

Effects of cross-tying horses during 24 h of road transport

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Summary

Transportation stress has been implicated as a predisposing factor to respiratory disease in horses. Cross-tying horses individually in stalls is common practice for transporting show and racehorses, but horses also travel in small groups or individually without being restricted by tying. The objective of this study was to compare physiological responses of horses travelling cross-tied or loose during 24 h of road transport. Ten horses were used in a cross-over design consisting of two 4 day trials. In the first trial, 6 horses were cross-tied, while 2 pairs of horses were loose in enclosed compartments. Treatments were reversed in the second trial. Baseline samples were collected on Day 1, horses transported on Day 2, and recovery data collected on Days 3 and 4. Blood samples were collected daily at 0800, 1100 and 2000 h. The mean responses in all horses of serum cortisol, lactate, glucose, α_1 -acid glycoprotein, and total protein concentrations, packed cell volume (PCV), white blood cell (WBC) counts and aminotransferase and creatine kinase were elevated significantly from baseline during the 4 day study. The response of white blood cell counts, neutrophil to lymphocyte ratios and glucose and cortisol concentrations was significantly elevated in the cross-tied compared to the loose group during transport and recovery. This study supports the recommendation of allowing horses during long-term transportation to travel loose in small compartments, without elevating their head by cross-tying.

Introduction

Transportation stress has been implicated as a predisposing factor to respiratory disease in horses (Crisman *et al.* 1992; Oikawa *et al.* 1994, 1995; Austin *et al.* 1995). The stress response during transport evokes changes in serum cortisol concentrations (Clark *et al.* 1993; Friend *et al.* 1998; Stull and Rodiek 2000), heart rate (Clark *et al.* 1993; Smith *et al.* 1994; Waran *et al.* 1996), immune parameters (Raidal *et al.* 1997; Stull 1999) and serum muscle enzyme activities (Codozza *et al.* 1974; Leadon *et al.* 1990; Stull and Rodiek 2000). Cross-tying horses individually in stalls is common practice for transporting show and racehorses, but horses also travel in small groups or individually without being restricted by tying. Elevation of the horse's head, causing restriction in the range of neck movements, has been documented to compromise

the immune system and increase the number of bacteria in transtracheal aspirates (Raidal *et al.* 1995, 1997). Separate studies examined physiological responses during long-term road transport in either riding horses individually cross-tied in a commercial show van (Stull and Rodiek 2000), or slaughter horses transported loose in small groups in tractor-trailer livestock trucks (Stull 1999). Interestingly, the cross-tied horses exhibited larger increases of selected stress parameters following 24 h of transport as compared to horses travelling loose.

The aim of this study was to compare the physiological responses of horses individually cross-tied with restriction in movements of the head, neck and stance to horses travelling loose with similar floor area during 24 h of road transport.

Materials and methods

Animals and study design

Ten mature horses (mares, $n = 7$; geldings, $n = 3$) age 5–18 years (mean \pm s.d. 10.5 ± 3.9 years) including American Saddlebred, Hackney Horse, Paint, Quarter Horse, Thoroughbred and Warmblood breeds were used. All horses had previous experience of transport in trailers; however, 1 week prior to the start of the study, horses were loaded and unloaded from the van to ensure that all horses loaded without hesitation. A crossover design consisted of two 4 day trials with a 3 week interval between trials. Horses were housed individually in partially covered pens (3.7 x 6.1 m). Prior to the study, horses were divided subjectively into 5 compatible pairs to minimise any potential injuries during transport. On Day 1 of each trial, data collection began by obtaining baseline measurements while horses were resting in the pens. On Day 2, horses were loaded onto a commercial equine van between 0700 and 0800 h. In the first trial, 6 horses were cross-tied in individual compartments, while 2 pairs were loose in 2 enclosed compartments in the van. During the second trial, treatments (cross-tied or loose) during transport were reversed but their placement in the van was similar. Transport commenced at 0800 h on Day 2 for a 24 h period. Horses were unloaded on Day 3 (0800 h) and housed in pens for a 48 h post-transport recovery period concluding on Day 5 at 0800 h.

