Journal of Equine Veterinary Science 126 (2023) 104268

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Effects of Transport and Feeding Strategies Before Transportation on Redox Homeostasis and Gastric Ulceration in Horses

Yashar Gharehaghajlou[a], Sharanne L. Raidal[b], Francesca Freccero[c], Barbara Padalino[d,*]

[a] Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
[b] School of Agricultural, Environmental and Veterinary Sciences, Charles Stuart University, Wagga, NSW, Australia
[c] Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia,Bologna, Italy
[d] Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy

A R T I C L E I N F O

Article history:
Received 28 October 2022
Received in revised form 14 February 2023
Accepted 28 February 2023
Available online 3 March 2023

Keywords:
Horse
Oxidative stress
Transport
Stomach ulcers

A B S T R A C T

Transportation may lead to oxidative stress (OS) and gastric ulceration in horses, and optimal feed management before, or during, transportation is unclear. This study aimed to evaluate the effects of transportation after three different feeding strategies on OS and to explore possible associations between OS and equine gastric ulcer syndrome (EGUS). Twenty-six mares were transported by truck for 12 hours without food or water. Horses were randomly divided into 3 groups; (1) fed 1 hour before departure (BD), (2) fed 6 hours BD, (3) fed 12 hours BD. Clinical examinations and blood collections were performed at approximately 4 hours BD (T0), at unloading (T1), 8 hours (T2) and 60 hours (T3) after unloading. Gastroscopy was conducted prior to departure, and at T1 and T3. Although OS parameters remained in the normal range, transportation was associated with increased reactive oxygen metabolites (ROMS) at unloading (P=0.004), with differences between horses fed 1 hour and 12 hours BD ($P < .05$). The level of total antioxidant (PTAS) was affected by both transportation and feeding strategy ($P = 0.019$), with horses fed 1 hour BD demonstrating greater PTAS at $T = 0$, and a different response in comparison with the other groups and the literature. Nine horses demonstrated clinically significant ulceration of the squamous mucosa at T1 but, although weak correlations were evident between OS parameters and ulcer scores, univariate logistic regression showed no associations. This study suggests that feed management prior to a long journey (12 hours) may affect oxidative balance. Further studies are needed to understand the nexus between feed management before and during transport, transport-related OS and EGUS.

1. Introduction

Horses are frequently transported worldwide by train, road, ship and air for reasons such as competing (e.g., racing, show, jumping), breeding, slaughtering, and veterinary treatments [1–3]. However, horses with and without prior transportation experience can be stressed by travelling [4] during both short and long-distance journeys [5]. Consequently, transport may contribute to poor health and welfare, causing injuries, health and behavioral problems [2,6–8].

Oxidative stress occurs as a consequence of an imbalance between the production of free radicals (ROMS) and protective antioxidant mechanisms [9]. The total plasma antioxidant status (PTAS) can be assessed with a single test [10,11] and, by calculating the associated oxidative stress index (OSI), defined as the ratio between ROMs and PTAS, it is possible to quantify oxidative stress [12,13]. Transportation has previously been associated with oxidative stress, and OS parameters were correlated with tracheal inflammation scores [2]. Oxidative stress has also been associated with gastric ulceration in people [14,15].

Equine gastric ulcer syndrome (EGUS) may involve the squamous or glandular mucosa and is the most common gastric disorder in this species [16]. Gastric ulceration has been associated with the administration of high doses of NSAIDs [17], transportation [18], and exercise such as show jumping, endurance, and racing [16]. Physiological and psychological responses to such stres-

Animal welfare/ethical statement: This research study has been approved by the Animal Care and Ethics Committee, Charles Sturt University, NSW, Australia (authorization no A17011).

Conflict of interest statement: None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

* Corresponding author at: Barbara Padalino, DVM, PhD, ECAWBM-AWSEL, Department of Agricultural and Food Sciences, University of Bologna, Bologna, 40127 Italy.

E-mail address: barbara.padalino@unibo.it (B. Padalino).

https://doi.org/10.1016/j.jevs.2023.104268

sors could change gastrointestinal physiology and predispose to oxidative injury [19].

