statistical analyses

The duration of clinical signs of disease was evaluated by calculating the median number of days with respiratory symptoms and/or increased body temperatures (>40.0 °C), and the duration of the acute phase responses was evaluated as median number of sampling days with increased concentrations of IL-6 (>0.3 U/ml), SAA (>40 mg/l) or haptoglobin (>1 g/l). The magnitudes of the acute phase responses are given as mean values ± S.E.M. in Figs. 3 and 4, whereas the median values and the range of the peak responses are given in Table 2. The summary measurements (Altman, 1991) were compared statistically using two-sample *t*-test (treated groups versus infected non-treated group).

3. Results

3.1. Clinical, bacteriological and pathological signs of infection

As seen in Fig. 2, all pigs experimentally infected with *A. pleuropneumoniae* developed clinical signs of disease including respiratory symptoms, increased body temperature (>40.0 °C) and loss of appetite (graded as less than five). The duration of respiratory symptoms and fever was shortened by the treatment with enrofloxacin (non-treated versus treated: $P = 0.0001$ and 0.002, respectively). Four of the 10 treated pigs and all of the infected but untreated pigs seroconverted to *A. pleuropneumoniae* by day 17. On that day, non-treated pigs had higher concentrations of serum antibodies to *A. pleuropneumoniae* ($P < 0.0001$)

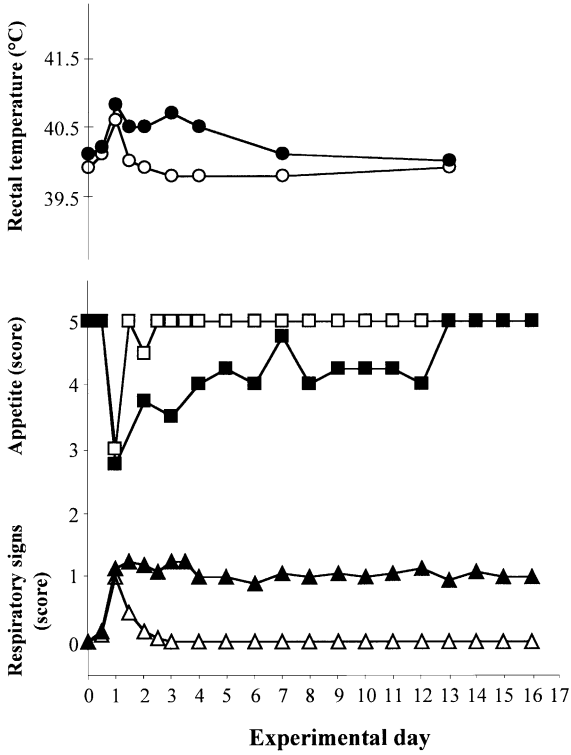

Fig. 2. Rectal temperature (circles), appetite (squares) and respiratory signs (triangles) on group level (median values) in pigs infected with *A. pleuropneumoniae* and treated with enrofloxacin (open symbols) or left untreated (closed symbols). The pigs were infected on day 0 and the treated pigs were injected with enrofloxacin (2.5 mg/kg body weight) once a day for 3 days starting at 20 h pi. Respiratory signs were scored from 0 (no signs) to 3 (severe respiratory signs). Appetite was scored from five (all consumed) to zero (refusal).

than the enrofloxacin-treated pigs; mean absorbance values 1.29 (range 0.59–1.73) versus 0.25 (range 0.03–0.52). One of the treated pigs had lesions of pneumonia whereas nine of the infected non-treated pigs had both pneumonia and pleuritis at necropsy. *A. pleuropneumoniae* could be reisolated from two treated pigs and from six non-treated pigs.

No clinical signs of disease were recorded in the control group. The control animals did not seroconvert to *A. pleuropneumoniae* and had no lesions of pneumonia or pleuritis at necropsy. *A. pleuropneumoniae* could not be isolated from the control pigs.