A commercial van (1990, Nichols, air-ride) designed to haul a maximum of 15 horses and pulled by a semi-tractor (1990, Freightliner, 3 axle, conventional) was used for the 24 h of transport. The van's length was divided into 5 areas, which were partitioned either as a single enclosure for the loose treatment or 2 individual stalls for the cross-tied treatment. During the second

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trial, the horses remained within the same location of the van, but the partitions were reconfigured to accommodate the appropriate assigned treatment. The walls for the enclosure of the pair of loose horses extended from the floor to the ceiling and provided a total floor area of 5.4 m² (2.7 m²/horse). Floor area for the cross-tied horses was 2.2 m²/horse, but they were able to extend their neck (approximately 0.7 m²) beyond the front chest bar. Each horse in the cross-tied treatment group was restrained by 2 chain cross-ties (attached to the wall approximately 1.3 m from the floor) clipped onto each side of their halter. Sawdust was placed on rubber mats for secure footing. Air movement was not measured, but windows and vents were fully open to allow ventilation. Distance travelled in the 24 h period was 1775 and 1823 km for trials 1 and 2, respectively.

Water in buckets was available *ad libitum* to horses in the pens. Alfalfa hay (approximately 1.5 to 2% bwt) was provided at 0700 and 1700 h during Days 1, 3, 4 and 5. During transport, alfalfa hay was placed in haynets accessible to each cross-tied horse and placed on the floor of the compartments containing pairs of loose horses. A supply of drinking water collected at the stabling area was transported with the horses, so horses were offered water which they were accustomed to during transport. The van stopped 4 times (1100, 1400, 2000 and 0200 h) to provide water via buckets for each horse and to obtain blood sample collections at 2 of the stops (1100 and 2000 h).

The study was conducted in July and August 1999 at Fresno, California. Temperature and relative humidity in the barn and van were recorded continuously by microcomputer data logger units (Models WTA32 and SRHA32, respectively)¹ placed near the centre of the barn during Days 1, 3, 4 and 5 and hung (10 cm) from the centre of the van's ceiling during transit (Day 2).

Collection of physiological data

Horses were weighed on Days 1, 3 and 4 at 0800 and 2000 h and 0800 h on Day 5 using an electronic portable scale (Model H90-3042)². On Day 2, horses were weighed prior to loading between 0700 and 0800 h. Rectal temperature was recorded using the same schedule as weighing. Blood (20 ml) was collected via jugular venipuncture into evacuated glass tubes at 0800, 1100 and 2000 h each day and an additional sample collected prior to loading at 0700 h on Day 2. Horses were restrained gently with a halter during blood collection. Blood samples analysed for concentrations of serum cortisol, lactate, glucose, α_1 -acid glycoprotein (AGP) and total protein were placed immediately on ice and allowed to clot. Serum was obtained and frozen at -56°C. Blood intended for haematology was collected in tubes containing EDTA. Serum for aminotransferase (AST) and creatine kinase (CPK) activities was refrigerated until analyses were performed on Day 5.

Laboratory analyses

A commercially available radioimmunoassay plate³ was used to quantify AGP concentration. Cortisol concentration was determined in duplicate by a microplate enzyme immunoassay technique (Munro and Stabenfeldt 1984; Munro and Lasley 1988). Intra- and intercoefficients of variation were less than 8 and 10%, respectively. Total serum protein concentration was determined using a commercially available enzymatic colorimetric assay (Procedure 541)⁴. Lactate and glucose concentrations were determined by use of an autoanalyser (YSI 2300 STAT Plus)⁵. The

AST and CPK activities were determined at the Veterinary Medicine Teaching Hospital (VMTH, Davis, California) using a clinical autoanalyser (Hitachi 717)⁶. The peripheral blood WBC counts were determined by an automated cell counter (System 9000)⁷. Whole blood was used for the determination of WBC differential counts using standard laboratory techniques (Heckner *et al.* 1988) and the neutrophil to lymphocyte ratio calculated.

