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The response of untamed (unbroken) ponies to conditions of road transport

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Abstract

The response to a one-hour road journey was observed in 145 semi-feral ponies unused to handling. Ponies were transported singly or in groups of four or eight at a typical commercial 'high' stocking density or at a slightly reduced 'low' density (range 139 to 316 kg m^{-2}). Blood samples were obtained for plasma biochemistry post transport. Behaviour during the journey of 40.2 miles was recorded using overhead video cameras. Analysis of these revealed that levels of aggression were lower in groups of four, as were the number of slips, falls, stumbles and collisions (SFSC). Within groups of four or eight, reduced stocking density was associated with lower levels of plasma cortisol and creatine kinase, reduced aggression and fewer SFSC. Levels of aggression pre-transit (observed over one hour the day before travel) were not predictive of aggression on the lorry. Single ponies were constrained to align at right angles to the direction of transport, whereas ponies transported in groups could also align forwards or backwards and were able to re-align themselves during transport especially at reduced stocking density. Of those transported singly, younger ponies showed separation anxiety, with highly-significantly greater levels of pawing and sniffing at the ground and increased packed cell volume. Thus, transporting young, untamed ponies singly is not recommended. A suitable maximum stocking density for ponies transported in groups would be approximately 200 kg m^{-2}. However, minimum stocking density for grouped and single untamed ponies transported by road requires future investigation. Whilst stocking density was correlated with many of the variables measured during transport, stocking rate (animals per unit floor area) was not.

Keywords: *animal welfare, group size, ponies, stocking density, transport, unbroken*

Introduction

This study was carried out to examine the effects of group size and stocking density during road transport on untamed (unbroken) pony behaviour and welfare. Until now, there has been no scientific work on the transport of untamed ponies, so that recommendations and legislation regarding their transport have been based on anecdotal information or studies on horses accustomed to handling. In the UK, during autumn and winter, semi-feral ponies from areas such as Dartmoor, Exmoor, the New Forest and Wales are gathered for annual sales at which young stock and unwanted older breeding animals are sold. The majority are transported to and from the sales and a substantial number are subsequently transported to slaughter at commercial abattoirs. Ponies are generally considered to be under 14.2 hands high (1 hand equal to 4 inches or 10.16 cm).

In the literature, loads are described in terms of stocking density (kg m^{-2}), stocking rate (animals m^{-2}) or as space allowance (m^2 per animal). In horses transported loose in groups there is evidence that higher stocking leads to more slips and falls and that horses that do fall find it harder to get up again (Collins *et al* 1999; Iacono *et al* 2007). Stull (1999) found that some physiological indices of stress (white blood cell count, total protein concentration and neutrophil:lymphocyte ratio) were significantly greater in adult horses stocked more tightly (1.14–1.31 m^2 per horse) than in those provided with greater floor area (1.40–1.54 m^2 per horse) when transported in trailers on long journeys (5 h 45 min to 30 h) to slaughter. However, Iacono *et al* (2007) found no influence of stocking density (in the range 221 to 397 kg m^{-2}) on blood cortisol or plasma chemistry profile for adult horses transported for 18–20 hours.

Horses need to expend physical energy to maintain their balance during transport (Waran & Cuddeford 1995; Doherty *et al* 1997; Giovagnoli *et al* 2002). A number of studies (eg Clark *et al* 1993; Smith *et al* 1994; Waran *et al* 1996; Gibbs & Friend 1999; Collins *et al* 2000; Toscano & Friend 2001) have examined the orientation, relative to the direction of travel, preferentially adopted by tame horses during transport. These studies gave ambiguous results owing to substantial variation between individuals, vehicle design and journey type and duration. However, some of the studies lend support to the anecdotal evidence that horses prefer or find it easier to balance when facing away from the

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direction of travel (Clark *et al* 1993; Smith *et al* 1994; Waran *et al* 1996; Doherty *et al* 1997).

The potential problems of aggression during transit have also been little studied. Grandin *et al* (1999), in a survey of horses arriving for slaughter in the USA, found that fighting was the major cause of injuries and recommend that aggressive mares and geldings that continually attack other horses should be segregated. In more recent work, Iacono *et al* (2007) found particular individual horses were aggressive, and that the incidence of aggressive behaviour during transport was not influenced by stocking density in the range 221 to 397 kg m^{-2}. The aim of the present study was to provide information upon which to base recommendations for transporting untamed ponies by road, in terms of group size and stocking density. Additionally, it was of interest to see if it would be possible to identify ponies that showed aggression during transport, before they were loaded.

Materials and methods

Eighteen groups of ponies unbroken to the halter were observed the day prior to transport by lorry, then before and during loading, transport and unloading. Details of these observations plus physical and physiological measurements are given below.

Study design

The critical part of the study was to compare and evaluate the behaviour and welfare of ponies transported in different group sizes and at different space allowances. Group sizes of one, four and eight were selected for study in combination with two stocking densities 'high' and 'low'. The 'high' density approximated that typically seen commercially, allowing sufficient room for ponies to stand, but with limited manoeuvrability, such that reducing the space by a further partition spacing would be unacceptable. In a preliminary survey of commercial practice in the UK, it was found that vehicles used for the commercial transport of ponies generally did not have the facility to allow fine adjustment of pen length. The positioning of the partitions was limited to align with the structural supports within the lorry body and in general this meant that pen length could be altered at approximately 62-cm intervals. Thus, the 'low' stocking density adopted was defined as the length that would have been used for the high stocking density plus one extra partition space of 62 cm.

To reduce the number of confounding variables, all ponies were transported from the same holding farm over the same route to the same abattoir, using an experienced driver and the same fixed-bed, air-sprung lorry representative of those commonly used within the industry (Figure 1).

Journeys took place during the 16-month period January 2006 to May 2007. Treatment combinations were used on each load in a near-balanced design, given in Figure 2, that varied the position on the lorry (front or rear) as well as group size and stocking rate. The number of ponies for each transport occasion thus varied according to this schedule between 5 and 12 animals. The total number of ponies in the study was 145.

Pre-transport day

The morning before transport each group of ponies was slowly and quietly driven in from grazing in a holding field (summer) or from a yard where hay and water were available *ad libitum* (winter). They were then put through a race system in small groups. This comprised a small holding pen with forcing gate, two sections before the weigh crate and then up to four sections ending in a head-restraint crush designed for cattle. The facilities used comprised high fencing throughout, well-lit areas and a solid, flat ground surface with reasonable grip. Ponies were never completely isolated in that at least one pony was always present immediately in front of or behind the ones being handled. Handling was performed quietly using highly-experienced animal handlers and was kept to a minimum. Each pony was weighed singly in the sheeted electronic weigh crate and then marked on its back from above either in the weigh crate or in the crush by applying water-soluble white emulsion paint using a 25-mm long-haired paintbrush. The sex of the pony was determined by visual examination and its height estimated. Brief descriptions of coat colour and white marks such as socks, stars and stripes were noted. Still photographs from the side and front were taken to aid identification. Ponies were never unduly restrained (the head crush was not used) and were released back to the group without delay.