3.2. IL-6 activity and concentrations of serum amyloid A and haptoglobin

The mean IL-6 activity of all animals in the three experimental groups is shown in Fig. 3a. As indicated by the large S.E.M. values, a considerable individual variation was observed. This variation was partly due to the short duration (<8 h) of the IL-6 response and that individual pigs reached peak values at different times. Therefore, the statistical comparisons between groups were carried out using median peak values. IL-6 activity was

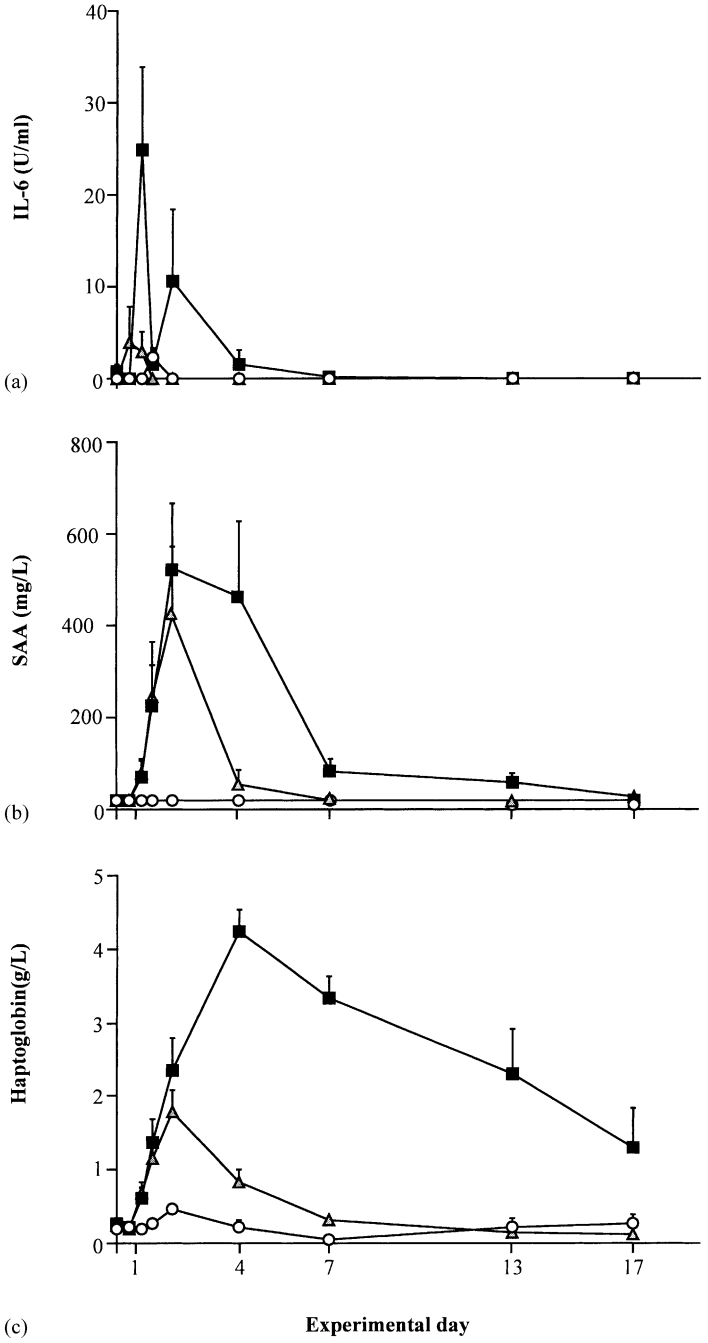

Fig. 3. Levels of IL-6 (a), SAA (b) and haptoglobin (c) in serum obtained from the experimental pigs. The pigs were infected day 0 with *A. pleuropneumoniae* and treated with enrofloxacin (shaded triangles) or left untreated (closed squares). The control pigs (open circles) were not infected and left untreated. Treated pigs were injected with enrofloxacin (2.5 mg/kg body weight) once a day for 3 days starting at 20 h pi. The results are given as mean values $\pm$ S.E.M., $n = 10$.

detected in serum from all infected but non-treated pigs (median peak value = 32.5 U/ml, range 8.5–50, $n = 10$). In all but one of these pigs, IL-6 was only detected at one occasion of sampling. The majority of the pigs ($n = 6$) were positive at 20 h pi, one pig 28 h pi, two pigs 44 h pi and one pig on day 4. Four of the infected and enrofloxacin-treated pigs displayed IL-6 in serum at one occasion; three of them at 20 h pi and one at 10 h pi (median peak value = 14 U/ml, range 3.0–40, $n = 4$). In the control group, IL-6 activity was demonstrated in serum collected from one pig 28 pi (24 U/ml).