Statistics

The effects of the treatment (loose or cross-tied) and loading (0700 and 0800 h, Day 2) were evaluated using repeated measures ANOVA (Anon 1989) with treatment as a between-subject factor and time as within-subject factor. A significant difference is claimed whenever $P < 0.05$.

Results

Environmental

The daily minimum and maximum environmental temperature ranges for the pretransit (Day 1) and recovery days (Days 3 and 4) for both trials was 14–16°C and 34–38°C, respectively. Maximum relative humidity was 76–88%, while minimum range was 14–27%. During transport, temperature range within the trailer was 16–34°C and 21–37°C for trials 1 and 2, respectively, while relative humidity was 30–79% for trial 1 and 18–87% for trial 2. The minimum humidity values generally corresponded to the maximal temperature values.

General

Mean responses of each parameter for the cross-tied and loose treatment groups to transportation and recovery are shown in Figure 1. Each parameter for all horses exhibited a significant time-related response over the 4 day study period. Overall, treatment differences in response over the 4 day period were significant for serum glucose and cortisol concentrations and for WBC counts and N:L values. Overall, treatment differences over the 4 day study were not significant for weight, rectal temperature, PCV, total protein, lactate, AST or CPK activities, or AGP. No significant treatment effects were observed in comparisons of parameters measured immediately before loading (0700 h, Day 2) and immediately after loading (0800 h, Day 2). However, the effect of loading was highly significantly different ($P < 0.005$) across all horses for serum cortisol and PCV, but not significantly different for total protein, glucose, lactate ($P = 0.054$), CPK, AST, AGP, WBC counts or N:L ratio.

Characteristics of each parameter

Bodyweight and rectal temperature were not recorded during transport. Mean rectal temperature did not exceed the normal range (37.5–38.5°C) for the resting horse at any time point during the study. Bodyweight immediately after unloading (515 ± 31 kg), showed a 4.5% loss, but was not significantly different from pretransit weight (539 ± 35 kg).

All mean PCV and total protein values were within the normal reference ranges of 32–53% (Jain 1993) and 52–79 g/l (Kaneko *et al.* 1997), respectively, peaking in the final 12 h of transit and then dropping to baseline levels, once horses had access to *ad libitum*

