SHORT COMMUNICATION

EFFECT OF TRANSPORT STRESS ON SERUM HAPTOGLOBIN AND PIG-MAP IN PIGS

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Abstract *Animal Welfare 2003, **12**: 403-409*

This study was undertaken in order to determine the variation of the acute phase proteins haptoglobin and Pig-MAP (major acute phase protein) in the serum of pigs affected by transport-related stress. Pigs were subjected to one of two pre-slaughter treatments: a) short-duration transport (1 h 15 min transport and 2 h lairage); or b) long-duration transport (6 h transport and 14 h lairage). There were 10 individuals in each treatment group, belonging to the NN (n = 5) or Nn (n = 5) genotypes for halothane susceptibility. Samples were taken before transport, just after transport, and on stunning at slaughter. We measured levels of serum haptoglobin, Pig-MAP and cortisol. Our results showed that the short-duration transport did not modify the levels of haptoglobin or of Pig-MAP in any of the three samples, whereas cortisol was increased just after transport. In contrast, there was an increase in haptoglobin and Pig-MAP in serum from animals after long-duration transport, as observed in the post-mortem samples (20–21 h after the beginning of transport); cortisol levels were not increased in these conditions. In this experiment, homozygotes for the halothane gene tended to have higher values of haptoglobin after slaughter than did heterozygotes. In conclusion, combined determination of acute phase proteins and cortisol levels could provide valuable information on welfare problems related to transport.

Keywords: *acute phase proteins, animal welfare, haptoglobin, Pig-MAP, pigs, transport stress*

Introduction

Acute phase proteins (APPs) are blood plasma proteins that change in concentration following infection or inflammation. The level of these proteins in serum has been recognised to be a valuable marker of clinical and even subclinical disease in farm animals such as cattle and pigs (Kent 1992; Gruys *et al* 1994; Kaneko 1997). Synthesis of APPs takes place in the liver after serum peaks of proinflammatory cytokines released by macrophages, one of the animal body's defence mechanisms against external agents (Gonzalez-Ramon *et al*

2000). In pigs, as well as in other animal species and humans, determination of the plasma concentration of these proteins gives valuable clinical information on infection and inflammatory lesions (Hall *et al* 1992; Heegaard *et al* 1998). Besides their well-established role as markers of disease, several reports in humans and in other animal species have suggested that some APPs could make good indicators for the assessment of animal welfare during animal production processes. Some recent preliminary reports indicate that levels of APPs can increase in pigs because of the stress caused by transportation (Eurell *et al* 1992; Murata & Miyamoto 1993; Toussaint *et al* 1995). There are substantial differences between species in the relative changes in APP production. Thus, while C-reactive protein (CRP) is a major APP in humans and dogs, haptoglobin, CRP and Pig-MAP (major acute phase protein) are the major APPs in pigs (Lampreave *et al* 1994; Eckersall *et al* 1996; Gonzalez-Ramon *et al* 1995; Toussaint *et al* 2000). Haptoglobin and Pig-MAP are interleukin-6 (IL6)-dependent (Gonzalez-Ramon *et al* 2000) and have been suggested to be indicators of stress in pigs (Piñeiro *et al* 2001a,b).

Pre-slaughter handling (ie transit from the production unit to the slaughter plant and lairage at the abattoir) has been identified as one of the most stressful periods in the lifespan of a pig. Many factors interact during the pre-slaughter period (eg ambient temperature, humidity, stocking density, vehicle motion, transport or lairage duration) and result in a final output in terms of meat quality and welfare. The halothane susceptibility genotype of pigs has been considered to play a role in individual responses to pre-slaughter conditions (Grandin & Deesing 1998); this genotype has now been associated with a mutation in the *ryr-1* gene, which encodes the muscle ryanodine receptor or calcium release channel (Fujii *et al* 1991). Pigs that are homozygous for the mutation (nn) are likely to develop a potentially lethal condition known as porcine stress syndrome (PSS) and produce poorer quality meat. Heterozygotes (Nn) have also been found to show higher pre-slaughter mortality rates and a higher incidence of pale, soft and exudative (PSE) meat (Murray & Johnson 1998; Fàbrega *et al* 2002).