Possible associations between transportation, OS and EGUS have not been previously evaluated. Moreover, how and if to feed horses before travelling is still a matter of debate [20]. Many equine industry members do not feed their horses before transportation [21], despite the demonstrated reduction in muscle problems following transportation in horses fed ad libitum hay prior to departure [22]. We have previously demonstrated that 12 hours transportation was associated with gastric ulceration, and that the severity of ulceration was reduced by feeding prior to departure [18]. The current study was designed to extend these findings by documenting the effects of 12 hours transportation following three different feeding strategies on OS parameters, and to explore possible associations between OS parameters and EGUS in transported horses. We hypothesized that 12 hours' transportation without feed and water would increase free radicals and OS parameters, that increased OS would be associated with transport-related gastric ulceration and that feeding before transportation would mitigate these negative effects.

2. Materials and Methods

All experimental procedures were approved by the Animal Care and Ethics Committee, Charles Sturt University, NSW, Australia (approval number A17011), and transportation details have been described previously [18,23].

2.1. Animals

All horses were CSU teaching or research horses and had been resident on-site for four or more weeks. Prior transport history was unknown for each horse, although all had been transported on at least one prior occasion. Details of horses and study design have been reported previously [18,23]. Briefly, 26 mares (Standardbred, n = 14; Thoroughbred, n = 10; Warmblood, n = 2) of mean age 9.9 years (range 4–20 years) and mean body weight 518.8 kg (range 416–658 kg) were used. Except during transportation days, horses were kept on pasture, fed alfalfa hay twice a day (8 AM; 5 PM), and had water ad libitum.

2.2. Experimental Design

The study was conducted as an interventional study to determine the influence of an overnight journey (6 PM–6 AM) on oxidative parameters and gastric ulceration. The mares were randomly allocated into three different groups based on different feeding management before departure: Group 1 (n = 7) was fed <60 minutes before departure (1 hour BD), Group 2 (n = 7) was fed 6h before departure (6 hours BD), and Group 3 (n = 12) fasted for 12 hours before departure (12 hours BD). All groups received 2.5 kg of alfalfa hay at the designated time.

As previously described [18], the horses travelled as two consignments, each of 13 horses, on consecutive nights (Trip 1, Trip 2). Group 1 horses travelled on Trip 1, Group 2 horses on Trip 2, and Group 3 horses travelled on both trips (n = 6 on each night). Both trips were completed over an identical route covering approximately 880 km. The driver and vehicle were same, departing at 6 PM and returning at 6 AM the following morning. Weather conditions on the day prior to departure, and during the journey, did not differ between trips. The transport vehicle was a 15-horse trailer attached to a prime mover (LF290 18T, DAF Trucks Australia, Bayswater, Victoria). Vehicle configuration and direction of travel have been reported previously [23], and treatment allocations were designed to control for different bay widths and directions of travel between groups.

Clinical examination and venous blood collection were performed for all groups prior to gastroscopy at 2 PM (T0) approximately 4 hours before departure, at unloading at 6 AM (T1), and 8 hours after the journey at 2 PM (T2) and 60 hours after the journey always at 2 PM (T3). Heparinized plasma and serum were obtained by centrifugation (1600 x g for 15 minutes). ROMs and plasma total antioxidant status (PTAS) were determined in plasma as described previously [2]. The oxidative stress index (OSI) was estimated using the ratio of ROMs/PTAS multiplied by 100 [12].

2.3. Gastroscopy

Gastroscopic examination of Group 1 and 2 horses was performed between 4 PM and 6 PM on the day before travelling, whilst gastroscopy of Group 3 horses was performed between 4 PM and 6 PM on the same day as the journey to allow for placement of an indwelling nasogastric tube, as previously described [18]. Gastroscopy was also performed on all horses after unloading (T1) between 7 AM and 10 AM, and after 60 hours recovery (T3) between 4 PM and 6 PM. All horses were fasted for a minimum of 10 hours prior to gastroscopy and were sedated (xylazine [Xylazil-100, Troy Laboratories Pty Ltd, Glendenning, New South Wales, Australia] 0.4 mg/kg bwt i.v. and acetylpromazine [Ilium Acepril-10 Injection, MD Solutions Pty Ltd, Williamstown North, Victoria, Australia] 0.02 mg/kg bwt i.v.) 5 to 10 minutes prior to gastroscopy. Endoscopy of gastric mucosa was performed with a 3 m endoscope (9 mm outer 9 diameters, Olympus Medical Systems Corporation, Tokyo, Japan), as previously described [24,25]. Squamous and glandular mucosa were visualized and scored as previously described [18] after insufflation of air to distend the stomach and, if necessary, lavage of gastric content adherent to the gastric mucosa. Gastroscopy examinations were recorded and stored with a unique, randomly generated four-digit identification number. Ulcers in horse's stomach were scored using a validated scoring system [25] with ulceration of the greater curvature (margo plicatus greater curvature, MPGC), lesser curvature and fundus scored separately and summed to give a squamous score, and fundic and pyloric glandular mucosa scored separately and summed to give a glandular ulcer score, as previously described [18]. Ulcer scores were independently assigned in real-time and by analysis of recorded examinations. Real-time results were analyzed because results were consistent between both methods, and recordings were missing or of inadequate quality for 11 examinations.

