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Long distance cattle transport: impact of variations of space allowance and/or group size on physiological and behavioural indicators of stress.

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Abstract
In the current context of interrogations and concern on transport conditions of living animals, three experimental 29h transports were realized to test the impacts of 2 variations of space allowance and group size on welfare of cattle. 26 young cattle (mean weight 396 Kg) were transported in each transport in 3 different compartments: SD (standard density, 1,14m^2/animal, 9 cattle), LSD (20% space allowance, 1,37m^2/animal, 7 cattle) and HSD (10% space allowance, 1,03m^2/animal, 10 cattle). Significant differences were observed in the behaviour of cattle in the LSD compartment compared to SD compartment: 10% less time spent standing, 30% more changes in position, and 2 to 3 times more losses of balances and free moves. No difference related to space allowance/group size was observed in biochemistry, in the amount of hay eaten, nor in the mean weight loss. These first results are equivocal to interpret, as they can describe a positive effect of the lower density, or a negative effect on cattle welfare because of the higher agitation of cattle in the low density compartment.
Keywords: welfare, space allowance, behaviour, density, transport

Résumé
Dans le contexte de la réflexion en cours sur les conditions de transport des animaux vivants, trois transports expérimentaux de 29h ont été réalisés pour tester les impacts de 2 variations de la densité de chargement et de l'effectif du groupe sur le bien-être des animaux. 26 broutards d'un poids moyen 396Kg ont été transportés à chaque transport dans 3 compartiments : SD (densité règlementaire, 1,14m^2/animal, 9 bovins), LSD (esapce disponible +20%, 1,37m^2/animal, 7 bovins) et HSD (espace disponible -10% , 1,03m^2/animal, 10 bovins). Des différences significatives du comportement des animaux ont été observées dans le compartiment LSD par rapport au compartiment SD : 10% de temps en moins passé debout, 30% de changements de position en plus, et 2 à 3 fois plus de pertes d'équilibre et de déplacements. Aucune différence liée à l'espace par animal n'a été observée dans les paramètres biochimiques dosés, dans la consommation de foin et dans les pertes de poids. L'interprétation de ces résultats est équivoque : elle peut indiquer des effets positifs de la diminution de la densité, mais également un risque pour le bien-être des animaux en raison de leur plus grande agitation dans le compartiment à faible densité.
Mots clés : bien-être animal, comportement, densité, espace disponible, transport

Introduction
The effects of transport on animal welfare have been studied from more than 20 years (review in Warris, 1998; Swanson et Morrow-Tesch, 2001) and several factors of stress have been identified (review in Grandin, 1997). The available space is determining for animal welfare during transport (Hall and Bradshaw, 1998), but few experimental studies were realized on the effects of stocking density (Earley and Riordan, 2006). Published results were also sometimes contradictory or difficult to interpret because of the high inter-individual variability and because of genetics influence (Grandin, 1997). In 2002, the SCAHAW report

recommended to increase of about 50% the available space for long distance transport with resting time. This increase in surface was more particularly proposed to allow the cattle to move more, and to better feed and drink during the resting time.
In the European Community, animal transport is regulated by the EC 1/2005 European regulation which controls means to organize and realize transport of all living animals. Space allowance is part of the regulation and the European Community has begun to consult about a decrease of stocking densities in long distance transports.

This experimental study is part of the current interrogations about possible evolution in the stocking density. It aimed to assess the impact of variations of space allowance, more particularly a 20% increase, on behavioural, and physiological indicators of stress during long distance transport of young cattle.

1. Materials and methods

This study was realized during regular commercial transports of young cattle between France and Italy.

1.1. The truck and special equipments

The half-trailer two-decks truck was divided in 3 compartments. Only one deck was loaded with cattle to allow for camera installation. Each compartment was 10.6 m^2 wide and provided with 2 to 3 retractable drinkers. For each ride, the truck floor was spread with litter (straw for the 1st transport, wood sawdust for the 2nd and the 3rd transports). The truck got all compulsory equipments for long distance transport.
Each of the 3 compartments was specially equipped with an infra-red video camera, and a video screen was installed in the driver cabin to allow direct observations of cattle behaviour alternatively in the three compartments.