Ponies were video-recorded during weighing and marking. These records, which included sound, were then scored for fractiousness using a subjective three-point score described as: 1 'quiet'; 2 'mildly fractious' and 3 'very fractious'. Quiet ponies were relatively easy to handle, showed little or no escape behaviour, and no overt response to being marked. Mildly fractious ponies were less easy to handle, often moving back and forth in the weigh crate during handling and marking and some made attempts to turn around to rejoin conspecifics or to escape. Fractious ponies typically vocalised, kicked the partitions, plunged, jumped, reared and responded aggressively (mainly kicking out but also rearing, plunging and squealing) to being touched with the paintbrush during marking.

Following weighing and marking, the ponies were allowed to settle down for approximately 30 min. Two portable video cameras were set up at either end of a rectangular covered yard and two observers were positioned on the top of the 2.5-m high railings on one long side of the yard (a solid wall comprised the other long side). A video camera with a very wide-angle lens was permanently fixed high above the yard and gave a view from above of the whole pen and this was attached to recording equipment at the beginning of each session. A small pile of hay was placed centrally in the yard and the ponies were then gently driven in and continuous video recording plus manual observations carried out for 1 h (1100 to 1200h, approximately). The observers aimed to record every interaction between ponies, identifying them using the paint marks and descriptions. The list of behaviours recorded is given in Table 1. The video recordings were made as back-up in case there were problems manually recording all interactions or identifying

Figure 1

External view of the lorry (Mercedes Atego 818, 7.5 tonne, rigid, 180 hp with body supplied by Williams Commercial Bodies) used throughout the study and a view of the load space showing the height of the pen partitions and the hard drive video recorder suspended from the roof.

Figure 2

Group size/stocking density/pen position combinations for each transport occasion.

	Load 1	Load 2	Load 3	Load 4	Load 5	Load 6
Back	**8**	**1**	**8**	8	**1**	**4**
Front	1	4	4	**1**	4	8

	Load 7	Load 8	Load 9	Load 10	Load 11	Load 12
Back	4	1	1	1	4	**4**
Front	**1**	**4**	**4**	**4**	**1**	1

	Load 13	Load 14	Load 15	Load 16	Load 17	Load 18
Back	8	**8**	**8**	**4**	**4**	1
Front	**1**	1	4	8	8	**8**

High SD (dark shading)
Low SD (light shading)

the ponies involved. Following observations the ponies were returned to their home pen or field to rest.

In practice, the most frequent forms of aggression (agonistic behaviour) recorded were bites and kicks. For statistical analysis, therefore, the number of bites and kicks made or received by individual ponies was tabulated by reference to the manual recording forms. An aggression score (the total number of bites and kicks to all ponies in the group during the hour) was then obtained for each pony. Many ponies did not show such physical aggression and therefore had a total of zero.

As the principal aim of observations prior to transport was the detection of ponies that might be aggressive during transport, those transported singly were excluded from the final analysis as they were unable to interact with others.

Transport day

The lorry used for transporting the ponies was fitted internally with four roof-mounted wide-angle cameras (Sony 1/3" CCD Color Bullet Camera VO6AO 4338, Tokyo, Japan) attached to a four channel hard drive, digital video recorder (Sitescape, Model MDVR-400, Kodicom, Hong Kong) which was switched on prior to transport. Tinytag Ultra temperature and relative humidity recorders (Gemini Data Loggers Ltd, Chichester, UK) were fitted to measure internal and external air temperature and humidity. The ponies were gathered into a pen adjacent to the loading bay and each had its body surface temperature on the rump and flank recorded using a non-contact, infra-red, portable thermometer (Raynger MX RS456-9165 Raytec, Santa Cruz, USA). Ponies were filmed during loading onto the lorry. The loading bay was at the same height as the lorry, leaving little to no slope on the lorry ramp. They were randomly allocated to two pens divided by high (160 cm) and solid partitions providing visual isolation. The floor of the pens was covered with wood shavings. The dimensions of each pen were recorded together with the identity of the ponies in each pen (treatment group). They were then transported (40.2 miles) to the abattoir using a standard route and the duration of the journey was noted.

At the abattoir, the ponies were slaughtered as soon as possible upon arrival, alternating treatment groups whenever feasible. A video recording was made of the unloading and again each pony's body surface temperature was recorded. Time of death was recorded. Immediately post slaughter, during bleed-out, the blood temperature was measured and recorded. Blood samples were collected and tubes were marked with the identity of the pony. The age of each pony was estimated from the teeth and this was recorded (range under 6 months to 25–30 years old, 25, 50 and 75 percentiles — 1, 1.5 and 6 years old, respectively).

Blood analysis

A 10-ml sample of blood was also collected at slaughter into heparinised tubes. The blood was stored on ice until it was centrifuged and the plasma collected and frozen in liquid nitrogen for subsequent analysis of cortisol, creatine phosphokinase (CPK) and lactate using the methods described in Warriss *et al* (1998). Packed cell volume (PCV) was measured by a microhaematocrit method.

Table 1 Behaviours recorded and analysed within the study.

Behaviour	Definition
Interactive behaviours recorded pre-transport	
Bite	Teeth grasp flesh, ears pinned back, lips retracted
Bite threat/snap	As bite but no contact by aggressor
Ears back	Ears pressed caudally against head (directed at another pony)
Kick	One or both hindlegs contact made
Kick threat	Similar to kick without sufficient extension to make contact
Paw	Repeated foreleg pawing (raking) at ground
Stamping	One foreleg raised and lowered firmly and repeatedly
Strike foreleg	Foreleg extended to make contact with recipient
Behaviours recorded from the video obtained during transport	
Paw	Repeated striking movement of the ground by either of the forefeet
Snap	As bite but no contact made
Bite	Teeth grasp flesh, ears pinned back, lips retracted
Stamp	Fore or hindleg raised and driven directly into ground
Kick attempt	Either hindleg kicked out but no contact made
Jump	All four feet off the ground at the same moment in time
Kick	Hindleg(s) kicked out with the contact made with either the lorry or another pony
Rear	Forefeet raised off the ground when the back hindfeet remain on the ground
Ears back	Ears pressed caudally against head (directed at another pony)
Nudge/head butt	Touching another pony with the nose/muzzle
Head tossing	Jerking movement of head up and down
Slip	Loss of balance involving one or more limbs sliding 'uncontrollably'
Fall	Loss of balance resulting in the pony's haunches or shoulders touching the ground
Collision with side, severity 1	Hitting of the side, no loss of balance
Collision with side, severity 2	Loss of balance resulting in the pony hitting the side of the pen
Collision with pony	Loss of balance resulting in hitting another pony
Stumble	Loss of balance resulting in pony having to take two steps or more in order to remain standing

Video analysis

Real-time analysis of the video recordings for each pen was carried out using the specialist software 'The Observer' v5.0 (Noldus, The Netherlands). Relevant behaviours were recorded as instantaneous (or momentary) and the software summed the number of occurrences (frequency) of each behaviour for the duration of the journey. Between the relevant behaviours (which were principally interactive and contact and are listed in Table 1), 'other' was used to complete the record. The duration of bouts of walking and positional changes were recorded. The orientation of each pony was recorded relative to the direction of travel.