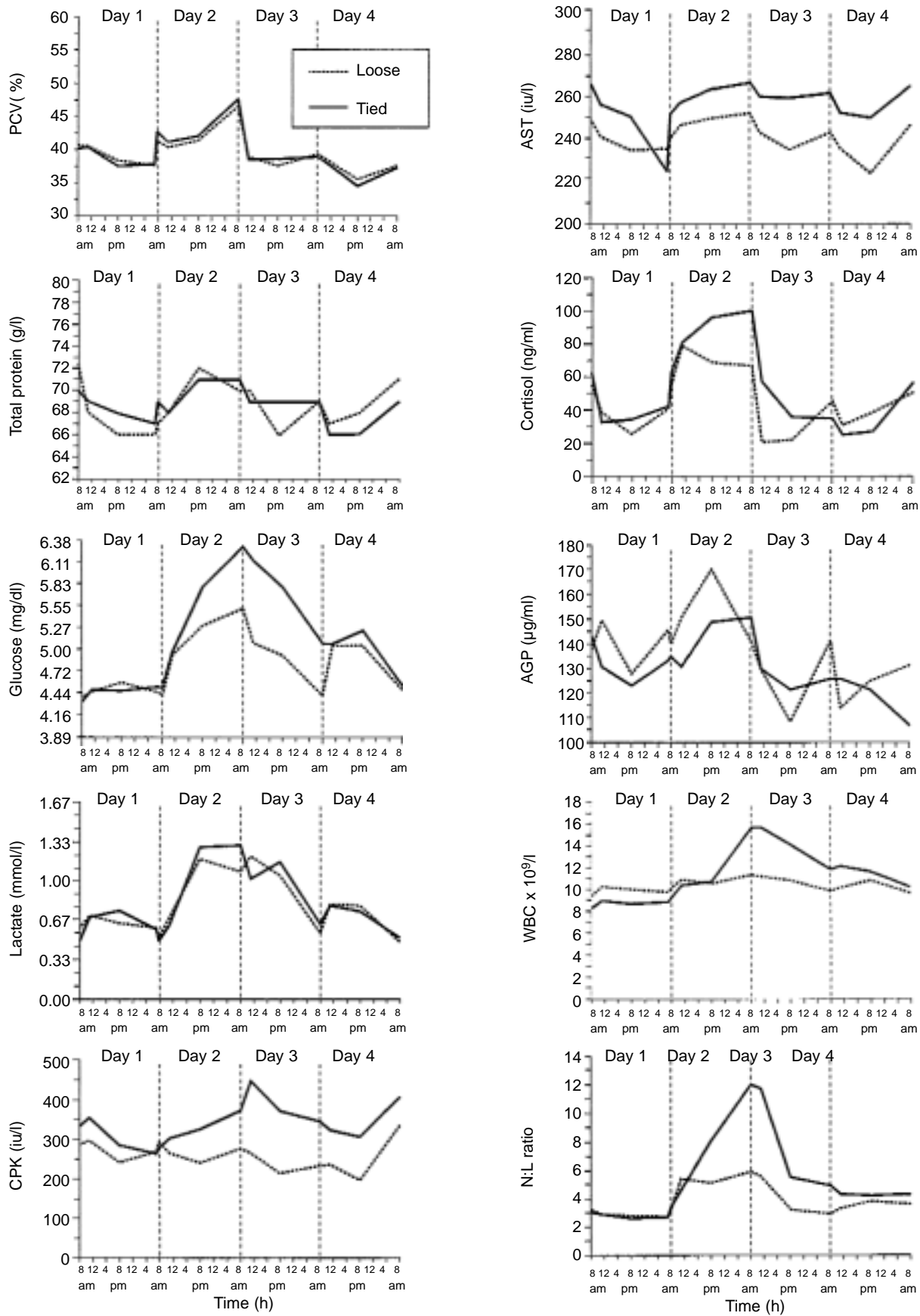


Fig 1: Response of packed cell volume (PCV), serum total protein, glucose, lactate, creatine kinase (CPK), aminotransferase (AST), cortisol, α_1 -acid-glycoprotein (AGP), white blood cell counts (WBC) and neutrophil:lymphocyte ratio (N:L) in loose and cross-tied treatment groups during the pretransit day (Day 1), transport day (Day 2) and recovery period (Days 3 and 4).

water in the pens during recovery. Horses were offered water 4 times (1100, 1400, 2000 and 0200 h) during transport and mean water consumption during transport was 13.1 ± 3.4 l for cross-tied horses and 21.4 ± 9.0 l for loose horses and 96.5% water consumption in both treatments occurred during the last 12 h of transport.

Mean glucose concentrations of the loose treatment were within the resting range (3.94–5.77 mmol/l) (Brobst and Parry 1987) during the 4 day study, with peak glucose concentration (mean \pm s.d. 5.50–0.61 mmol/l) occurring at the termination of transport. However, in the cross-tied treatment, glucose concentration was elevated above resting range during the last 12 h of transport and the first 12 h of recovery. Peak glucose concentration (mean \pm s.d. 6.27–0.78 mmol/l) occurred at the termination of transport and returned to baseline (2000 h, Day 1) values after 12 h recovery (2000 h, Day 3) in the loose treatment, compared to 48 h recovery (0800 h, Day 5) needed to return to concentrations at 2000 h, Day 1, in the cross-tied treatment.

Lactate concentration of all horses, from 12 h into transport through 12 h recovery, was above the reference range for resting horses of 0.56–1.11 mmol/l (Brobst and Parry 1987; Stull and Rodiek 1995) and significantly elevated above pretransit concentration.

