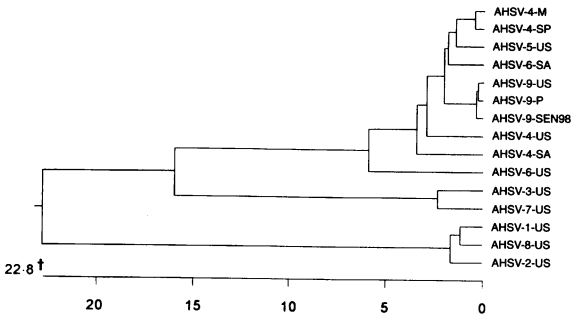

FIG 1: Phylogenetic tree (Clustal method) showing the relationships between the nine serotypes of African horse sickness virus (AHSV) based on amino acid sequence analysis of the protein NS3. SP Spain, M Morocco, US United States Department of Agriculture, Plum Island, New York, SA South Africa, P Institute for Animal Health, Pirbright, UK, SEN98 1998 isolate from Senegal (the sources of sequence data are described in Martin and others 1998). † Units indicate the number of substitution events

logically for topotyping viruses (Sailleau and others 1997), although further studies will be necessary to obtain significant interpretative data for different AHSV strains.

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Notices and divisional events

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Transport stress in cattle as reflected by an increase in faecal cortisol metabolite concentrations

R. PALME, C. ROBIA, W. BAUMGARTNER, E. MÖSTL

ALMOST all cattle are transported at some time during their lives, so improving methods for the transportation of animals is a major concern throughout the world, both from an animal welfare and an economic point of view. This is reflected in the many experiments which have been undertaken (Knowles 1999), and it is agreed that a combination of both physiological and behavioural measures for the assessment of stress and discomfort should be taken into account (Grandin 1997, Terlouw and others 1997). Cortisol values in the blood are widely recognised as a physiological parameter of stress; however, due to blood sampling, additional stress may be superimposed. To overcome this problem, an enzyme immunoassay (EIA) for measuring a group of faecal cortisol metabolites has been established (Palme and Möstl 1997). The biological relevance of this non-invasive method has been proven in ruminants such as cattle and sheep, following stimulation (using adrenocorticotropic hormone [ACTH]) or suppression (using dexamethasone) of cortisol release by the adrenal cortex (Palme and others 1999). The aim of this study was to examine the usefulness of this method in transported cattle.

Sixteen lactating cows, mainly Austrian brown and Fleckvieh and aged three to nine years, were used in this study. They were housed in tether stalls, and had all experienced two previous transportations. They were also used to being handled. The animals were divided into three groups: a transportation group (T), a stationary group (S) and a control group (C). Group T consisted of eight cows which were divided into two sets of four animals. Each set was loaded onto a lorry and transported on country roads for two hours at the same time. After transportation, they were returned to their familiar environment in the stable; neighbouring cows were not transported. The loading and unloading took about 15 minutes, respectively. The four cows of group S were loaded and handled in the same way but with the exception that the lorry remained stationary for three hours. The four control (C) cows stayed in their familiar environment for the whole experimental period.

Samples were collected from faeces voided by the cattle immediately before loading and at every spontaneous defecation over a period of 48 hours afterwards. The only time no samples were collected was when the cows were in the lorries. The samples were frozen immediately and stored at –20°C until analysis. The cortisol metabolites, that is 11,17-dioxoandrostanes (11,17-DOA), in the faeces were determined as described by Palme and Möstl (1997). Faeces (0·5 g) were extracted with 5 ml 80 per cent methanol, and after centrifugation at 2500 *g* for 15 minutes, an aliquot of the supernatant was measured using the EIA. Values of all faecal samples from transported cows were grouped according to time, at intervals of eight hours (Fig 1). To determine the basal values of cortisol metabolites in faeces, the median of the measured concentrations during the first eight hours of the experiment was calculated.

Median basal values of 11,17-DOA in faeces were 88 (range 51 to 282), 99 (range 71 to 106) and 88 (range 39 to 104) nmol/kg in groups T, S and C, respectively. In group C, the

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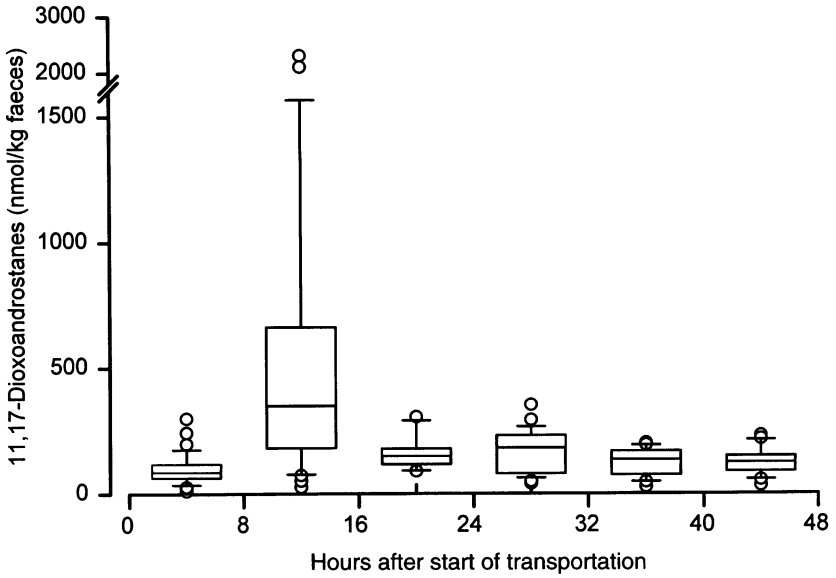