The orientations measured were:

- Facing forwards, 0° in the direction of travel;
- 45° to the direction of travel — both clockwise and anti-clockwise;
- 90° to the direction of travel — both clockwise and anti-clockwise;
- 135° to the direction of travel — both clockwise and anti-clockwise;
- Facing backwards, 180° to the direction of travel.

Statistical analysis

The amount of time ponies spent in different orientations within the pens was analysed by means of radar plots. An overall, formal analysis of the gross differences between the treatment groups was not necessary as interpretation of the radar plots was unambiguous. Multilevel models, as described below, were used to investigate more subtle questions concerning the time spent in different orientations. The effect of the combinations of the transport treatments on the response variables (the behavioural data, the blood variables and the blood and body temperatures) were investigated using the multilevel software MLwiN v2.10 (Centre for Multilevel Modelling 2008). This type of model allowed the best use of the data, in terms of power, to detect any differences between the treatments as it is able to use the data at the level of each individual animal whilst allowing for the correlations between animals within each pen and the correlation between pens within the lorry. The multilevel structure pony within pen, within journey, was used in the models and the effects of the factors pen position (front or back pen), group size and stocking density and their interaction, were fitted as independent variables in the models. Where appropriate, covariates were also tested and fitted in the models to further investigate the response of the animals during transport and also to reduce error variation. For example, besides the effects of the transport treatments on plasma CPK, we also tested for a difference in plasma CPK levels between ponies recorded as quiet or as fractious on the day prior to transport.

For the analysis, different behaviours that were correlated, and that were assumed to be associated, were summed. Thus, for each pony, the number of bites, snaps and occasions on which ears were back were summed and the new variable used as a measure of aggression during transport. For each pony, the number of slips, falls, stumbles and collisions (SFSC) with the sides of the pen and with other ponies were summed and the new variable used as a measure of the stability of the ponies within the pens. The number of sniffs at the ground and the number of paws at the ground were each analysed separately. The behavioural measures were treated as continuous response variables but were natural log-transformed to meet the distributional

Table 2 The mean, minimum and maximum stocking density, space allowance and pen length for each of the group size/stocking density treatment combinations.

Category	SD	Group size								
		1			4			8		
		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
SD (m^2 per 100 kg)	H	0.81	0.61	1.35	0.42	0.29	0.72	0.37	0.32	0.44
	L	1.32	0.93	1.55	0.53	0.41	0.59	0.49	0.39	0.61
SA (m^2 per animal)	H	1.80	1.75	1.82	0.70	0.65	0.88	0.62	0.60	0.66
	L	3.84	2.45	4.03	1.05	1.01	1.19	0.83	0.76	0.87
Pen length (cm)	H	75	73	76	117	108	147	208	199	220
	L	160	102	168	175	168	198	278	255	291

Note that in this Table alone, to maintain consistency within the Min and Max columns, stocking density (SD) is reported as m^2 per 100 kg. Thus, the Max column relates to the maximum area per animal and the Min, the minimum area per animal. Elsewhere in this report SD is reported in units of kg per m^2. All reference to an increase in stocking density (or high SD) throughout the report refers to a decrease in space allowance and vice versa.

assumptions of the analyses. The blood variables plasma cortisol, CPK and lactate were also natural log-transformed to meet the distributional assumptions of the analyses.

Home Office licence

The project was carried out under licence from the Home Office as EU legislation had recently introduced a maximum limit of four unbroken ponies to be transported within a pen.

Results

Table 2, above, summarises the mean, minimum and maximum stocking density, space allowance and pen length occurring within the study for each of the six transport conditions investigated (group size one, four and eight each at high and low stocking density [H and L]). Note that in Table 2 only, stocking density is reported in m^2 per 100 kg so that both an increase in the magnitude of the numbers for stocking density and an increase in the magnitude of space allowance actually mean a decrease in stocking density (low stocking density, more room for the animals). Elsewhere, stocking density is reported in units of kg m^{-2} and references to an increase in stocking density (or high SD), throughout, refer to a decrease in space allowance (and vice versa). Given these numbers alone, it is difficult to envisage how the stocking densities/space allowances tested actually appear in terms of space allowance and crowding for the ponies. Figure 3 shows representative still images taken from each of the six treatment combinations of group size and stocking density. These convey immediately the difference in the amount of space and the difference between transporting ponies as a group or singly. Given the width of a standard lorry used for transport, a single pony intrinsically has a greater space allowance, however, it is constrained to align across the lorry. Even at the lower stocking density, whilst it is possible for the pony to turn, there is insufficient space to align with the direction of travel. Similarly, for groups of 4, at the high stocking density, it is not possible to align with the direction of travel. The images also demonstrate that the lower stocking densities for groups of 4 and 8 allow large clear spaces within the pen in which it is possible for small numbers of animals to manoeuvre if the remaining animals are bunched.

Figure 4 shows the weight profile of the animals in the study by sex and Figure 5 shows the age profile, as estimated by dental inspection, by sex. Although the study profile was of cull animals transported to slaughter, it broadly reflects the types of animal most likely to be transported. There were females from across the weight range, but their distribution had a slightly bimodal pattern reflecting the exchange and movement of younger females and the movement of older, generally cull females. The majority of males transported were young males, which are generally of lower value and less desirable. The few larger males were cull stallions.