Mean CPK activity for the cross-tied treatment was above reference range (119–287 iu/l; VMTH) during the 4 day study, with the exception of one sample collection (0700 h, Day 2, mean \pm s.d. 261 ± 88 iu/l). The CPK activity for the cross-tied horses increased during transport and recovery but was not significant over pretransit values (Day 1). The CPK activity of the loose treatment also showed no significant difference during transport and recovery when compared to pretransit samples. In contrast, the activity of AST in both treatments remained within the normal reference range (138–409 iu/l; VMTH) during the 4 day study and was not significantly different during transport or recovery compared to pretransit values.

Mean \pm s.e. serum cortisol exhibits a daily circadian rhythm, 65 ± 2 ng/ml (0600 to 1200 h) and 25 ± 2 ng/ml (1300–1800 h), with mean level 45 ± 1 ng/ml (Stull and Rodiek 1988). Cortisol increased gradually throughout transportation for the cross-tied horses with mean \pm s.d. peak concentration 100 ± 33 ng/ml measured at the termination of transport (0800 h, Day 3). In the loose treatment, peak concentration (79 ± 32 ng/ml) was measured at 3 h (1100 h) into transportation and then declined with subsequent samples during transport to mean \pm s.d. 67 ± 13 ng/ml at termination of transport. When compared to pretransit concentrations, cortisol showed elevations at 1100 and 2000 h of Day 2 for each treatment group. Additionally, in the cross-tied group, cortisol was elevated significantly over baseline at 0800 h on Day 3. All other recovery values for cortisol were not significantly different from pretransit values within each treatment group.

Mean AGP concentration of all horses during transport was elevated over normal range (75–125 μ g/ml) established for healthy, mature horses (Taira *et al.* 1992).

Mean WBC counts of loose horses remained in the reference range (5.5 – 14.3×10^9 /l) for healthy horses during the entire 4 day period, whereas counts in the cross-tied horses rose gradually during the last 12 h of transport and peaked at levels (mean \pm s.d. $15.6 \pm 4.4 \times 10^9$ /l) above the reference range during the first 3 h of recovery. In the cross-tied treatment at 24 h into recovery (Day 4, 0800 h), WBC counts ($11.9 \pm 3.3 \times 10^9$ /l) remained significantly elevated compared to pretransit (Day 1, 0800 h) values ($8.3 \pm 1.7 \times 10^9$ /l). The WBC counts ($10.2 \pm 2.2 \times 10^9$ /l) at

the conclusion of the 48 h recovery period were similar to pretransit counts. Mean N:L ratios in both groups exceeded the normal range of 0.8–2.8 for healthy horses (Morris and Large 1990) during 24 h transport and peaked in the cross-tied and loose groups (mean \pm s.d. 12.0 ± 7.4 and 5.9 ± 2.9 , respectively) at the termination of transport. Ratios in both treatments remained significantly elevated over baseline values at the 1100 h sampling following transport on Day 3 and then declined to baseline values for the remaining recovery period.

Discussion

Thermal stress has been documented in ponies exposed to ambient temperatures above 30°C, whereas 21°C is considered thermoneutral (Kaminski *et al.* 1985). The environmental temperature in the van exceeded 30°C each transport day and contributed to the stress of transport. Bodyweight response showed no difference between treatment groups and the 4.5% decrease immediately following transport may have been due to sweat loss, heat dissipation or reduced gut fill during transit. The weight loss was similar to that observed in other studies conducted under summer conditions; a 6% weight loss was observed in horses cross-tied in a van for 24 h of transit (Stull and Rodiek 2000) and a 3–4% decrease in slaughter horses transported 6–30 h (van den Berg *et al.* 1998; Stull 1999) when compared to pretransit bodyweight.