FIG 1: Boxplot of grouped concentrations (intervals of eight hours) of cortisol metabolites for all transported cows. The boxes show the median value and the upper and lower quartile values, while the whiskers show the 10th and 90th percentiles of the values of faecal samples (n = 33, 30, 24, 21, 16 and 16 respectively for the different intervals). The circles represent data points that fall outside these percentiles

concentrations were within that range throughout the whole experiment. In groups T and S, the maximal concentrations occurred 12 (2) hours after the start of the experiment (Fig 2). They ranged from 344 to 2301 (median 964) and from 217 to 654 (median 407) nmol/kg and exceeded basal values by 5·5 to 39·1 (median 6·9) and 2·1 to 6·8 (median 5·0) times, respectively. In two cows of group S, a smaller second peak occurred about four hours after the maximum (Fig 2b). Concentrations of 11,17-DOA from eight to 16 hours after the start of transport (Fig 1) were significantly higher ($P<0·001$; Mann-Whitney rank sum test) than during all the other intervals. About 26 to 48 (median 29·5) hours after the start of the transport, the concentration of cortisol metabolites in faeces reached pretransport values again.

Patterns and concentrations of faecal cortisol metabolites were comparable with results obtained in cattle following ACTH administration (Palme and others 1999). Similar individual differences in faecal 11,17-DOA concentrations (basal and peak values) were observed, which were probably due to the fact that an animal's response is determined by a complex interaction of genetics and previous experiences (Grandin 1997). As discussed earlier (Palme and others 1999), the influence of individual variation can be reduced if an animal acts as its own control (response expressed as per cent increase above basal values).

One of the most stressful aspects of the transportation chain for cattle is confinement in a moving vehicle (Tarrant 1990). A marked increase in faecal cortisol metabolites after transportation of the cows was seen in this study, reflecting the release of cortisol by the adrenal gland. However, the small increase of faecal 11,17-DOA after confinement on a stationary truck suggested that although the cows were stressed, it was not to the same extent. This may be at least partly due to the loading and unloading, as indicated by the second, smaller peak following maximum concentrations of faecal 11,17-DOA in two cows. In addition, it must be considered that all cows had had previous experience of transportation. Therefore, cows which are transported for the first time may show an even greater response, as the novelty of a situation is regarded to induce a strong, psychological stress (Grandin 1997).

The non-invasive method used to measure faecal cortisol metabolites seems well suited as an additional tool to further elucidate the complex reactions that occur during stressful events such as transportation. The delay times of faecal excretion in ruminants (Palme and others 1999) must be taken into account, which allow for the monitoring of disturbances which occurred about 12 hours before sampling. Due to the simple, non-disturbing sample collection, it is possible to conduct large-scale studies. Therefore, this method may be especially helpful in areas which require further work, such as optimum stocking densities, the benefits of rest stops during transport in cattle or maximum journey times (Knowles 1999).

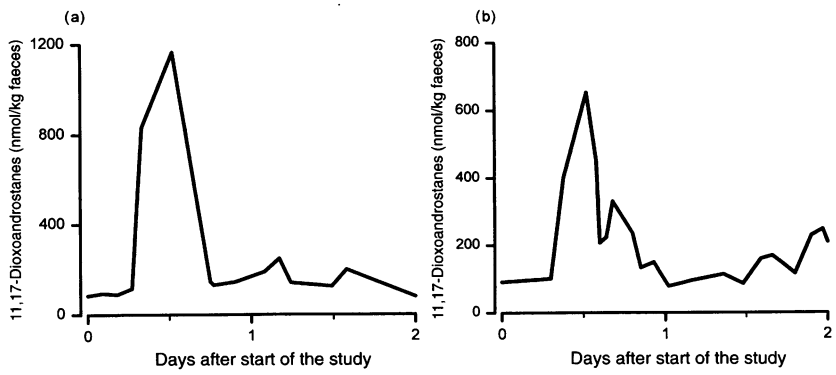

FIG 2: Concentrations of cortisol metabolites in (a) one cow following transportation for two hours and (b) another cow kept on a stationary lorry for three hours

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