The mean SAA concentrations in serum of animals in the three experimental groups are illustrated in Fig. 3b. In the following text, however, the onset of the SAA response is given on an individual basis, and median peak concentrations are used to allow comparisons with the kinetics and magnitude of the IL-6 response. SAA was detected in serum from all infected but non-treated pig (median peak value = 884 mg/l, range 258–1250, $n = 10$). In general, SAA responses above 100 mg/l appeared somewhat later than the IL-6 response (20 h pi, $n = 2$; 28 h pi, $n = 4$; 44 h pi, $n = 2$; day 4, $n = 2$) and lasted longer, from 1 up to 4 days. The highest SAA concentrations were recorded at 44 h pi (5/10) or at day 4 (3/10). SAA was demonstrated in serum from 7 out of 10 infected and enrofloxacin-treated pigs (median peak value = 572 mg/l, range 168–1250, $n = 7$). Among these animals, SAA was first recorded 20 h pi ($n = 2$), 28 h pi ($n = 1$) and 44 h pi ($n = 4$) and in most cases these SAA responses were below detectable levels within 1 day. SAA was not demonstrated in serum collected from pigs in the control group.

The mean haptoglobin concentrations in serum of animals in the three experimenal groups are illustrated in Fig. 3c. Haptoglobin responses above 1.0 g/l were detected in serum from all infected non-treated pigs (median peak value = 4.5 g/l, range 3.2–5.8, $n = 10$). The responses were first observed at 20 h ($n = 1$), 28 h ($n = 5$), 44 h ($n = 2$) or 4 days ($n = 2$) pi. In one pig the highest haptoglobin level was recorded at 44 h pi, but most individual peak haptoglobin levels appeared late (day 4, $n = 6$; day 7, $n = 2$; and day 13, $n = 1$). The haptoglobin responses among these pigs were long-lasting, from 6 to 16 days. Two of the enrofloxacin-treated pigs did not display haptoglobin levels exceeding 1.0 g/l serum and these pigs were also negative for SAA. Among the other enrofloxacin-treated pigs, haptoglobin was first demonstrated 20 h pi ($n = 2$), 28 h pi ($n = 5$) and 44 h pi ($n = 1$). Peak values (median peak value = 2.3 g/l, range 1.0–2.8, $n = 8$) were recorded at 28 h pi ($n = 1$) or at 44 h pi ($n = 7$), and among these pigs the response only lasted for 1–3 days. Thus, the haptoglobin response in the non-treated pigs lasted longer ($P = 0.001$) and reached higher ($P < 0.001$) levels than in the enrofloxacin-treated pigs. In the control group, the haptoglobin concentrations remained below 1 g/l (range 0.01–0.86 g/l) throughout the experimental period.

3.3. Clinical effects of treatment with various antibiotics during an experimental infection with A. pleuropneumoniae

The results above indicated that both the duration and the magnitude of the acute phase responses to *A. pleuropneumoniae* were influenced by an effective antibiotic treatment. Therefore, serum samples collected from pigs infected with *A. pleuropneumoniae* and treated with antimicrobial substances of varying efficacy were selected for further studies. The clinical effects of the different treatment regimens have previously been described

Table 1
Clinical, bacteriological and pathological findings in pigs experimentally infected with *A. pleuropneumoniae* (*A. pp*) and treated with various antimicrobial drugs[a]

Antimicrobial drug	Respiratory signs		Findings at necropsy (day 17)			
	Affected pigs	Duration (days)	Pneumonia (%)	Pleuritis (%)	Isolation of *A. pp*	Seropositive pigs
None	8/8	16.3 ± 0.5	16 ± 8	18 ± 10	6/8	8/8
Enrofloxacin	8/8	2.5 ± 0.8	5 ± 10	4 ± 9	0/8	0/8
Ceftiofur	8/8	4.6 ± 2.1	6 ± 6	7 ± 6	5/8	5/8
Penicillin G	8/8	14.8 ± 2.2	16 ± 10	22 ± 20	5/8	8/8
Tiamulin	8/8	15.5 ± 1.9	15 ± 8	19 ± 17	6/8	8/8

[a] The results (mean values ±S.D.) are summarised from previously published data (Wallgren et al., 1999).

in detail (Wallgren et al., 1999), and are briefly summarized in Table 1. Treatment with enrofloxacin and ceftiofur were effective whereas penicillin and tiamulin less efficiently eliminated the bacteria, as determined by cultivation and development of antibodies to *A. pleuropneumoniae*.

