Development of infection models to assess subclinical disease in pigs through the use of acute phase proteins as markers

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Introduction Sub-clinical disease is a major contributor to a lower than expected pig performance, economic losses and reduction in pig welfare. Currently there are no markers to objectively assess the presence and extent of sub-clinical disease in live pigs, which hampers the development of strategies to overcome the consequences of subclinical disease, including increased environmental burdens. Acute phase proteins (APP) are components of the innate immune response and their response to infection pathogens is generic rather than pathogen specific (Eckersall 2000); for this reason they have the potential to characterise pig disease status. Here we describe two studies which aimed to establish infection models to assess the presence of sub-clinical disease in young pigs through changes in their APP profiles.

Materials and methods In Experiment 1 (Exp1), 24 8-week old male pigs were used (live weight (LW) 22.16±0.44 kg) and were either sham infected (n=2 controls for each infection protocol) or trickle infected (n=4) with one of the following pathogens: *Enterotoxigenic E. coli* (ETEC) and *Brachyspira pilosicoli* (models for local intestinal disease; *per os* dose: 10^8 cfu/pig/day) and *Haemophilus parasuis* and *Listeria monocytogenes* (models for systemic disease; doses: *per os* 10^4 and subcutaneously 10^9 cfu/pig/day respectively). In Experiment 2 (Exp2), 18 4-week old male pigs were used (LW 8.45±0.8 kg) and were infected with either 10^9, 10^8 and 10^6 cfu/pig/day of *ETEC*, *B. pilosicoli* and *H. parasuis* respectively (n=4) or were sham infected controls (n=2 controls for each infection protocol). Bacterial challenge was mixed with the food for *ETEC* and *B. pilosicoli*, whereas it was administered intranasally for *H. parasuis*. Animals were challenged 3 times per week for either two or one weeks for Exp1 and Exp2 respectively. Blood samples were obtained at regular intervals to monitor the response on 3 APP (haptoglobin (Hp), C-reactive protein (CRP) and serum amyloid A (SAA)). Food intake was recorded daily; weight gain, health scores, bacteria excretion and temperature were monitored at regular intervals. The two studies were analysed separately, using a one-way ANOVA which included APP results prior to infection as covariate. Contrast statements were used to compare each infection group to the control (Genstat 9).

Results In Exp1, subclinical disease was evident in *L. monocytogenes* infected pigs, with an increase in their rectal temperature (P<0.001) and a reduced, although not significantly different, LW gain (Table 1). None of the other infected pigs showed any signs of subclinical disease throughout the experiment. All measured APP were significantly upregulated in *L.monocytogenes* infected pigs (Table 1), whereas they were not affected by infection in any of the other groups. In Exp2, ETEC infected pigs showed a reduced, although not significantly different food intake and LW gain. This was accompanied with a significant increase in Hp and SAA. There was no effect of infection on performance or APP profiles in the remaining infected pigs. The consequences of infection were evident only during the first week of the infections.

Table 1 Effects of infection with different pathogens on log-transformed APP, feed intake (FI) and LW gain LWG) in growing pigs during the first week of infection. Superscripts denote significant difference from the control at P<0.05; [(1)] pooled across all controls as results were not affected by sham-infection protocols

	Exp1					Exp2				
	Hp g/L	CRP mg/L	SAA mg/L	FI g/day	LWG g/day	Hp g/L	CRP mg/L	SAA mg/L	FI g/day	LWG g/day
Control[(1)]	0.09	2.10	0.37	1290	880	0.09	2.14	0.81	503	434
ETEC	0.09	2.19	0.19	1234	842	0.21[d]	2.32	1.23[e]	433	300
B. pilosicoli	0.08	2.12	0.16	1250	820	0.13	2.12	0.66	488	429
H. parasuis	0.07	2.13	0.43	1210	923	0.04	2.37	0.94	527	472
L. monocytogenes	0.32[a]	2.63[b]	2.11[c]	1197	772	NA	NA	NA	NA	NA
s.e.d.	0.03	0.07	0.19	58.5	124	0.04	0.15	0.19	112	147

Conclusions *L. monocytogenes* infection resulted in systemic effects (pyrexia), upregulation of all three APP measured and limited penalties in performance. These make it a good candidate model for assessing the use of APP as markers for subclinical, systemic infection in pigs. ETEC challenge resulted in local infection (no pyrexia) and upregulation of two APP and these, in combination with studies where penalties in performance were confirmed (Wellock *et al.* 2008), make it a good model for assessing the use of APP as markers for subclinical localised infection. Four-week old animals were considered better candidates than 8-week old pigs to test these models at the tested infection doses. The lack of evidence for subclinical disease from *B. pilosicoli* and *H. parasuis* infections under the protocols tested prohibits their use as models to study subclinical pig infections at this stage.

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