In general, pigs are among the species that are most sensitive to stress, and this fact is directly related to the quality of meat products. Therefore, it is necessary to search for better tools to monitor pig welfare during all stages of production. The fact that carriers of the halothane susceptibility gene have been demonstrated to show high stress susceptibility makes these individuals a good model for testing the potential of new stress markers.

In the present study, two groups of pigs with different halothane susceptibility genotypes were subjected to two different types of transport, and then blood samples were taken at intervals in order to measure haptoglobin, Pig-MAP and cortisol and to evaluate them as stress indicators.

Methods

Subjects and procedure

NN females from a cross of halothane-susceptibility-free Large White × Landrace sows, and Nn Piétrain or Large White × Piétrain boars, weighing 90–100 kg, were subjected to one of two types of pre-slaughter treatment: a) short-duration treatment, comprising 1 h 15 min transport and 2 h lairage; and b) long-duration treatment, comprising 6 h transport and 14 h lairage. There were 10 individuals in each treatment, belonging to the NN (n = 5) or Nn (n = 5) genotypes for halothane susceptibility. Blood samples were taken before transport, just after transport, and after stunning for slaughter. Blood from the caudal vein was collected in polystyrene tubes without anticoagulant and maintained at 4ºC. Serum was obtained after centrifugation of the blood at 3000 rpm for 10 min.

Biochemical determinations

Haptoglobin was analysed using the kit from Tridelta (Tridelta Development Ltd, Co Wicklow, Ireland). The assay, performed in a Cobas Mira autoanalyser, is based on binding of haptoglobin to haemoglobin, which increases the peroxidase activity of haemoglobin at low pH. The analytical performance of the assay was a between-run coefficient of variation (CV) of 1.6% and a between-day CV of 3.8%. The sensitivity of the assay was 0.05 mg ml^{-1} and the linearity was very good, being in the range 0–4 mg ml^{-1}.

Serum Pig-MAP was determined after diluting the samples 1/20 in saline solution by radial immunodiffusion in 1% agarose gels containing the specific rabbit antiserum against this protein, as described in González-Ramon *et al* (1995). Purified Pig-MAP was used as a reference standard. Serum cortisol was determined using a competitive ELISA assay using a kit (EIA-1887) from DRG Diagnostics (Marburg, Germany).

Data analysis

Serum concentration after different transport conditions was analysed using one-tailed Student's *t*-tests.

Results

Our results showed that the short-duration transport condition (transport 1 h 15 min) did not modify the levels of haptoglobin or the levels of Pig-MAP just after transport or before slaughter (total time since start of transport: 3 h 15 min–4 h). In contrast, an increase was found in haptoglobin and Pig-MAP levels after long-duration (6 h) transport; however, this increase was observed only in the *post-mortem* samples (20–21 h after the beginning of the transport; see Figure 1). Plasma cortisol, the most widely used marker for stress, was increased just after short transport, but it had returned to normal levels in the samples taken after slaughter. In the long-duration transport condition, cortisol levels were not increased after transport and were even found to decrease after slaughter.

A significant correlation was found between Pig-MAP and haptoglobin levels ($r^2 = 0.7646$), whereas no correlation was found between haptoglobin and cortisol ($r^2 = –0.0648$) or between Pig-MAP and cortisol ($r^2 = –0.2435$, $P < 0.05$; Figure 2).

In the long transport experiment, pigs that were homozygous for the halothane gene (NN) had significantly higher values of haptoglobin after slaughter (*post-mortem* samples) than did heterozygotes (Figure 3). After short transport, differences between homozygotes and heterozygotes were not significant.

Discussion

The goal of this study was to evaluate the usefulness of determination of two APPs, haptoglobin and Pig-MAP, for assessing the degree of physical and psychological stress in pigs during transport. Transit to abattoirs can be an important source of stress, resulting in reductions in carcass weight and meat quality.