3.4. Effects of treatment with different antibiotic substances on the acute phase response to A. pleuropneumoniae

The archived serum samples had previously been analysed for IL-6 activity (Fossum et al., 1998) and were now analysed for SAA and haptoglobin. In general, infection with *A. pleuropneumoniae* induced an immediate IL-6 response of short duration followed by SAA and haptoglobin responses (Fig. 4 and Table 2). The IL-6 response was significantly shortened ($P = 0.02$) in groups treated with enrofloxacin or ceftiofur when compared with the infected control group.

Increased levels of SAA were observed within 24 h pi, but in all groups the peak values were recorded at 44 h pi (Fig. 4b). Four days post-infection, SAA could still be detected in serum from some pigs in all infected groups, but a substantial SAA response was at that time only observed in serum obtained from pigs treated with tiamulin or from untreated pigs. In these groups, as well as in the group treated with penicillin, SAA was detected in serum from one out of eight pigs also on day 7. The SAA response was significantly shortened ($P = 0.04$) in groups treated with enrofloxacin or ceftiofur when compared with the infected control group.

A slight increase in haptoglobin levels was detected in serum obtained 20 h pi, but the peak values were reached later and the response lasted longer than for IL-6 and SAA (Fig. 4c). The haptoglobin response was highest in untreated and tiamulin treated pigs with a similar kinetic during the first 2 weeks post-infection. Pigs in the penicillin treated group had elevated levels of haptoglobin throughout the experimental period. In comparison, the haptoglobin response was clearly reduced in pigs treated with enrofloxacin or ceftiofur from the second day pi. The haptoglobin response was significantly shortened in groups treated with enrofloxacin ($P = 0.01$) or ceftiofur ($P = 0.05$) when compared with the infected control group. Furthermore, the peak concentrations of haptoglobin obtained in the infected