Horses were accustomed to the drinking water provided during transport, since it was collected from the same source as water provided in the stabling area. Water during transport was offered in buckets to each horse 4 times while in transit, but excessive sweat losses may be reflected in the PCV and total protein concentrations. No difference between treatments was evident for the response of PCV and total protein concentration to transport and the elevations exhibited with transport never exceeded the reference range. PCV levels dropped noticeably in early recovery with the availability of *ad libitum* water in the pens. The inability to dissipate heat during transit under hot summer conditions may be assessed by an elevated rectal temperature. Mean rectal temperature was within the normal range immediately following transport and during recovery. These indices indicate the ability of the horses to handle the thermal load during transport, primarily through sweating and respiratory mechanisms and the consumption of water.

Fatigue is a common observation in horses following long-term transport, probably due to the efforts required to maintain balance during acceleration and deceleration in a moving trailer (Clark *et al.* 1993; Smith *et al.* 1994; Waran *et al.* 1996). Muscle fatigue in exercising horses is commonly evaluated using serum lactate concentration, an anaerobic metabolite. Both treatment groups showed similar lactate response during transport and recovery. Therefore, the restricted movement of the head, neck and stance of the cross-tied horses did not increase muscle fatigue, as measured by lactate response. Lactate levels as high as 22.2 mmol/l in horses have been documented following racing (Snow *et al.* 1983), while 0.56–1.11 mmol/l were considered resting levels by Stull and Rodiek (1995). Lactate response during transport never exceeded 1.33 mmol/l, suggesting that 24 h of transport elicits mild anaerobic fatigue.

Muscular diseases in horses are often evaluated clinically using the enzyme activities of CPK and AST (Hodgson 1990). Moderate exercise or the initiation of a training programme can evoke small increases in CPK activity (400–500 iu/l), whereas fatiguing, long-term exercise bouts are associated with levels over 1000 iu/l. In

equine myopathies such as exertional rhabdomyolysis, levels in the hundred of thousands iu/l are often found. The activity of CPK rose above normal reference range (119–287 iu/l) in the cross-tied treatment group, but only to levels associated with moderate exercise. Both the loose and cross-tied groups showed similar AST activities throughout transport and recovery. These data indicate that both treatment groups of horses experienced minimal muscular insult due to the 24 h transport period.

Cortisol is secreted in response to activation of the hypothalamic-pituitary-adrenal axis during stressful situations and may impact broad-based biological functions such as increasing blood glucose concentration for energy availability, suppressing immune competence and impairing reproductive functions (Moberg 2000). Cortisol concentration dramatically increased with the initiation of loading and transport. The treatment groups exhibited different responses to transport, with the cortisol concentration of the cross-tied group rising to a peak of mean \pm s.d. 100 ± 33 ng/ml at the termination of transport, while the peak concentration in the loose group (79 ± 32 ng/ml) occurred 3 h into transport. Pretransit concentrations following transit were reached at 0800 and 1100 h on Day 3 for the loose and cross-tied groups, respectively. The sharp decrease of cortisol following unloading in both groups is due to the elimination of the stressor (transport) and cortisol's short half-life of 1 to 1.5 h (Lassourd *et al.* 1996). Glucose concentration was elevated in the cross-tied treatment during transport and peak elevation in each treatment group occurred at termination of transport. The return of glucose to pretransit concentrations occurred more slowly than cortisol, with the loose treatment obtaining pretransit levels by 2000 h on Day 3, while the cross-tied treatment did not return to baseline until 48 h recovery. Elevated glucose in the cross-tied horses, after 24 h recovery, may alter energy metabolism and availability for post-transit athletic performance.

Cortisol may also initiate the acute phase response, with the production of acute phase reactive proteins in the liver such as AGP (Stone and Mauer 1987; Itoh *et al.* 1992; Taira *et al.* 1992). Different types of stress, disease or inflammation may activate the acute phase response and AGP functions to aid in tissue repair or has immunosuppressive effects (Taira *et al.* 1992). Mean AGP concentration in all horses during transport was above normal range (mean \pm s.d. 99 ± 26 μ g/ml) established for healthy, mature horses (Taira *et al.* 1992), but no difference was shown between treatments.