2.4. Statistical Analyses

Data (ROMs, PTAS and OSI) were evaluated using descriptive statistics and checked for normal distribution by the Kolmogorov-Smirnov test. Results for OSI ($P > .05$), but not ROMs or PTAS ($P < .05$), were normally distributed. Therefore, ROMs and PTAS data were normalized by the fractional rank. OS data were analyzed by a mixed linear model using PROC mixed procedure and each OS parameter was analyzed as a separate outcome variable. The model evaluated the three different feeding strategies (Group 1, 2, and 3–respectively, feeding 1, 6 , or 12 hours before departure), time (T0, T1, T2, T3) and their interaction as fixed effects, with horse and replicate (trip 1, trip 2) as random factors. A Tukey test was used for post hoc testing. Spearman correlations were calculated among OS and gastroscopy parameters registered at T0, T1, and T3. Univariate logistic regression analysis was used to evaluate possible associations between PAT, ROMS, and OSI levels at T1 with clinically significant equine squamous gastric ulcer disease (ESGD, defined as horses with a squamous ulcer score of ≥ 3 in any single location and/or a summed score of ≥ 5) as the binary outcome. Statistical analyses were performed using SPSS 25.0 (SPSS, an IBM

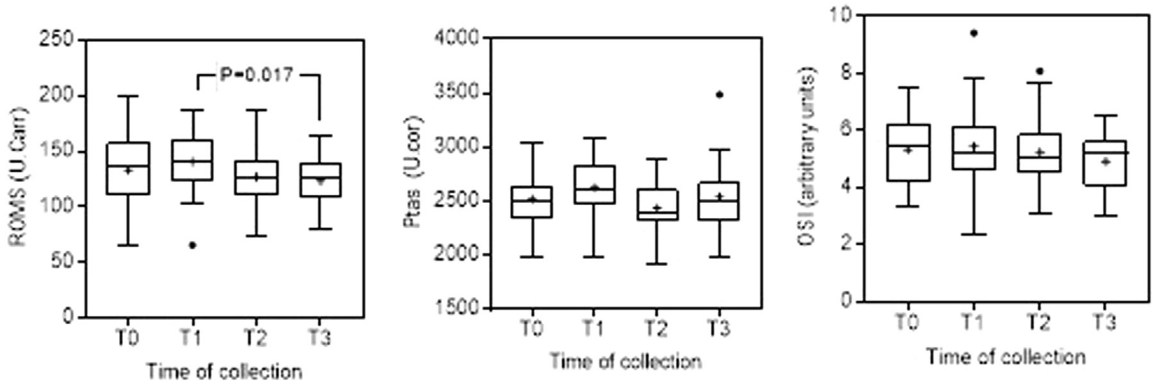

Fig. 1. Effect of time on ROMs, PTAS and OSI in 26 horses subjected to 12 hours' transportation without food and water. Results are shown as mean (+), median and quartile, with whiskers and outliers calculated by Tukey method; significant difference between results are shown.

Company, Chicago, IL) and Graph-Pad Prism (GraphPad Software version 7.0, San Diego, CA), was used for visualization. For all statistical analyses, a *P*-value < .05 was considered significant.