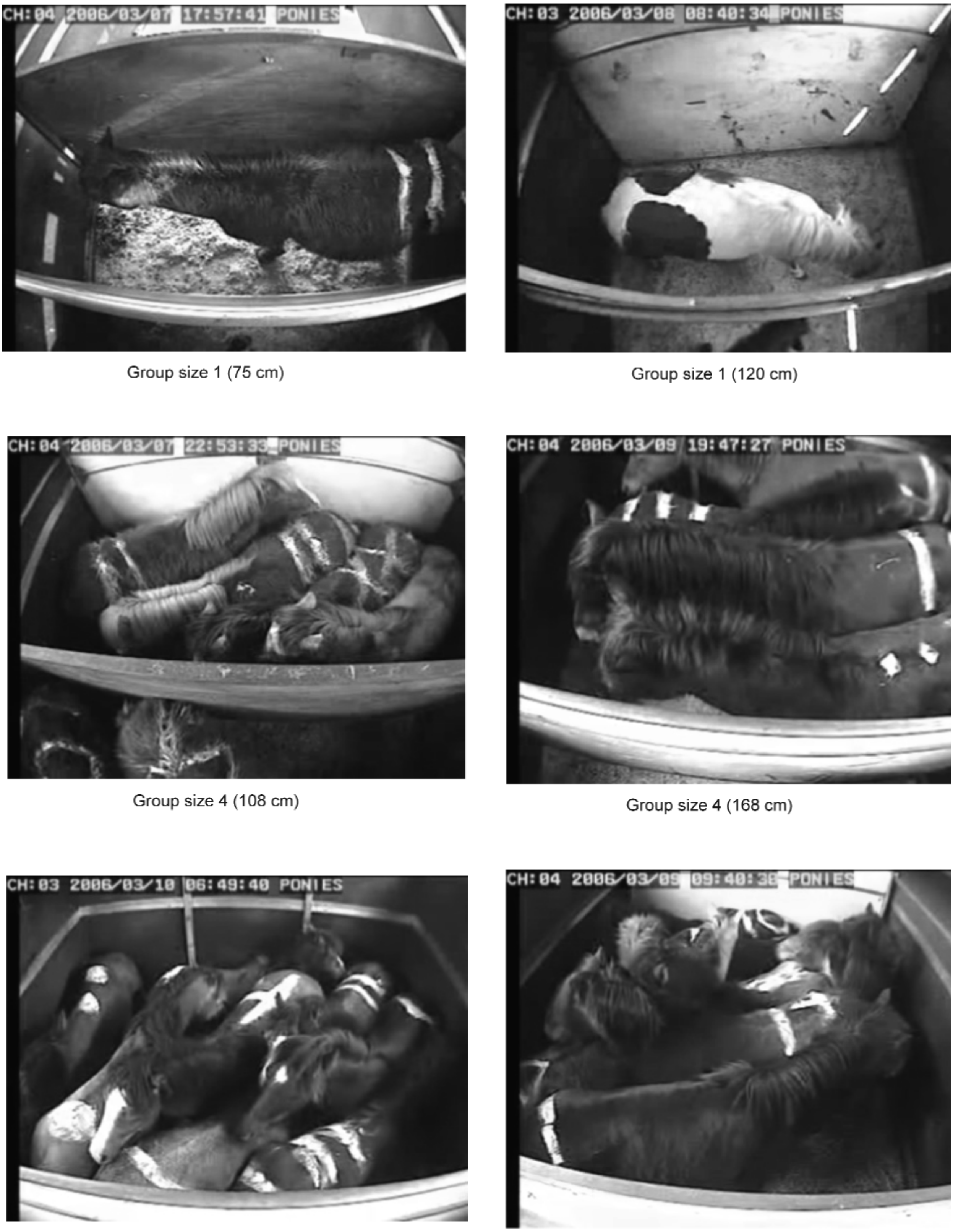
Whether ponies were transported in the front pen or the rear pen on the lorry was a design factor that was tested within all of the analyses. There was no detectable effect due to transport in the front or rear pen in any of the analyses ($P > 0.05$).

Orientation within the pens during transport

Figure 6 shows the percentage of time that individual ponies were facing in the different orientations during transport, broken down by the different group sizes, and the high and low stocking densities. The data are displayed as radar plots with the direction of travel of the lorry set at 0° and the percentage of time facing a given direction (to within 45°) shown along a radial axis. Figure 6 shows that single ponies at the high stocking density were essentially

Figure 3

High stocking density | Low stocking density



Group size 1 (75 cm) | Group size 1 (120 cm)

Group size 4 (108 cm) | Group size 4 (168 cm)

Group size 8 (199 cm) | Group size 8 (276 cm)

Examples of the three group sizes at high and low stocking densities within the lorry pen. The length of the pen is given in brackets. The width of the pens was 240 cm (the lorry width).

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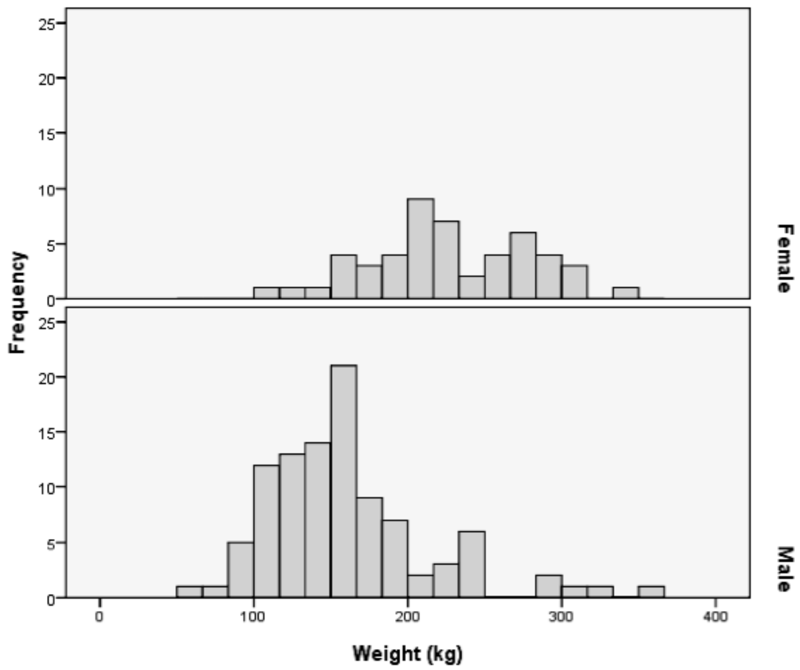
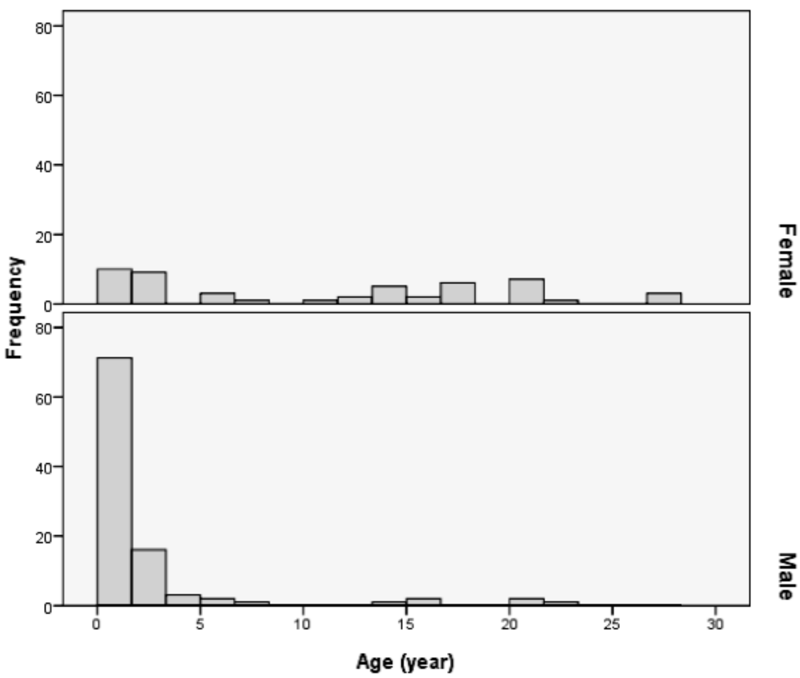
Figure 4



The weight distributions of the ponies, by sex.