1.2. The journey

Three experimental transports were realized in May and June 2009. Each transport was organized from France to Italy on a standardized journey from Lentilly, France (commercial farm) to the control center of Cadeo (Piacenza, Italy). Most of the journey was realized on highways to minimize any transport events. The transport duration was set to the maximum allowed, 29 hours, as following :

- **14 hours** of transport from Lentilly to the South of Italy then back North to Cadeo,
- **1 hour resting time** at Cadeo to water and feed cattle without unloading them,
- **14 hours** of transport from Cadeo to the South of Italy and back North to Cadeo. Cattle were finally unloaded at Cadeo at the end of the 29h of transport.

During the 1 hour resting time, cattle were watered *ad libitum*. They were fed with hay put in horse filets (10 kg/compartment). Water and food intake in each compartment was measured.
In addition to the resting time for animals, several breaks were realized by drivers to respect the work regulation for transportation.

1.3. Animals and the 3 stocking densities

Each experimental transport was realized with 26 young cattle representative of the main activity of export of cattle between France and Italy (Table 1). In each transport, 3 available space/animal were tested, adjusted by the number of animal in the 10.6 m^2 compartment:

– **Standard density** (SD) which corresponds to a 1.14 m^2 space allowance by cattle, that is 9 cattle in the compartment.

– **20% higher space allowance** (LSD) which corresponds to a 1,37 m^2 space allowance by cattle, that is 7 cattle in the compartment.

– **10% lower space allowance** (HSD) which corresponds to a 1.03 m^2 space allowance by cattle, that is 10 cattle in the compartment.

	Compartments			Description of cattle
	front	middle	back	
Transport 1 May 5/6	SD	HSD	LSD	Female Crossed breed : Limousine/Charolais Mean weight = 393 kg
Transport 2 May 26/27	LSD	SD	HSD	Male Charolais Mean weight = 395 kg
Transport 3 June 23/24	HSD	LSD	SD	Male Charolais Mean weight = 400 kg

***Table 1. Description of cattle transported during the study**. For commercial reasons, we could not get Charolais male cattle during the 1st transport. Each cattle was randomly allocated to a compartment.*

During these experimental transports, we actually tested the confounded effects of both the space allowance and the group size in each compartment.

1.4. Behavioural observations

Two observers took turns during the 29 hours to observe cattle behaviour from the video screen in the cabin.

An ethogram was first realized to define relevant behaviours to observe during transport. Then, observations were made each hour using two procedures:

- 30 minutes of instantaneous scan sampling (ISS) where several behaviours and positions were numbered every 5 minutes in each compartment,
- 5 minutes of *ad libitum* continuous observations of all behaviours in each compartment.

Only part of ISS observations will be presented here.

For this presentation, the posture (standing/lying), the position (perpendicular, parallel, diagonal) and free and restricted (pushing congener) moves were analyzed. For each transport, the time-budgets of each behaviour were calculated as well as the frequency of changes in position in between 2 ISS.

1.5. Physiological and technical indicators

Blood samples were collected before the loading and after the unloading to measure the variation of selected biochemical indicators of muscular fatigue (Aspartate Amino Transferase ASAT and Creatine Kinase CK), of dehydration (total protein blood content, Na, Cl and K), of mobilization of carbohydrate reserves (fructosamine, glucose), of a dysfunction of hepatic system (urea and Alanine Amino Transferase ALAT) and a stress acute phase protein (haptoglobin).

All samples were stocked in heparin tube or on glucose inhibitor substrate, below 4℃ and analyzed within 24 hours after the sampling (National Veterinary School, Lyon, France). For each transport, mean variation after/before transport by compartment was calculated for each indicator.

Cattle were individually weighed before loading and after unloading. The mean loss of weight by compartment was calculated for each transport.

1.6. Statistical analysis

All data (time-budgets, losses of weight, hay consumption, and variation of biochemical indicators) have been compared using Mixed procedure, with Transport as a random effect, and “Density” as a fixed effect. A Dunnet test ($p<0.01$) was then realized to compare observations in LSD and HSD to observations in SD.