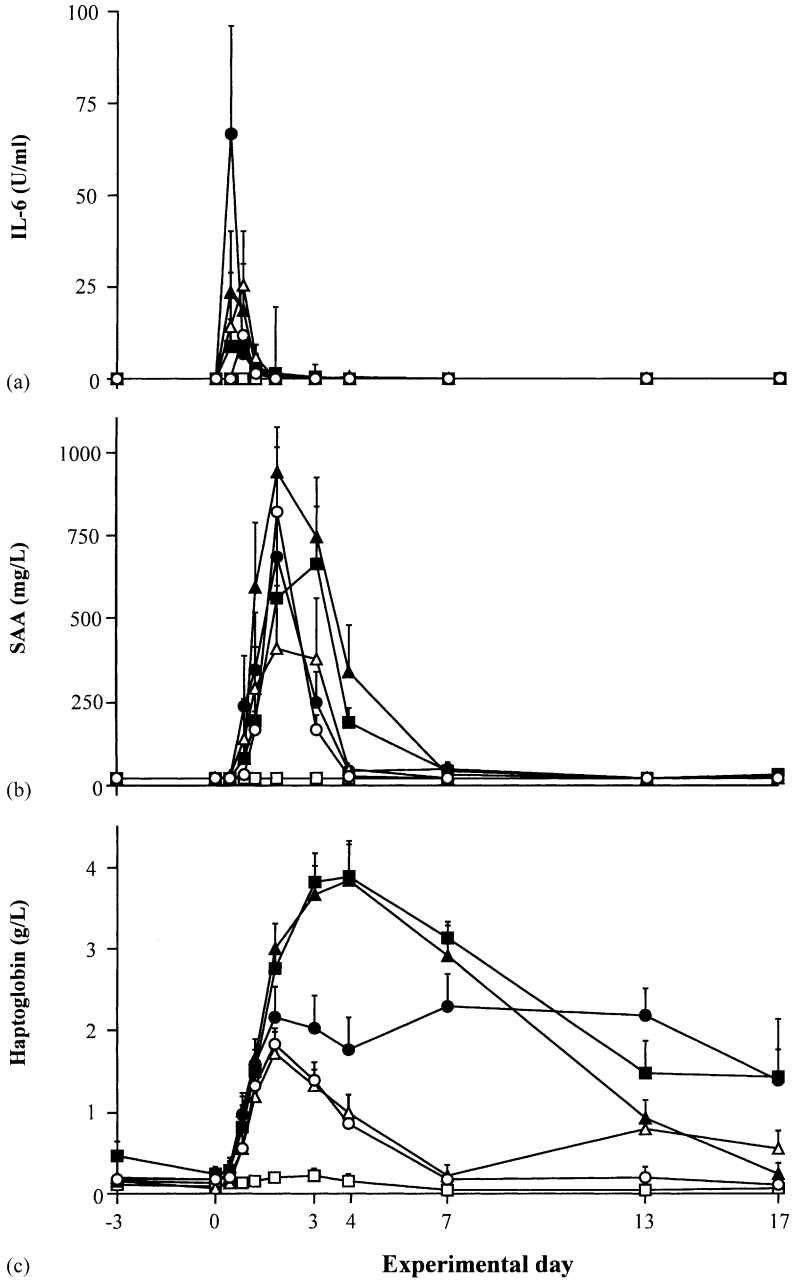

Fig. 4. Levels of IL-6 (a), SAA (b) and haptoglobin (c) in serum obtained from pigs infected with *A. pleuropneumoniae* and treated with various anibiotics. The pigs were infected on day 0 and treated once a day for 3 consecutive days with enrofloxacin (open triangles), ceftiofur (open circles), penicillin (closed circles) or tiamulin (closed triangles). One group of pigs was infected but not treated (closed squares) and one group served as non-infected, untreated pigs (open squares). The results are given as mean values ± S.E.M., $n = 8$.

Table 2
Duration[a] and magnitude[b] of the acute phase response (IL-6, SAA and haptoglobin) in serum from pigs experimentally infected with *A. pleuropneumoniae* and treated with different antimicrobials for 3 consecutive days, beginning 20 h post-infection

Treatment group	IL-6			SAA			Haptoglobin		
	No. of positive pigs	Duration (days)	Peak value (U/ml)	No. of positive pigs	Duration (days)	Peak value (mg/l)	No. of positive pigs	Duration (days)	Peak value (g/l)
Not infected, non-treated	0/8	–	–	0/8	–	–	0/8	–	–
Enrofloxacin	7/8	1 (1–2)	12 (2.4–28)	7/8	2 (1–3)	1141 (64–1250)	7/8	2 (1–3)	2.0 (1.1–2.6)
Ceftiofur	6/8	1 (1)	39.5 (6–119)	6/8	2 (1–3)	606 (109–1250)	7/8	3 (1–6)	2.3 (1.6–2.7)
Pencillin	8/8	2.5 (1–3)	16 (0.5–191)	8/8	2 (1–4)	688 (45–1250)	8/8	12 (6–16)	2.9 (2.1–3.3)
Tiamulin	6/8	1.5 (1–7)	70 (6–106)	8/8	3 (3–7)	1250 (338–1250)	8/8	6 (5–16)	3.9 (2.5–5.4)
Infected, non-treated	7/8	2 (1–3)	11.5 (4–59)	8/8	3 (2–7)	815 (362–1250)	8/8	12 (5–16)	4.1 (2.9–5.1)

[a] The duration (days) is given as median value with the range in parenthesis.
[b] The magnitude (peak value) is given as median value with the range in parenthesis.

control group was higher than in groups treated with enrofloxacin ($P < 0.001$), ceftiofur ($P < 0.001$) or penicillin ($P = 0.003$).

3.5. Relationship between the acute phase responses and treatment effects

As judged from clinical recordings (Table 1) treatments with enrofloxacin or ceftiofur were more efficient than treatments with penicillin or tiamulin. As summarized in Table 2 and Fig. 4, these treatment effects were also reflected by the acute phase response. All infected pigs that responded with IL-6 production later also produced SAA and haptoglobin. Six infected pigs did not mount a detectable IL-6 response. Three of these pigs were either treated with enrofloxacin or ceftiofur and did not show any SAA or haptoglobin responses during the first 2 weeks after infection. The other three pigs were treated with penicillin or tiamulin and developed SAA and haptoglobin responses that were similar to those of their group mates.