Figure 5



The age distributions of the ponies, by sex (as estimated by dental inspection).

constrained to face at 90 or 270° to the direction of travel once penned. Not one pony in this treatment group turned within the pen and the majority of single ponies at the high stocking density were facing at 90° because this was the direction in which they entered the pen, with the pen partitions hinged on the right-hand side of the lorry. At the low stocking density, single ponies were able to turn within the pen and align themselves diagonally to the direction of travel, but not generally along the direction of travel, because of the short length of the pen. It could be seen in

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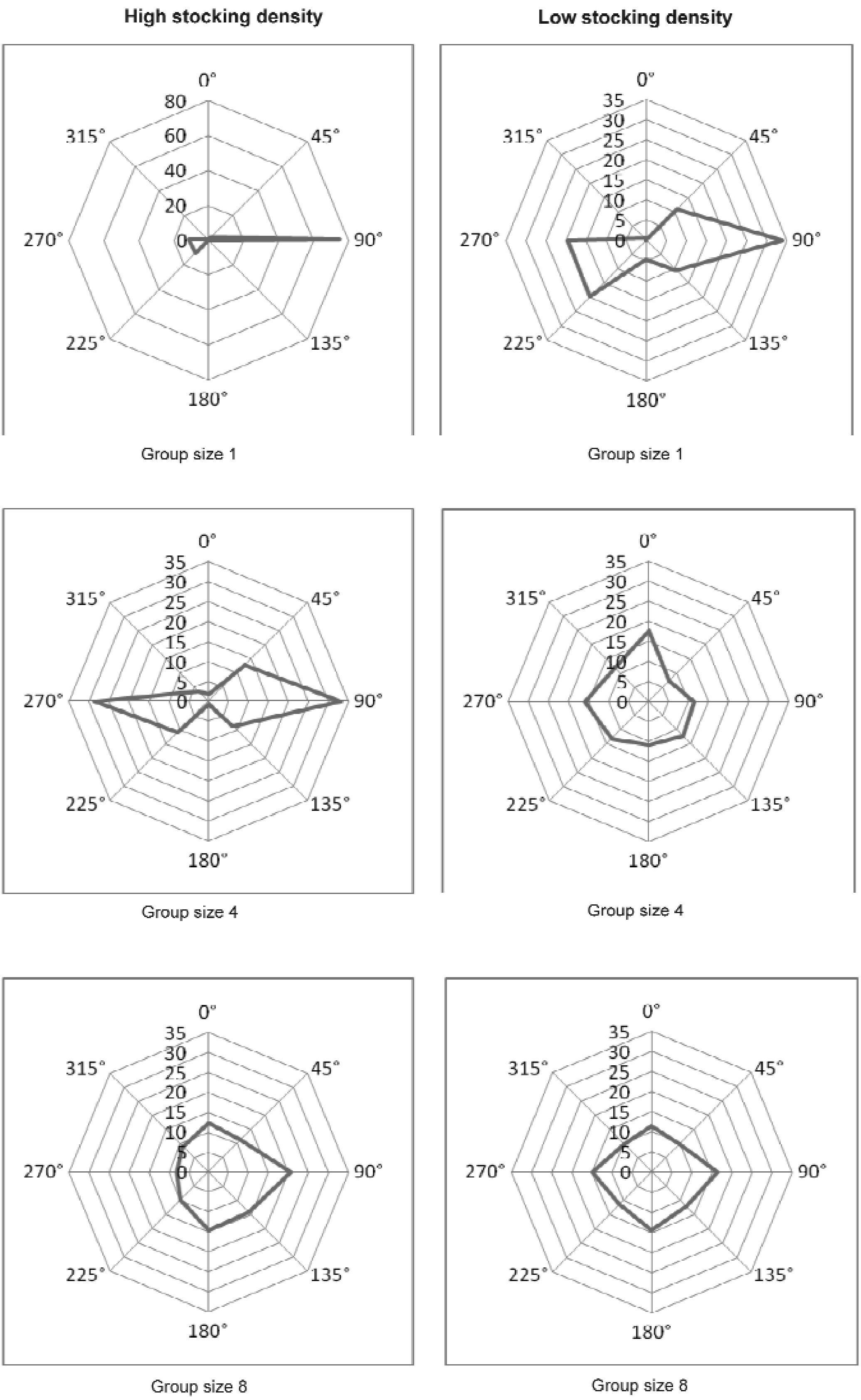
Figure 6



Radar graphs showing the mean percentage time spent in different orientations relative to the direction of travel by ponies in the three group sizes, at both high and low stocking densities. Zero degrees is the direction of travel. Note that the radial scale (percent of time) for single ponies at the high stocking density is different from that of the other graphs.

Figure 7

A single pony at low stocking density (L) bracing against a forward corner of the pen during transport.

the video recording that single ponies tended to brace themselves by placing their rear against a corner of the pen. When doing this there was a tendency to prefer a forward corner of the pen and this is reflected in the radar plots for the single ponies which show a greater area below the 270–90° line than above. Figure 7, above, shows a single pony braced against a forward corner of the pen during transport.

In ponies that were recorded as at some time facing directly backwards or forwards, a test of the time spent facing forwards (315, 0 or 45°) against that spent facing backwards (135, 180 or 225°) perhaps showed a trend towards a preference for facing backwards, but this was not significant ($P = 0.13$). Where ponies could turn easily through 360°, in groups 8L, 8H and 4L, there was no preference for aligning along the direction of travel or transversely across the direction of travel, but there was a highly-significant ($P = 0.001$) propensity for alignment with the pen walls (0, 90, 180 and 270°) as opposed to aligning diagonally across a pen (45, 135, 225 and 315°). This is also apparent in the radar plots in Figure 6.

Behaviour during transport — pawing and sniffing

The mean frequency of pawing at the ground was high in individually-penned ponies (5.3 bouts pre-journey) compared with ponies transported in groups (0.04 or 0.02 bouts per journey, pens of four and eight, respectively; $P < 0.001$). Sniffing at the ground was also more frequent in individually-penned ponies ($P < 0.001$) with a mean frequency of 13.7, 2.1 and 1.2 per pony per journey in the pens of one, four and eight, respectively. There was no detectable effect of stocking density category on the amount of pawing or sniffing at the ground. The younger or lighter a pony the greater was the amount of sniffing ($P = 0.070$, $P = 0.004$, respectively) and the younger a pony the greater was the amount of pawing at the ground ($P = 0.034$), however, there was no significant relationship between pawing and weight ($P = 0.310$). In the statistical analyses, either age or weight was fitted within a model, not both at the same time.