2. Results

2.1. Posture and position: a similar trend in time

Time-budget analysis shows that cattle have spent most of the journey standing (figure 1). During Transport 1, cattle stood almost 100% of the time before the resting time, and lying cattle were mostly observed after the resting time, preferentially with spread legs. In both other transports, changes in posture were observed before and after the resting time, with an increase of lying cattle after 5 to 6 hours. In these 2 transports, cattle laid down preferentially in a decubitus position. In all cases, cattle stood up as soon as the truck stopped and eventually laid down when it moved again. In all transports, after a few hours, standard deviation were large.

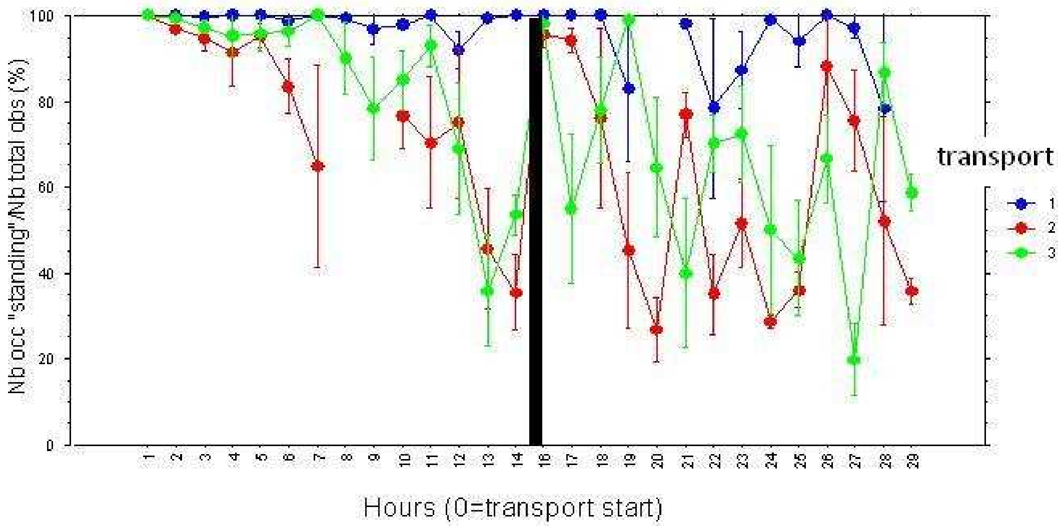

Figure 1: Time spent standing during the 3 transports*. Grey bar shows the 1 hour resting time to feed and water cattle.*

Position time-budgets had the same time trend as posture: standing cattle travelled in a perpendicular position, but variations were observed after the resting time (figure 2). Transport 1 differed from both others with a higher occurrence of perpendicular position, even after the resting time (more than 70% of standing cattle in perpendicular position). In the 3 transports, standard deviations increased, particularly after the resting time.

A positive correlation was observed between standing posture and perpendicular position (R^2=0.60) and a negative correlation was observed between standing posture and parallel position (R^2= -0.62).