Our results show that plasma levels of haptoglobin and Pig-MAP increased markedly after long-duration transportation, but this change was not observed after short-duration transport. This result is not unexpected, as the increase of APPs results from an increase in transcription rate of the genes caused by release of cytokines such as IL-6, a mechanism that usually takes several hours (Gonzalez-Ramon *et al* 2000). In the present experiment, the short treatment

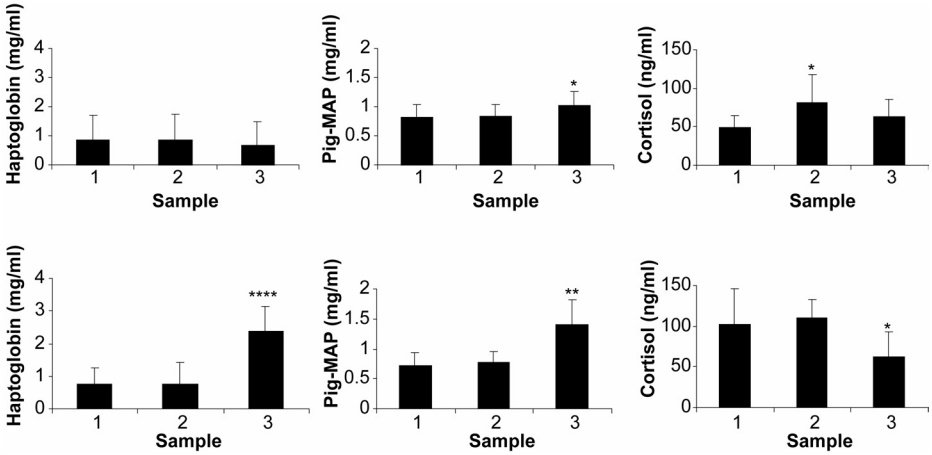

Figure 1 **Haptoglobin, Pig-MAP and cortisol levels in serum of pigs subjected to short-duration transport (top row) or long-duration transport (bottom row). Samples were taken (1) before transport, (2) just after transport, and (3) after stunning. *$P < 0.05$; **$P < 0.01$; ****$P < 0.0001$.**

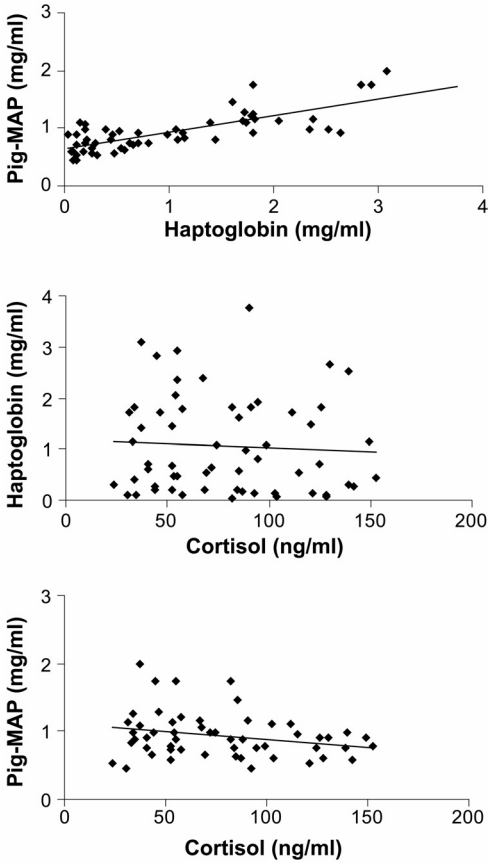

Figure 2 **Correlations between haptoglobin, Pig-MAP and cortisol levels in pigs subjected to short- or long-duration transport.**