The duration of the acute phase responses in pigs treated with penicillin or tiamulin was not significantly different from that of infected, untreated pigs. In contrast, significantly ($P < 0.05$) shorter IL-6, SAA and haptoglobin responses were recorded for pigs treated with enrofloxacin or ceftiofur. The magnitude of the responses, however, showed large individual variation so that significant differences between treatment groups were only obtained for haptoglobin. In that case, the maximal concentration was significantly ($P < 0.001$) higher for infected and untreated than for those treated with penicillin, enrofloxacin or ceftiofur whereas no effect was recorded after treatment with tiamulin.

4. Discussion

Acute phase proteins have extensively been used to monitor treatment effects in both infectious and non-infectious diseases in humans (reviewed by Van Leeuwen and Van Rijswijk, 1994; Whicher et al., 1993). In the present study, effective medical treatments initiated early during the course of infection with *A. pleuropneumoniae* in pigs reduced the duration of all three acute phase responses measured, and also reduced the maximal concentrations of haptoglobin. Thus, the present results indicate that acute phase responses can be used to monitor the therapeutic effect of antimicrobial substances in the pig. Because the differences in acute phase response recorded for the various treatment groups were most prominent for haptoglobin and least evident for IL-6, acute phase proteins that develop comparatively late and last for a certain period of time seem to best reflect the efficiency of an antimicrobial substance.

In the first part of the study, sera from three experimental groups of pigs were analysed. In accordance with previous results (Fossum et al., 1998; Johansson et al., 2001) the experimental infection with *A. pleuropneumoniae* caused a rapid onset of IL-6 production in non-treated pigs, but the individual responses were short-lived.

The SAA responses were prominent during the first week of infection, appearing when the IL-6 responses were declining. Detection of SAA during the first week of infection with *A. pleuropneumoniae* corresponded well with earlier results (Heegaard et al., 1998). In the present study, however, the SAA response was described quantitatively, and peak

values were registered on the second day post-infection. Thus, the porcine SAA response resembled that described in cattle with acute respiratory disease (Horadagoda et al., 1993, 1999) and horses affected by *Rhodococcus equi* and coincided with signs of clinical disease (Hultén and Demmers, 2002).

Infection with *A. pleuropneumoniae* has previously been shown to elicit production of haptoglobin (Hall et al., 1992; Agersø et al., 1998) and the magnitude and duration of the response observed in the present study correspond to previous reports (Heegaard et al., 1998). Thus, the haptoglobin response is extended over time and appears to cover the more chronic period of the disease process, as previously shown in cattle with various chronic inflammatory diseases (Horadagoda et al., 1999). Similarly, serum haptoglobin concentrations increase during growth in conventional fattening pigs without overt disease but not in SPF pigs (Petersen et al., 2002).

The results of the first trial also revealed that treatment with enrofloxacin reduced the clinical signs of disease and limited the development of serum antibodies to *A. pleuropneumoniae* (Wallgren et al., 1999). Interestingly, enrofloxacin treatment initiated 20 h post-infection also reduced the magnitude and duration of the acute phase reactants. This effect was most evident for haptoglobin concentrations when non-treated and enrofloxacin-treated pigs were compared.

Although a relation between the antimicrobial effect and the acute phase response was observed, it should be noted that the bacteria do not need to be intact or replicating in order to induce cytokine production. Indeed, fluoroquinolones reduce the serum levels of IL-6 and TNF-α in LPS treated mice (Kahn et al., 2000), and tetracyclines can inhibit the secretion of TNF-α by porcine Kupffer cells exposed to LPS in vitro (Akunda et al., 2001). The underlying mechanisms are not clear but in vitro studies on human monocytes have demonstrated that tetracyclines inhibit the LPS-induced TNF-α and IL-1β secretion on a post-transcriptional level (Shapira et al., 1996), which could explain the anti-inflammatory effect of tetracyclines.

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References

Aarden, L.A., De Groot, E.R., Schaap, O.L., Landsorp, P.M., 1987. Production of hybridoma growth factor by human monocytes. Eur. J. Immunol. 17, 1411–1416.

Agersø, H., Friis, C., Nielsen, J.P., 1998. Penetration of amoxycillin to the respiratory tract tissues and secretions in *Actinobacillus pleuropneumoniae* infected pigs. Res. Vet. Sci. 64, 251–257.