Behaviour during transport — slips, falls, stumbles and collisions

The mean, minimum and maximum number of slips, falls, stumbles and collisions (SFSC) per pony per journey by stocking density and group size are shown in Table 3. A multilevel model of the natural log of the number of SFSC showed a greater number of SFSC in groups of eight than in singles and groups of four ($P < 0.001$) but no effect of stocking density category. A model of only the ponies in groups of four and eight, showed a similarly higher level of SFSC in groups of eight, above groups of four, but also showed a significantly-increased number of SFSC with increasing stocking density, when the actual calculated value of the stocking density for a pen was used within the model rather than just the category of stocking density. For every 1 kg m^{-2} increase in stocking density within the groups of four or eight there was an additional 0.0037 increase in the natural log of SFSC (Ln[SFSC]). The use of the model based on the reduced number of animals (those in groups) is justified as it allowed a more sensitive measure of stocking density to be used. The singly-penned animals could not be satisfactorily included in the model due to the lack of overlap in the stocking densities seen in the single pens compared with the grouped pens (Table 2). The data in Table 3 seem to suggest that the single ponies at the lower stocking density may have suffered a greater number of SFSC, however, this was not statistically significant when modelled either as the stocking density category ($P = 0.56$) or as the actual pen stocking density ($P = 0.57$) for single ponies, alone. During one transport journey one pony did fall completely to the ground and this pony was then unable to get up. This was in a pen of eight transported at a high stocking density.

Behaviour during transport — aggression

The statistical analysis used only those animals transported in groups where aggression was a possible occurrence. There was a significant effect of both group size ($P < 0.001$) and stocking density category ($P = 0.010$) on the number of aggressive behaviours (bites, kicks, snaps, ears back) per pony per journey. The predicted number of these behaviours (from the statistical model) during the journey was 22 and 28 within a group of four at low and high stocking density, respectively, and 36 and 48 within a group of eight at low and high stocking density, respectively. The weight and sex of an animal were also found to be significant within the statistical model, with a significant weight by sex interaction ($P = 0.016$). The number of overtly aggressive behaviours increased with the weight of an animal, however, below approximately 200 kg, females on average showed more physical aggression than males. Above 200 kg liveweight, males tended to be more aggressive than females.

Table 3 The mean, minimum and maximum number of cumulative slips, falls, stumbles and collisions (SFSC) per pony per journey by group size and stocking density category.

	Group size								
	1			4			8		
SD	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
H	3.8	1.0	8.0	3.8	0.0	19.0	6.3	0.0	33.0
L	7.3	0.0	22.0	1.8	0.0	8.0	3.3	0.0	19.0

Table 4 The mean, minimum and maximum levels of post-transport plasma cortisol, CPK and lactate within each group size (unadjusted values [calculated from the raw data]).

	Group size								
	1			4			8		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Cortisol (ng ml^{-1})	137.1	38.5	469.3	108.4	26.9	472.2	138.7	33.4	563.8
CPK (U L^{-1})	43.3	12.4	86.7	62.9	8.3	288.9	88.8	12.4	567.5
Lactate (mg per 100 ml)	34.9	15.8	61.8	40.4	13.3	91.2	41.1	9.0	78.7

Plasma cortisol — post transport

The data for singly-penned ponies were analysed separately from that of the groups of four and eight as, together with a lack of overlap in the stocking density, different stressors appeared to be operating for ponies penned singly. Overall levels of cortisol were similar in range in singly-penned animals compared with levels seen in the groups of four and eight (Table 4). An initial analysis of the data for all the pens combined showed that the levels of cortisol measured varied with the ambient temperature on the day, the live weight of the pony and the delay between the end of transport and slaughter. An increase of 1°C was associated with an increase of 0.03 natural log units of plasma cortisol ($P = 0.007$) across the range 2 to 24°C. An increase of 1 unit of liveweight measured as the natural log of weight in kilograms was associated with an increase of 0.38 natural log units of plasma cortisol ($P = 0.009$) across the range 63 to 361 kg. An increase in the delay between the end of transport and slaughter of 1 unit of time, measured as the natural log of time in minutes, was associated with a 0.30 decrease in natural log units of plasma cortisol ($P < 0.001$) across the range 5 to 115 minutes. Ambient temperature, liveweight and delay were used as covariates in the separate models for the single animals and a model for the two groups. Within the pens of single animals the more an animal pawed at the ground the higher were its levels of plasma cortisol ($P = 0.030$). An analysis of just the group pens showed overall levels of plasma cortisol to be higher within the groups of eight than within the groups of four ($P = 0.025$). Within both groups of four and eight, with every 1 kg m^{-2} increase in stocking density across the range 139 to 316 kg m^{-2} there was an associated 0.002 rise in the levels of plasma cortisol measured as the natural log of cortisol in ng ml^{-1} ($P = 0.036$).

Plasma creatine phosphokinase (CPK) — post transport

Levels of plasma CPK were higher in groups of eight ponies than in single ponies or groups of four ($P = 0.008$) (Table 4). For the reasons given above, the data for single ponies were then analysed separately from that of the grouped ponies. There was no measurable effect of stocking density on the levels of plasma CPK in the single ponies ($P = 0.42$). In the single ponies after transport, those that were recorded as fractious during handling the day before transport had levels of plasma CPK 1.91 U L^{-1} higher than those recorded as quiet ($P < 0.001$). In groups of both four and eight ponies an increase in stocking density of 1 kg m^{-2} was associated with a 0.003 rise in the levels of plasma CPK measured as the natural log of CPK in U L^{-1} ($P = 0.011$). There was a suggestion that levels of CPK activity were higher in the groups of eight than groups of four but this difference did not reach statistical significance ($P = 0.074$).

Packed cell volume (PCV) — post transport

An initial analysis of the data for all the pens combined showed that the PCV measured varied with the live weight of the pony and the delay between the end of transport and slaughter. An increase of 1 unit of liveweight measured as the natural log of weight in kilograms was associated with an increase of 4.6% PCV ($P < 0.001$) across the range 63 to 361 kg. An increase in the delay between the end of transport and slaughter of 1 min, was associated with a 0.07 increase in percentage PCV ($P < 0.001$) across the range 5 to 115 min. Liveweight and delay were used as covariates in the models that were tested of PCV. The post-transport PCV of singly-penned ponies was higher than that of those in groups of four and groups of eight of single sex, with predicted values of 36.1, 30.3 and 28.9%, respectively ($P = 0.021$). However, if the group of four or eight was of