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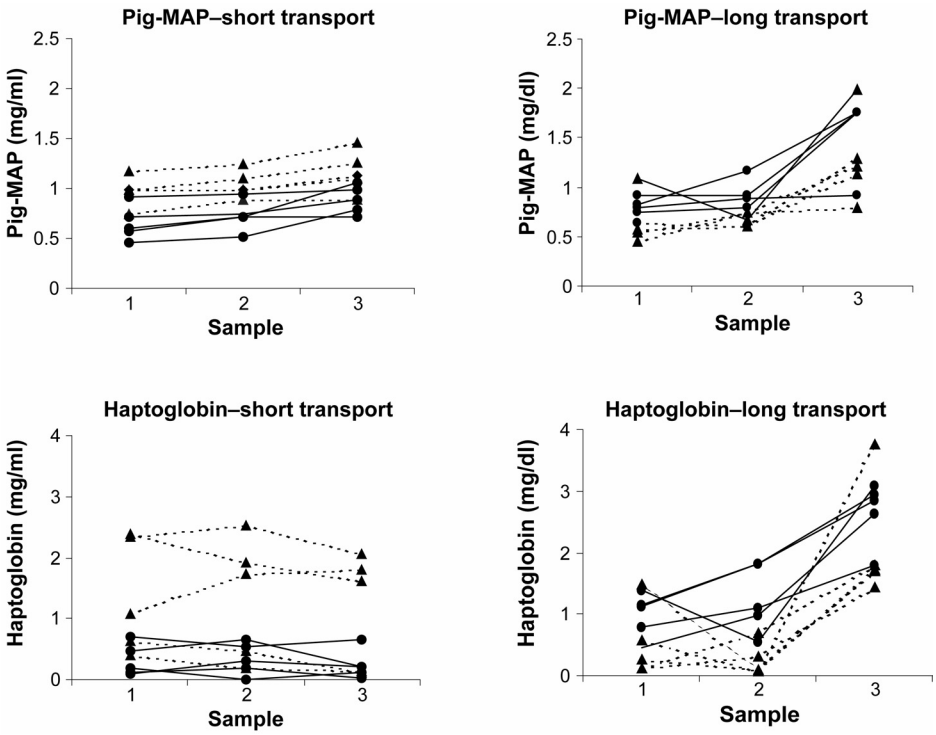

Figure 3 **Influence of the genotype for halothane susceptibility on haptoglobin and Pig-MAP levels in pigs subjected to short- or long-duration transport. Pigs belonged to the NN (solid line) or Nn (dotted line) genotype for halothane susceptibility. Samples were taken (1) before transport, (2) just after transport, and (3) after stunning.**

comprising 1 h 15 min transport and 2 h lairage may not have been long enough to detect a rise in APPs in plasma, whereas the long treatment (6 h transport and 14 h lairage) was of sufficient duration to allow us to find this change. Another possible explanation is that long-duration transport is needed to cause an increase in APPs, perhaps because of tissue damage resulting from the animals trying to maintain their balance for a prolonged period of time.

In relation to cortisol level, which is widely used as a marker for psychological stress, although a significant increase was found after short-duration transport, this difference was not seen in the long-duration treatment. Stress assessment using cortisol levels is likely to raise problems when interpreting the data, because the acute cortisol response is subject to multiple sources of variation such as time or duration of stressor application, circadian rhythm, and age of the individual (Ruis *et al* 1997). Furthermore, individual variability in cortisol response has been found (von Borell & Ladewig 1992) and there is relatively little information about the exact time-responses and half-lives of this hormone in the pig. These practical constraints limit the usefulness of cortisol in assessing stress responses and may partially explain the results of the present experiment.

It is also interesting to note the high correlation between haptoglobin and Pig-MAP, which showed a parallel increase in their levels. This is in agreement with the findings of Piñeiro

et al (2001a), who suggested that APPs could be good indicators of stress. Our results, together with the fact that these proteins may be less subject to individual variability and circadian fluctuations than cortisol, support the idea put forward by Piñeiro *et al* (2001b) and enhance the potential for their use in welfare assessment.

Finally, we have compared the levels of haptoglobin and Pig-MAP in the plasma of pigs homozygous and heterozygous for the halothane susceptibility gene. Although preliminary, our results indicate that homozygotes have higher values of both parameters after long-duration transport, which probably indicates an unexpectedly higher degree of stress. Further studies using a larger number of animals are required for this relationship to be fully established.

Conclusions and animal welfare implications

Our results show that stress resulting from transport conditions can substantially affect the concentration of serum APPs, and that they could become useful stress markers. This could be particularly true if individual variability and external sources of variation were found to have only a minor influence on these parameters. However, the present data also show that caution must be taken in some aspects of interpretation of these parameters. First, acute stresses may not be detected until several hours after the trigger of the stressful episode because of the release mechanism of these proteins. Second, transportation is a stimulus leading to both physical and psychological stress; an increase in APPs found after this stressor may, therefore, be attributable to either, or both, of these types of stress, and further research would be required if they are to be distinguished. If care is taken with regard to these considerations, our results reinforce the great potential of APPs as tools for assessing animal welfare.

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