Akunda, J.K., Johnson, E., Ahrens, F.A., Kramer, T., 2001. Chlorotetracycline modulates acute phase response of ex vivo perfused pig livers, and inhibits TNF-α secretion by isolated Kupffer cells. Comp. Immunol. Microbiol. Infect. Dis. 24, 81–89.

Altman, D.G., 1991. Comparing groups—continuos data. In: Practical Statistics for Medical Research. Chapman and Hall, London, pp. 194–197.

Anderson, M.D., Williams, J.A., 1990. Effects of tiamuline base administered intramuscularly to pigs for treatment of pneumonia associated with *Actinobacillus* (*Haemophilus*) *pleuropneumoniae*. Proc. Int. Pig. Vet. Soc. Congr. 11, 15.

Baarsch, M.J., Foss, D.L., Murtaugh, M.P., 2000. Pathophysiologic correlates of acute porcine pleuropneumonia. Am. J. Vet. Res. 61, 684–690.

Biberstein, E.L., Gunnarsson, A., Hurvell, B., 1977. Cultural and biochemical criteria for the identification of *Haemophilus* spp from swine. Am. J. Vet. Res. 38, 7–11.

Boosman, R., Niewold, T.A., Mutsaers, C.W.A.A.M., Gruys, E., 1989. Serum amyloid A concentrations in cows given endotoxin as an acute-phase stimulant. Am. J. Vet. Res. 50, 1690–1694.

Christensen, G., Sørensen, V., Mousing, J., 1999. Diseases of the respiratory system. In: Straw, B.E., D'Allaire, S., Mengeling, W.L., Taylor, D.J. (Eds.), Diseases of Swine, eighth ed. Iowa State University Press, Ames, USA, pp. 913–940.

Fossum, C., Wattrang, E., Fuxler, L., Jensen, K.T., Wallgren, P., 1998. Evaluation of various cytokines (IL-6, IFN-α, IFN-γ TNF-α) as markers for acute bacterial infection in swine—a possible role for serum interleukin-6. Vet. Immunol. Immunopathol. 64, 161–172.

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.

Gunnarsson, A., Biberstein, E.L., Hurvell, B., 1977. Serological studies on porcine strains of *Haemophilus parahemolyticus* (*pleuropneumoniae*): agglutination reactions. Am. J. Vet. Res. 38, 1111–1114.

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

Heegaard, P.M.H., Klausen, J., Nielsen, J.P., González-Ramón, N., Piñeiro, M., Lampraeve, 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. 119B, 365–373.

Henderson, B., Wilson, M., 1996. Cytokine induction by bacteria: beyond lipopolysaccharide. Cytokine 8, 269–282.

Horadagoda, A., Eckersall, P.D., Alsemgeest, S.P.M., Gibbs, H.A., 1993. Purification and quantitative measurement of bovine serum amyloid-A. Res. Vet. Sci. 55, 317–325.

Horadagoda, N.U., Knox, K.M., Gibbs, H.A., Reid, S.W., Horadagoda, A., Edwards, S.E., Eckersall, P.D., 1999. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. Vet. Rec. 144, 437–441.

Hsu, F.S., Chou, S.M., Fang, F.W.S., Lin, S.B.S., 1990. Effects of ceftiofur and a pleuromutilin derivate in the treatment of experimentally induced *Actinobacillus* (*Haemophilus*) *pleuropneumoniae* pneumonia in swine. Proc. Int. Pig. Vet. Soc. Congr. 11, 14.

Huang, H., Potter, A.A., Campos, M., Leighton, F.A., Willson, P.J., Haines, D.M., Yates, W.D., 1999. Pathogenesis of porcine *Actinobacillus pleuropneumoniae*. Part II. Roles of proinflammatory cytokines. Can. J. Vet. Res. 63, 69–78.

Hultén, C., Demmers, S., 2002. Serum amyloid A (SAA) as an aid in the management of infectious disease in the foal: comparison with total leukocyte count, neutrophil count and fibrinogen concentration. Equine Vet. J. 34, 693–698.

Johansson, E., Fossum, C., Fuxler, L., Wallgren, P., 2001. Effects of an experimental infection with *Actinobacillus pleuropneumoniae* on the interferon-α and interleukin-6 producing capacity of porcine peripheral blood mononuclear cells stimulated with bacteria, virus or plasmid DNA. Vet. Microbiol. 79, 171–182.

