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Short communication

Utility of acute phase proteins as biomarkers of transport stress in ewes

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ABSTRACT

The aim of this study was to evaluate the effect of transport stress on the values of serum amyloid A (SAA), haptoglobin (Hp), fibrinogen (Fbg) and white blood cells (WBCs) in 20 healthy Comisana ewes. The animals were divided into two groups: A ($n = 10$) was transported by road for 6 h over a distance about 490 km with an average speed of 80 km/h (experimental group) and B ($n = 10$) was not subject to road transport (control group). Blood samples, collected via jugular venipuncture, were obtained before and after the road transport as well as after 8, 12, 24 and 48 h rest time in experimental group and at the same time point in control group. The application of two-way repeated measures analysis of variance (ANOVA) showed a significant effect of sampling time on SAA ($P = 0.001$), Hp ($P < 0.0001$) and WBCs ($P = 0.005$) in Group A, and it showed statistically significant differences between the Groups A and B on SAA ($P = 0.0004$) and Hp ($P < 0.0001$). Time × Groups interaction effects were not significant for any of the blood parameters studied ($P > 0.05$). On the basis of these modifications, Hp and SAA levels, together with WBCs, could be considered as effective biomarkers useful to evaluate magnitude and time response of those APPs in order to validate their use which could be useful to improve the ovine transporting conditions.

1. Introduction

The transport, an inevitable husbandry practice which animals unexpectedly encounter in the livestock industry, can have major implications for their welfare. Its importance in livestock production lies in the effect of transport on production and reproduction, and how animals adapt to transport (Fisher et al., 2010). Transport is considered as one of the main causes of stress raising considerable interest (Maejima et al., 2006). Transport stress may be more or less severe, depending on a number of different stress factors. The effects of transport stress on animal health and welfare have been evaluated through behavioural, physiological and haematological variables (Adenkola and Ayo, 2010; Broom, 2003, 2008), mobilization of energy and protein metabolism (Todd et al., 2000), and activity of enzymes and hormones (Adenkola and Ayo, 2010; Stull and Rodiek, 2000). Particularly, road transport of livestock is perceived as an acute stressor that evokes changes in some plasma variables, for instance by increasing glucose, creatine kinase, catecholamines and cortisol (Ali et al., 2006). In sheep previous researchers have examined the effects of transport durations of up to 24 h (Knowles et al., 1995) and of a long distance transport stress (Hall et al., 1999). There are limited scientific data available to demonstrate the extent to which short transport stress affects the responses of sheep (Knowles et al., 1995; Ruiz-de-la-Torre et al., 2001). It is well known that long term stress affects a large number of systems in ewes including the reproductive (Smith et al., 2003) and immune systems (Fisher et al., 2010). Recently, there is great scientific interest aimed at

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ensuring that the welfare of transported animals is optimal and identifying easily obtainable biomarkers in relation to transport stress. Investigations over the last decade have shown that there exists a linkage between stress and acute phase protein response (Murata, 2007). Acute-phase protein synthesis and their actions appertain to systemic and tardy period of acute phase response (Tîrziu, 2009). Acute-phase proteins (APPs) are a group of blood proteins that change in concentration in animals subjected to external or internal challenges, such as infection, inflammation, surgical trauma, or stress (Gonzàlez et al., 2008). The APP response is thus a robust indicator of disease and easily measurable in a blood sample (Heegaard et al., 2011). In fact, it is used increasingly as a very useful diagnostic tool in veterinary medicine for several animal species such as goats and sheep (Cray et al., 2009; Gonzàlez et al., 2008; Eckersall and Bell, 2010; Petersen et al., 2004). In sheep serum amyloid A (SAA) and haptoglobin (Hp) are known to be major acute phase proteins increasing up to 10- and 100-fold respectively on stimulation, while fibrinogen (Fbg) is considered as a minor APP increasing around 4 times (Braun et al., 2010; Eckersall et al., 2007; Eckersall and Bell, 2010; Jain et al., 2011; Murata et al., 2004; Petersen et al., 2004). These measurements in health monitoring programmes on a herd basis in livestock are useful both for the identification of individual animals with disease and as a means to identify animals with subclinical diseases (Eckersall, 2004; Winter et al., 2006).

Considering the potential role of APPs as indicators of stress response, the aim of this study was to evaluate the modifications of serum concentrations of Hp, SAA and Fbg, together with white blood cells (WBCs), in order to identify the impact of transport on biomarkers that will be increasingly useful in the future for health and welfare of Comisana ewe.