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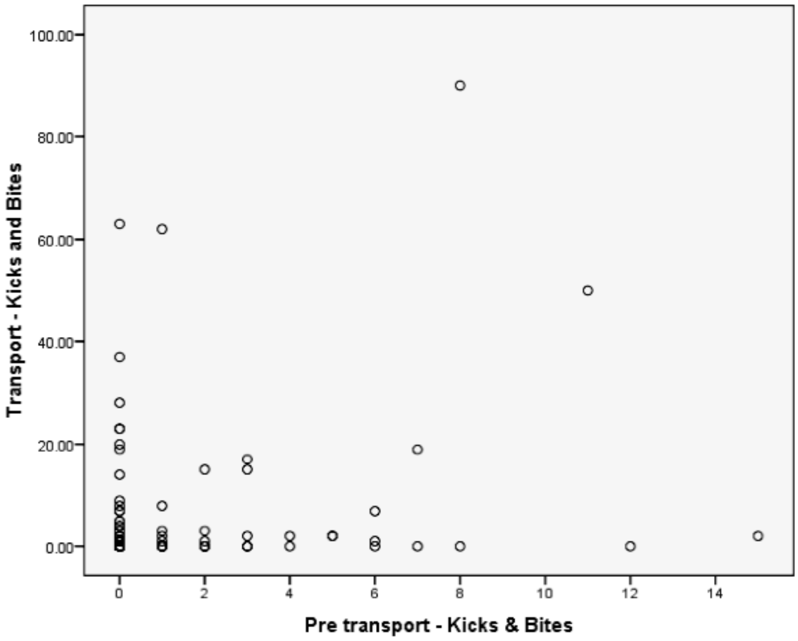
Figure 8



The total number of kicks and bites recorded for ponies during the one-hour observation period before transport plotted against the total number of kicks and bites recorded during transport.

mixed sex, the mean level of PCV in these groups was increased by 8.4% (P = 0.040). There was no detectable effect of stocking density on PCV either when the data were analysed as a whole or by single and grouped animals. An analysis of PCV using only data from single ponies showed that the younger a pony the higher was its PCV ($P < 0.001$), this was as adjusted for weight and delay.

Post-transport lactate, blood temperature and surface temperature

No relationship was identified between any of post-transport plasma lactate, blood temperature or body surface temperature and the transport conditions. Nor was any relationship identified with any of the possible covariates measured.

Identifying aggressive ponies prior to transport

The approach taken to developing a methodology for predicting ponies which would be aggressive during transport was to treat any method as a diagnostic test. Thus, ideally, a test would have a high sensitivity (it would correctly identify nearly all of those ponies that would be aggressive during transport) and it would have a high specificity (it would not misclassify many unaggressive ponies as aggressive). Further, a test could still be considered useful if it had a poor sensitivity but a high specificity, that is, if it were only able to identify a few of the aggressive ponies but it did not incorrectly identify non-aggressive ponies as aggressive. Pre-transport, aggressive behaviour was recorded as the total number of bites and kicks made towards another animal. Aggressive behaviour during transport was recorded as bite, bite attempt, kick, kick attempt, ears back and snap. Only ponies transported within the groups of four or eight could be used in the analysis (n = 132) as single ponies were unable to display any potential aggression towards other animals during transport.

The majority of animals displayed at least some combination of aggressive behaviours during transport. For example, if the criterion were that a pony was classified as aggressive during transport if it displayed any of bite, kick, kick attempt or snap, then 80% of animals were classified as aggressive. To refine this to identify only the most aggressive animals, only the potentially injurious behaviours, kick and bite, were used as a categorisation. This still categorised 32.5% of ponies as aggressive. Plots were inspected of pre-transport behaviours against aggressive behaviour during transport. These showed that there were no strong relationships between the degree of aggressive behaviour during transport and any of the pre-transport behaviours recorded. For example, a plot of the total number of aggressive kicks and bites displayed before transport against the number shown during transport is given in Figure 8. It is apparent that a complete prediction of aggressive behaviour using these criteria would not be possible. Many animals displaying high levels of aggression during transport displayed little pre-transport aggression and vice versa.

Different combinations of predictors and measures of aggression during transport were formally evaluated. For example, defining aggressive animals during transport as those displaying greater than five bites or kicks during transport (16% of animals) and attempting to predict them using different cut-offs of the amount of pre-transport aggressive behaviour. These verified what can be seen from Figure 8, that aggressive ponies during transport could not be satisfactorily identified from the pre-transport observations.

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Discussion

Overall levels of cortisol and, marginally, also CPK, were lower in groups of four than eight and levels of aggression were lower in groups of four, as were the number of slips, falls, stumbles and collisions (SFSC). Within groups of four and groups of eight a reduction in stocking density was associated with lower levels of plasma cortisol, lower levels of plasma CPK, reduced aggression and a reduction in SFSCs.

Evaluating the welfare consequences of individual transport compared with transport in groups of four is less straightforward. Given the wide variability in measurements between individuals within the treatment groups, measures of cortisol and CPK were not significantly different, neither were the numbers of SFSCs. Stocking densities between the two types of transport were not comparable as single ponies intrinsically had a much lower stocking density, given the whole width of the lorry. They were, however, constrained to align across the direction of travel unlike groups of four at the lower stocking densities. The groups of four at the lower stocking densities showed no preference for alignment with or across the direction of travel. There was aggression within the groups of four and none for an individually-penned pony. Individually-penned ponies showed very high levels of pawing and sniffing at the ground near the partition separating them from the other ponies and raised PCV, most probably indicative of a degree of separation distress. Visser *et al* (2008) have described pawing and snorting as stress-related behaviour in young horses stabled for the first time. Levels of sniffing, pawing and PCV were all higher amongst the younger or lighter animals. We interpret these results to show that preferably, unbroken ponies should be transported in small groups, especially when the animals are young. Transporting young, unbroken ponies isolated in a single pen is especially undesirable.

Only groups of size one, four and eight were investigated in this study. It is interesting to speculate on the suitability of other sizes of group. It would seem likely that the intermediate group sizes between four and eight, and probably greater than eight to some limit, would simply result in a proportional change in the responses seen between groups of four to eight. The relative suitability of transport in groups of two or three is more difficult to predict. It is possible that different underlying behavioural mechanisms would come into play with groups of only two or three, creating better or worse conditions. A separate study is probably required to properly answer this question.

In January 2007 legislation was changed to limit to four the number of 'unbroken' equidae transported within a pen. The change in legislation was primarily introduced to curb the cruelty reported in the trade of horses into the EU from Eastern Europe. The results reported here show that the new legislation should result in an improvement in the welfare of untamed ponies. That is, given the proviso that welfare in groups of two or three is equivalent to, or better than that, within groups of four, as the change in legislation will likely lead to an increase in ponies transported in groups of these sizes.

We should comment on the difference in the 'open-field' behaviour of untamed ponies to those of equidae accustomed to handling, and accustomed to living not as a social group. The grouped behaviour of the unbroken ponies was very similar to that of sheep (Hutson 2007). When herded they moved closely as a group and were thus easy to drive, direct, load and unload. Separating them into their transport groups for the study was sometimes difficult, especially as individual animals had to be formally randomised to one treatment group. The younger animals were particularly difficult to separate from the older animals as their motivation to remain with an older animal was strong. Although relatively calm when in a large pen with room to move away from a handler, ponies preferred to keep a distance of several metres between themselves and a handler. When closely confined, for example, whilst in the race when being marked, respiration rate was elevated and individual animals could be extremely fractious, often kicking out or rearing (authors' personal observations).