3. Results

All horses travelled well, with no clinical signs of injury or illness attributable to transportation. Results of gastric ulceration scores have been published previously [18]. There was no difference between groups prior to departure. The median summed squamous ulcer scores were higher after transportation (T1 and T3), particularly for group 3 horses (fasted 12 hours prior to departure). In total, 14 (of 26) horses demonstrated moderate or severe (grade ≥3) ulceration of the squamous mucosa at T1, and 9 (of 26) horses were treated for ulceration of the squamous mucosa of grade ≥3 at the end of the trial.

All OS parameters remained within normal ranges reported previously for horses [9] at all times during the current study. The effect of time (i.e., transportation) was significant only on ROMs ($P = .004$), with an increase at unloading (T1) relative to T3 (Fig. 1). Significant effects were observed due to interactions between feeding strategies and time for ROMs and PTAS. At T0, Group 1 horses (fed 1 hour BD) had the highest value of PTAS, significantly greater than that of Group 3 horses (fed 12 hours BD). After transportation, at T2, mean ROMS of Group 1 horses (fed 1 hour BD) was significantly greater than that observed for Group 3 horses (fed 12 hours BD). Within each group, the highest value of ROMs was found immediately or 8 hours after transportation (T1 or T2), while increased PTAS was found after transportation (T1) in horses of Group 2 and 3 (fed 12 and 6 hours BD). PTAS had indeed a different trend in the Group 1 horses (fed 1 hour BD), as this was the only group that had the highest value at T0 and did not exhibit an increase following transportation but a significant decrease was present at unloading (T1) (Table 1).

Correlations between OS parameters and gastric squamous ulceration scores are shown in Table 2. ROMs correlated positively with ulcer scores for the margo plicatus greater curvature (MPGC) ($r = 0.294$, $P = .009$) and lesser curvature ($r = 0.386$, $P = .000$); PTAS correlated positively with ulcer scores for the MPGC ($r = 0.235$, $P = .039$), fundus ($r = 0.224$, $P = .048$), summed squamous ($r = 0.235$, $P = .039$); OSI correlated positively with ul-

Table 1
Effect of feeding management before departure (feeding 12 hours, 6 hours or 1 hour before departure) and transportation of 12 hours without feed and water on oxidative responses. Data are expressed as the least square mean and standard error (SE), with *P* values determined by linear mixed models and Tukey post hoc testing. Means on the same line with different superscript differ significantly (A, B, $P < .01$; a,b $P < .05$); means on the same column with different superscript differ significantly (E,F < .01; e,f $P < .05$).

Group	T0	T1	T2	T3	S.E.	P Time	*P* Feeding	*P* T*F
ROMS								
Group 1 (fed 1 h BD)	131.3	142.2^{A}	145.6^{Af}	116.7^{B}	10.6			
Group 2 (fed 6 h BD)	142.4^{a}	146.3^{A}	130.8	120.1^{bB}	10.3	.004	.688	.030
Group 3 (fed 12 h BD)	129.6^{a}	137.1^{a}	113.9^{be}	128.7	7.4			
PTAS								
Group 1 (fed 1 h BD)	2748.7^{aF}	2470.8^{b}	2497.8	2584.5	93.4	.107	.521	.019
Group 2 (fed 6 h BD)	2554.7	2703.4^{a}	2460.3^{b}	2452.9^{b}	93.4			
Group 3 (fed 12 h BD)	2371.6^{aE}	2673.8^{b}	2388.7^{a}	2570.4^{b}	71.3			
OSI								
Group 1 (fed 1h BD)	4.87	5.92	5.88	4.56	0.45	.091	.840	.065
Group 2 (fed 6 h BD)	5.59	5.40	4.36	4.89	0.45			
Group 3 (fed 12 h BD)	5.44	5.17	4.76	5.08	0.33			

Table 2
Spearman correlations among oxidative stress parameters and squamous ulcer scores recorded before departure, at unloading and 60 hours after journey.

		MPGC	LC	Fund	SumSqua
ROMs	r value	0.294	0.386	0.052	0.320
	P value	**.009**	**.000**	**.654**	**.004**
PTAS	r value	0.235	0.173	0.224	0.235
	P value	**.039**	.129	**.048**	**.039**
OSI	r value	0.133	0.234	-0.075	0.153
	P value	.246	**.040**	.516	.181

Abbreviations: LC, lesser curvature; MPGC, margo plicatus greater curvature; SumSqua, summed squamous ulcer score.

cer scores for the lesser curvature (r = 0.234, P = .040) (Fig. 2). A clear relationship was not evident between oxidative parameters and glandular ulcer scores (data not shown). The univariate logistic regression showed that there was no significant association between oxidative parameters and clinical ulcers scores (P > .05).