2. Materials and methods

The study was carried out on 20 non pregnant clinically healthy Comisana ewes. The animals were divided into two groups: A ($n = 10$, aged 2–3 years old and weighing 54.1 ± 2.8 kg), designated as experimental group, was transported by road for 6 h over a distance about 490 km with an average speed of 80 km/h, and B ($n = 10$, aged 2–3 years old and weighing 55.2 ± 3.2 kg), designated as the control group, was not subject to road transport. Before the road transport, clinical examination as well as haematology and serum biochemistry was used to ensure that the animals were healthy (Table 1).

The animals of Group A were transported by road involving a combination of road surfaces ranging from small country lanes (10 km), secondary roads (60 km) to motorways (420 km). They had no previous experience of road transport. After the transport the animals were confined to paddock where they were fed hay (2 kg), wheat straw (1 kg) and wheat concentrate (0.5 kg). Water was available *ad libitum*. All animals were transported in accordance with Directive 1/2005 CEE.

The journey started at 08:00 h and lasted 6 h. The animals were transported in April at the ambient temperature of 21 °C and relative humidity of 68% at loading time and during the first stage of the transport, and at 20 °C and 73% in the final stage of the transport and unloading. Temperature inside the vehicle at start and after transport ranged from 23 to 24 °C. For all experimental period temperature and relative humidity were continuously recorded with a data logger (Gemini, Chichester, West Sussex, UK).

The animals of Group B were located at the destination site of transported sheep in the same environmental conditions.

Blood samples were collected from each animal by jugular venipuncture into evacuated glass tubes (Venoject, Terumo Europe, Leuven, Belgium) and stored in three different types of vacutainer tubes: with no additive, containing 3.8% sodium citrate (one part citrate and nine parts blood), and 4.2 mg ethylenediaminetetraacetic acid (EDTA). Blood samples were obtained before and after the road transport as well as after 8, 12, 24 and 48 h rest time to determine SAA, Hp, Fbg content and WBCs. In particular, blood samples were collected between 07:30 and 08:00 under basal conditions and immediately after journey (between 14:00 and 14:30). Sheep were then allocated to the pen and subsequently sampled at 22:00 h, 02:00 h, 14:00 h and 14:00 h. Each ewe was sampled by experienced and skilled operators in less than 1 min to minimize handling stress affecting the results. Serum SAA concentration was determined and analysed by method of sandwich enzyme linked immunosorbent assay using commercial ELISA kits (Tridelta Development, Maynooth, Ireland). The concentration of Hp was assessed using commercial colorimetric kits (Tridelta Development, Maynooth, Ireland) in microplates, based on Hp–haemoglobin binding and preservation of the peroxidase activity of the bound haemoglobin at low pH. The reading of absorbancies and the consecutive calculation of final concentrations of both acute phase proteins were performed on automatic microplate reader Opsys MR (Dynex Technologies, Chantilly, USA).

The concentration of Fbg was assessed in blood samples containing citrated sodium, after centrifugation, using a coagulometer (Clot 2S, SEAC). The WBC count was assessed in blood samples containing EDTA using a multiparametric automatic analyser (HecoVet, SEAC). Moreover, in order to exclude the haemoconcentration which potentially causes small changes in concentrations of proteins, packed cell volume (PCV) was also assessed.

Protocols of animal husbandry and experimentation were reviewed and approved in accordance with the standards recommended by the Guide for the Care and Use of Laboratory Animals and by Directive 86/609 CEE.

All the results were expressed as mean values ± standard deviation (SD). Data were normally distributed ($P < 0.05$, Kolmogorov–Smirnov's test). Two-way repeated measure analysis of variance (ANOVA) was performed to determine the statistically significant effect of sampling time, the significant differences between the Group A and Group B, and the interaction term Time × Groups. The level of significance was set at <0.05. Bonferroni's multiple post hoc comparison test was applied. The data were analysed using the software STATISTICA 8 (Stat Soft Inc.).

3. Results

The application of two-way repeated measure ANOVA showed a significant effect of sampling time on SAA ($P = 0.001$), Hp ($P < 0.0001$) and WBCs ($P = 0.005$) in Group A, and it showed statistically significant differences between the Groups A and B on SAA ($P = 0.0004$) and Hp ($P < 0.0001$). Moreover, PCV values were not statistically significant both in Group A and Group B ($P > 0.05$) during the experimental period, this excludes that the observed changes on studied parameters are due to haemoconcentration. Fig. 1 shows the mean pattern (±SD), together with statistical significances, of studied acute phase proteins in Groups A and B.

4. Discussion

The obtained data of all studied parameters were within the physiological range referred in literature for ovine species (Eckersall and Bell, 2010; Jain et al., 2011). The results of our study showed a statistically significant increase of SAA and Hp values in Group A in comparison with Group B. Time × Groups interaction effects were not significant for any of the blood parameters studied ($P > 0.05$). In Group A the values of SAA and Hp increased during the hours rest time relative to changes from pre-transportation values (Fig. 1). In particular SAA increased