The behaviour of groups of untamed ponies transported by road contrasts with that anecdotally reported in tame horses transported in groups by road. Commercial hauliers reported that they transported tame horses in individual pens as experience had shown that very serious aggression and considerable injury could be common when these types of animal, often unaccustomed to the close proximity of conspecifics, were transported in groups.

European legislation appears to be drafted primarily for tame animals transported in individual pens. When groups of animals are transported, as group size increases at a set stocking density, more apparent free space is potentially available for an individual within the group. For ponies (under 144 cm) transported by road, EU legislation specifies a minimum of 1 m^2 per animal which may be reduced by up to 20% under favourable conditions. Additionally, it specifies the minimum dimensions for an individual pen. This is done only for equidae and not for any of the other species, for which tables of space allowances are presented. A pony of 144-cm height weighs approximately 350 kg, well above the weight of the majority of the ponies in this study (Figure 4). The increase in apparent space with group size is demonstrated in the space allowances seen within this study as, when using the working approach to loading animals that was adopted for the protocol, the area per animal was always less within groups of eight than within groups of four (Table 2). However, there will be an upper limit on the amount of free space that is desirable (Knowles 1999), as during emergency braking or other rapid changes in velocity an animal should not have too far to move before it encounters a physical object as it could fall or collide with the pen wall or another animal at an injurious speed.

As this study demonstrated, untamed ponies do require sufficient room in which to be able to balance and adjust their posture or they are prone to slips, falls stumbles and collisions given too little area (Collins *et al* 1999; Iacono *et al* 2007). In this study we were only able to investigate an acceptable limit for minimum space allowance (maximum stocking density). The space allowance within groups of four and eight in the study ranged from less than that in the legislation, to overlap with the minimum space allowance within the legislation. In the statistical analyses, the relationship of both stocking density and space allowance were investigated in relation to the outcome measures. Stocking density always showed a much stronger relationship with the outcome measures than space allowance. So, although space allowance is a much easier measure to work with for both the legislators and the hauliers, if the animals are not of a uniform weight and size, as is the case when unbroken ponies are transported, space allowance may be an inappropriate measure. The correlation between stocking density and space allowance in the 23 pens of grouped animals was poor at $r = -0.396$ and was marginally not statistically significant ($P = 0.062$).

The reason for the poor agreement between stocking density and space allowance was the variability in the weights of animals transported in a group. The majority of other types of commercial livestock transported in groups are of a more uniform weight and for these animals a space allowance is a reasonable method of specification. A characteristic of untamed, semi-feral ponies is that they are generally taken from free-living social groups containing animals at all stages of development and that a relatively uniform weight distribution within a transport group is unlikely. This is compounded by the desire of the younger animals to remain with older animals.

Taken together, these results suggest that for groups of untamed ponies, legislation to protect their welfare needs to be drafted in terms of stocking density rather than as a space allowance, unless the ponies are to be transported in groups of relatively uniform weights. Given that most of the measures of welfare improved as stocking density was decreased, across the range used in the study, the results suggest that the maximum stocking density should be set toward these lower stocking densities. For the groups of four this would suggest a maximum of approximately 200 kg m^{-2}, given the range seen within the study of 140 to 340 kg m^{-2}. For the reasons given above, there should also be a lower limit to the stocking density that should be specified. This limit cannot be readily discerned from this study. Thus, the suggested limit of 200 kg m^{-2} reflects a compromise between the improvements seen in welfare (in terms of reduced aggression, lower cortisol and decreased CPK activity) across the stocking densities investigated and our lack of knowledge of the cost/benefit of further lowering stocking density.

A minority of ponies displayed high levels of aggression during transport. Although this did not result in any obvious physical injury when the ponies were unloaded after these short journeys, it would be desirable to identify and remove potentially aggressive ponies before loading. We found that pre-transport behaviour could not be used to identify ponies that were later aggressive during transport. It can be seen in Figure 8, from the different scale used for each axis, that levels of aggression, when it occurred, were generally higher during transport when animals were penned in close proximity and unable to readily move away. Aggression can be defensive as well as offensive (Blanchard *et al* 2003). Much of the aggression seen pre-transport was defensive and ponies also had the opportunity to move away from aggressors. During transport there was less scope for escape and thus aggressive interactions were increased in number, both defensive and offensive. The study did show that during transport a decrease in stocking density was associated with a general decrease in aggressive behaviour between animals, most probably because it allowed more distance between animals and would have given victims of aggression greater room to manoeuvre for escape.

The spacious pen used for pre-transport observations did not elicit behaviour which could be used to predict behaviour during transport. Had more restricted conditions been used it is possible that the behaviour would have been more akin to that occurring during transport and thus a better predictor. However, this set up would not have replicated the jostling and interactions caused by the motion of the lorry. Further, there would be some cost, in terms of additional stress, had this approach been adopted.

Some of the journeys were carried out during the spring months, a time that the unbroken ponies would be breeding. Their behaviour was markedly different during these weeks than at other times of year, with far greater interaction amongst animals of different sexes and of different sizes, and higher levels of aggressive behaviour. The study sample size was too small to be able to provide any directly useful information but it does suggest that an investigation into the possible need for a different set of special guidelines for mixing and transport during this time could be required.

There was marginal evidence that ponies within a group did prefer to face away from the direction of travel, when they had sufficient room to turn. Additionally, single ponies tended to brace themselves by wedging their rump into a corner of the pen (Figure 7) and there was a preference for choosing a corner at the front rather than the rear of the lorry. The deceleration of a lorry is likely to be more severe than acceleration and facing away from the direction of travel means that the ponies would be able to brace themselves using their rump rather than their head or chest.

Animal welfare implications

The evidence from this study indicates that, at least for short journeys, unbroken ponies should not be transported singly, that group sizes of four are preferable to larger groups of eight and welfare is improved by lower stocking density. Whereas a maximum stocking density of 200 kg m^{-2} is suggested, it is not known whether this is suitable for longer journeys, nor what the minimum density should be.

Conclusion

For the journeys of approximately one hour duration studied here, transport of unbroken ponies in group sizes of four appears to be preferable to transport in group sizes of eight. We infer that transport in groups of four is preferable to transport in groups of greater than four. Some ponies appear distressed when penned alone, especially the younger animals. Unbroken ponies have a strong herding instinct and individual animals can be difficult to separate from a group. For these reasons we suggest that, where possible, unbroken ponies should be transported in small groups rather than individually penned.

It did not appear possible to predict which ponies would be aggressive during transport from observations of the group's behaviour in a large pen prior to transport.

Within the ponies transported as groups there was a general trend for an improvement in the response to transport as stocking density was decreased. We suggest that, given the range of stocking densities investigated here, a suitable maximum stocking density for ponies transported in groups would be approximately 200 kg m^{-2}. Space allowance showed little correlation with the response variables measured in the study and poor correlation with stocking density. Thus, unless the animals within a group are of a uniform weight, space allowance is not a reasonable way to define transport conditions for the purposes of legislation.

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