4. Discussion

The current study was conducted to evaluate the effects of transportation after three different feeding strategies on OS parameters in horses and to explore possible associations between OS and EGUS. The results partially supported our hypotheses. Transportation did cause an increase in free radicals and EGUS, and the feeding strategies before departure mitigate the effects. Horses that fasted for 12 hours before departure had the highest summed ulcer scores and the lowest value of antioxidants in their plasma, while horses fed 1 hour before departure had the highest PTAS prior to departure which was also higher than the value at unloading. While the horses fed hay 6 hours before departure seemed to have an intermedium level of antioxidants. Horses fed 1 hour before departure had more feed retention in the stomach and less ulceration [18]. However, OS did not result in association with EGUS. Our findings suggest that prolonged fasting before travelling is not recommended, since may affect the antioxidant capacity of the horses and the risk for EGUS, but the tested OS parameters cannot predict EGUS.

Twelve hours of transportation was associated with increased ROMS, as has been previously reported [2,4]. This increase persisted until at least 8 hours after arrival (T2) for horses fed 1 hour prior to departure, in contrast to the response seen in horses that fasted prior to departure. In contrast to previous studies [2,4], an effect of transportation on PTAS was not found. The expected significant increase in PTAS at unloading was not observed in this study; this finding might have been influenced by divergent responses associated with feeding strategies. PTAS responses for horses fed 6 hours or 12 hours prior to departure increased, although differences were not significant. By contrast, results from horses fed within 1 hour of departure showed a significant reduction in PTAS levels. This may be due to the fact that PTAS were adsorbed in the gut from the ingesta and immediately used at the blood level [4], and the animals did not need to metabolize the antioxidant reserve from the tissues. The findings of the current study suggest that feeding strategies prior to transportation might have influenced the PTAS level and have mitigated the need for mobilization of the antioxidant reserve due to transport stress reported in horses [6] and in other species [26,27].

Fasting during transport is considered a risk factor for transport-related disease [20], and feeding management is regulated differently in different jurisdictions. In Europe live animal is regulated by the EC 1/2005 and horses must be fed and watered en route every 8 hours, and recently the European Food safety authority suggested feeding the horses every 4 hours while the truck is stationary during the compulsory rest of the driver [20]. The Australian Code of standards and guidelines instead recommend feeding and watering the animals every 6 hours in transit but allows a maximum journey duration without feeding and watering of 24 hours. None of the codes related to animal transportation gives clear instructions on feeding management before transport. Horses fed before travelling showed retention of feed within their stomachs, and the presence of ingesta appeared to minimize the development of transport-related stomach ulcers [18]. Padalino et al [28] found that giving water and food ad libitum before travelling decreased the risk of muscular problems postjourney and suggested that the presence of ingesta may increase the water and electrolytes reservoir available within the gastrointestinal lumen. Food deprivation has been used to induce gastric ulceration in equine studies [29,30], and may lead to negative welfare consequences, such as prolonged hunger [20]. EFSA recently reviewed the literature and reported that travelling for 12 hours in fasting conditions and travelling for 6 hours and taking part in sports competitions increased the risk of EGUS in horses while travelling for more than 20 hours without feed increase the risk of gastroenteric disorders [20]. Consequently, EFSA suggested avoiding prolonged fasting before and during the journey in horses and making sure that the animals have access to fresh water and appetible feed before the journey and regularly in transit to protect their welfare [20]. In the current study, horses fed within 1 hour before travelling had the hay and water available until departure, as suggested by EFSA. This group of horses had a higher antioxidant level in their blood prior to departure, and this may have helped in balancing the production of free radicals associated with transportation. Further studies are needed to deeply understand the effects of feeding management before and during transport on oxidative stress, health and welfare of the animals.