An increase in cortisol concentration during long-term stress may lead to neutrophilia and lymphopenia and, therefore, an increase in the N:L ratio. The N:L ratio may be a more reliable indicator of chronic stress than cortisol concentration (Gross and Siegel 1983). A substantial increase during transport was shown for the cross-tied treatment as compared to the loose treatment. Mean N:L peak for cross-tied horses was comparable to previous studies using individually tied horses for 24 h transport (mean \pm s.d. 11.9 ± 12.2) (Stull and Rodiek 2000), while the mean N:L peak in the loose horses was similar to slaughter horses travelling loose in small groups (Stull 1999).

Studies assessing the pathology of respiratory disease following long-term road transport have reported peripheral blood leucocytosis and neutrophilia (Oikawa *et al.* 1995; Raidal *et al.* 1997). Comparison of mean WBC counts between treatments showed an elevated response in the cross-tied group due to transport, with peak WBC counts in recovery ($15.7 \pm 4.4 \times 10^9/l$) which slightly exceeded the normal reference range (5.5 to $14.3 \times 10^9/l$) counts. Clearly, a larger impact on their immune system was

shown in the cross-tied horses than horses travelling loose. A series of research studies examined the influence of an elevated head posture on tracheal bacteria (Racklyeft and Love 1990; Raidal *et al.* 1995, 1996, 1997) in both confined and transported horses. The presence of an increased number of bacteria was proposed to be the result of a decrease in clearance rate of the bacteria from the tracheobronchial secretions in horses confined to stocks and unable to lower their heads for 24–48 h (Racklyeft and Love 1990). In a subsequent study, this increase in number of bacteria and accumulation of mucopurulent secretions of the lower airway was shown to occur after only 12 h confinement with an elevated head posture. Clearance of accumulated secretions and bacteria occurred 8–12 h following a 24 h confinement with an elevated head posture (Raidal *et al.* 1995). The lowering of the head to increase the tracheal mucociliary clearance was confirmed using a radiopharmaceutical colloid method (Raidal *et al.* 1996). These factors of increased mucociliary clearance and bacterial burden in horses with an elevated head carriage for prolonged periods also occurred in horses undergoing transport for 12 h with their head restricted by cross-tying (Raidal *et al.* 1997). Data from the previous and current studies suggest that several factors, including the practice of cross-tying, may predispose horses to respiratory disorders following long duration transport.

The practical implications of the recovery data are that at least 48 h are necessary at rest for values to return to baseline after long-term transport with cross-tied horses, whereas horses travelling loose may recover with 24 h rest. This leads to further questions on the athletic potential and disease susceptibility of the horse during the recovery period and post-transit complications from other stressors such as social stress, thermal stress, housing or pathogen challenges.

The data collected in this study support the recommendation of allowing horses undergoing long-term road transportation to travel loose in small compartments without elevating their head and neck by cross-tying. Compatible pairs of horses travelling loose in this study did not receive any noticeable injuries due to aggressive behaviours, such as kicking or biting. The limited floor area for the loose pair may have restricted some interactions between the horses. No efforts were made in this study to document the balancing movements of either the cross-tied or loose group of horses during transport, but no horses were observed during the frequent stops in a recumbent position or with signs of falling or previous recumbency. From the data collected in this study, along with other previous reports, it is recommended that a small box stall is preferable to cross-tying during long-distance road transport.

Manufacturers' addresses

¹Onset Instruments, Pocasset, Maine, USA.

²Fairbanks, St Johnsbury, Vermont, USA.

³Development Technologies International, Fredrick, Maryland, USA.

⁴Sigma Diagnostics, St. Louis, Missouri, USA.

⁵YSI, Yellow Springs, Ohio, USA.

⁶Boehringer Mannheim, Indianapolis, Indiana, USA.

⁷Serono-Baker Diagnostics, Allentown, Pennsylvania, USA.

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