Kahn, A.A., Slifer, T.R., Araujo, F.G., Suzuki, Y., Remington, J.S., 2000. Protection against lipopolysaccharide-induced death by fluoroquinolones. Antimicrob. Agents Chemother. 44, 3169–3173.

Madsen, M.E., Carnahan, K.G., Thwaits, R.N., 1995. Evaluation of pig lungs following an experimental challenge with *Actinobacillus pleuropneumoniae* serotype 1 and 5 in pigs inoculated with either hemolysin protein and/or outer membrane proteins. F.E.M.S. 131, 329–335.

Murtaugh, M.P., Baarsch, M.J., Zhou, Y., Scamurra, R.W., Lin, G., 1996. Inflammatory cytokines in animal health and disease. Vet. Immunol. Immunopathol. 54, 45–55.

Petersen, H.H., Ersbøll, A.K., Jensen, C.S., Nielsen, J.P., 2002. Serum-haptoglobin concentration in Danish slaughter pigs of different health status. Prev. Vet. Med. 54, 325–335.

Rygg, M., Husby, G., Marhaug, G., 1993a. Differential expression of rabbit serum amyloid A genes in response to various inflammatory agents. Scand. J. Immunol. 38, 417–422.

Shapira, L., Soskolone, W.A., Houri, Y., Barak, V., Halabi, A., Stabholz, A., 1996. Protection against endotoxic shock and lipopolysaccharide-induced local inflammation by tetracycline: correlation with inhibition of cytokine secretion. Infect. Immun. 64, 825–828.

Stephano, A., Diaz, C., Vazques-Rojas, F., Navarro-Fierro, R., 1988. Efficacy of a new antimicrobial (enrofloxacin) against experimental infection with *Haemophilus pleuropneumoniae* in pigs. Proc. Int. Pig. Vet. Soc. Congr. 10, 95.

Syversen, P.V., Juul, J., Marhaug, G., Husby, G., Sletten, K., 1994. The primary structure of serum amyloid A protein in the sheep: comparison with serum amyloid A in other species. Scand. J. Immunol. 39, 88–94.

Taylor, D.J., 1999. *Actinobacillus pleuropneumoniae*. In: Straw, B.E., D'Allaire, S., Mengeling, W.L., Taylor, D.J. (Eds.), Diseases of Swine, eighth ed. Iowa State University Press, Ames, USA, pp. 343–354.

Van Leeuwen, M.A., Van Rijswijk, M.H., 1994. Acute phase proteins in the monitoring of inflammatory disorders. Bailliere's Clin. Rheumatol. 8, 531–552.

Van Reeth, K., van Gucht, S., Penseart, M., 2002. In vivo studies on cytokine involvment during acute viral respiratory disease of swine: troublesome but rewarding. Vet. Immunol. Immunopathol. 87, 161–168.

Wallgren, P., Persson, M., 2000. Relationship between amounts of antibodies to *Actinobacillus pleuropneumoniae* serotype 2 detected in blood serum and in fluids collected from muscles of pigs. J. Vet. Med. B. 47, 727–737.

Wallgren, P., Segall, T., Mörner, A.P., Gunnarsson, A., 1999. Experimental infections with *Actinobacillus pleuropneumoniae* in pigs. I. Comparison of five different parenteral antibiotic treatments. J. Vet. Med. B. 46, 249–260.

Whicher, J.T., Banks, R.E., Thompson, D., Evans, S.W., 1993. The measurement of acute phase proteins as disease markers. In: Mackiewicz, A., Kushner, I., Baumann, H. (Eds.), Acute Phase Proteins. Molecular Biology, Biochemistry, and Clinical Applications. CRC Press, Boca Raton, pp. 633–650.

Willson, P.J., Osborne, A.D., 1985. Comparison of common antibiotic therapies for *Haemophilus pleuropneumoniaein* pigs. Can. Vet. J. 26, 312–316.

Woo, G.H., Chae, C.H., Kim, J.H., Lee, O.S., Park, J.M., 1996. Sequential pulmonary lesions induced by *Actinobacillus pleuropneumoniae* serotype 5Apx toxins in pigs. J. Agri. Sci. Vet. 38, 649–662.