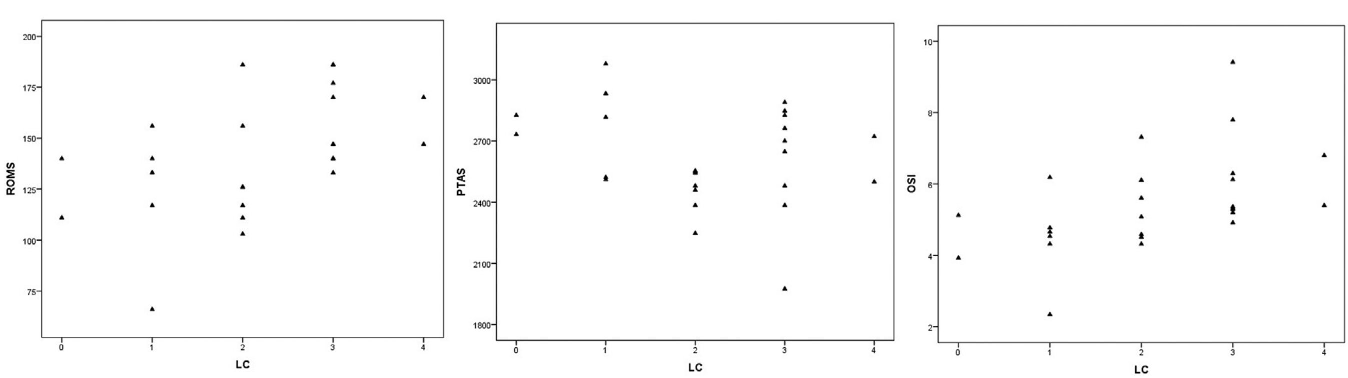

Fig. 2. 2D graph of the Spearman correlation between LC (less Curvature Ulcer score) and ROMS, PTAS and OSI recorded before departure, at unloading and 60 hours after journey**.**

Although in human and murine studies, oxidative stress has been associated with gastric ulceration [15], this could not be substantiated in the current study, as only weak correlations were found. This could be due to the limited sample size used which could have affected the regression model, or also for the fact that only ROMs and PTAS were tested, while in the literature often other markers of oxidative stress such as markers of lipid peroxidation, protein oxidation or other antioxidants, like glutathione or superoxide dismutase, were used. A large cohort study on the effects of EGUS on oxidative stress parameters and the welfare of horses may be more suitable for responding to the research question. The findings of this study need to be interpreted with caution because there are some other limitations. The effects of the three feeding strategies have been tested only before transport, so there is not a 'control group' just for the effect of the feeding regimes on the oxidative stress parameters. Moreover, it was impossible to standardize the timing of gastroscopy between groups and this difference may have in impact on the results observed. Before gastroscopy, the horses were sedated, and we cannot exclude an effect of sedation on the OS parameters studied. Phenothiazine and detomidine in horses and xylazine in goats had proven to have antioxidant and anti-inflammatory propriety in horses and may have led to a lower production of ROMs [31,32]. Finally, only mares were used in the current study, and further studies with a larger number of horses, and including colts and geldings, would be useful to determine whether responses observed in the current study are repeatable Notwithstanding these limitations, this study has increased our understanding on the effects of feeding practice before travelling, suggesting that this should be investigated further to give evidence to the animal welfare policy makers.

5. Conclusions

This study has suggested that feed management prior to a long journey (12 hours) may affect oxidative balance in transported horses. Feeding prior to travelling might be recommended as it might be helpful to increase the plasma antioxidant status and reduce the risk of EGUS. However, more studies are needed to understand which feeding practices before and during journeys may protect the health and welfare of the horses in the best way.

Acknowledgments

The authors are grateful to Austvet Endoscopy for providing a 3m endoscope for gastroscopy during the study. The authors thank Ruby Costello, Zali Clark, Martina Sardo, Fauve Buckley, Lexi Burgemeestre, Rhys Powell and Paloma Ivanyi for assistance with the care of horses, Drs Surita du Preez, Edwina Wilkes and Meredith Platt for assistance with gastroscopy, and Dr Martina Felici for helping with formatting the text.

Financial disclosure

This research was funded by Virbac (Australia) Pty Ltd, Milperra, Australia; Virbac (Australia) Pty Ltd; Goldners Horse Transport, Len Waters Estate, Australia; and World Horse Welfare, World Horse Welfare, Norfolk, England.

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