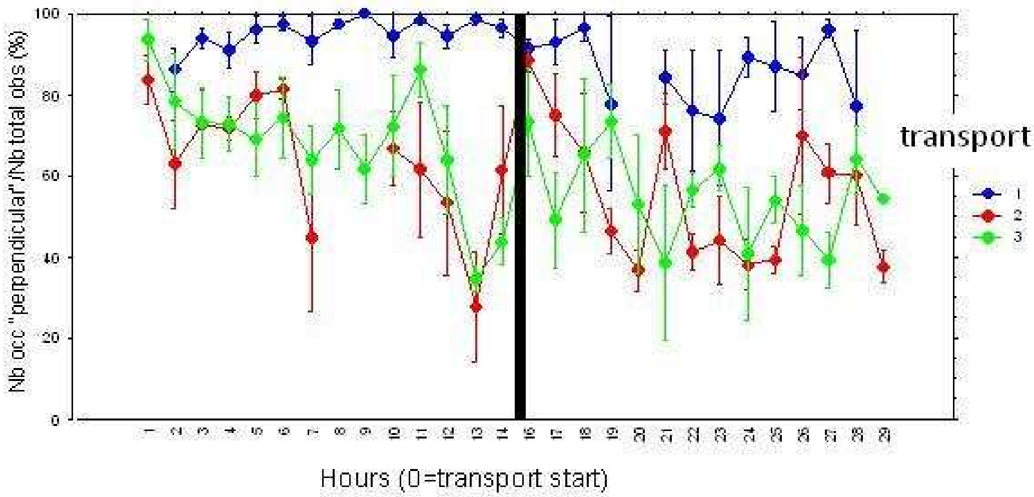

***Figure 2: Time spent in perpendicular position during the 3 transports**. Grey bar shows the 1 hour resting time to feed and water cattle.*

2.2. More losses of balance after the resting time

Like for position and posture, losses of balance showed a trend in time: an increase of the number of losses of balance was observed after a few hours, but strong variations were observed in time and between transports (figure 3). A higher proportion of losses of balance was recorded during the 3 first hours of Transport 1, but in both other transports, most losses of balance were observed after the 10^{th} hour and after the resting time.

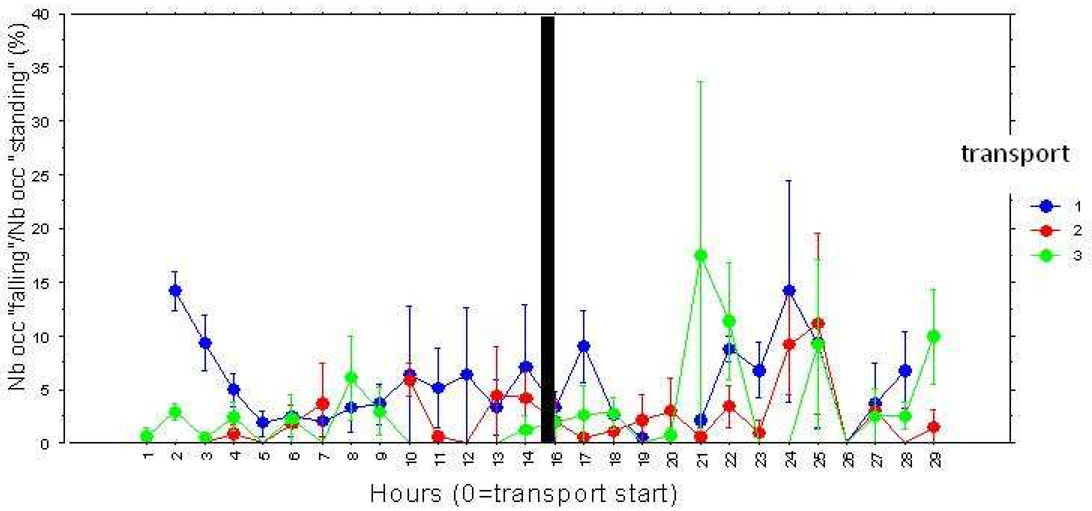

***Figure 3: Losses of balance during the 3 transports**. Grey bar shows the 1 hour resting time to feed and water cattle.*

In all cases, the proportion of losses of balance reached almost 15% of the standing time.

2.2. More losses of balance after the resting time

In the LSD compartment, cattle spent significantly less time standing, before and after the resting time, than cattle in the SD compartment. They also spent less time in the perpendicular position, but no significant difference was observed in the parallel position, which may indicate frequent changes of position in this compartment. Actually, before the resting time, the frequency of changes of position between 2 successive 5 minutes scans was 30% higher in the LSD compartment. After the resting time, no significant difference was observed compared to SD compartment, which may be related to the higher number of lying cattle in the LSD compartment.

The proportion of losses of balance was up to 3 times higher in the LSD compartment compared to cattle in SD compartment, before and after the resting time.
Finally, moves differed in SLD compartment with a significant increase of free moves (twice as much as in SD compartment) but no difference in restricted moves.

No significant difference was observed in any cattle behaviour between HSD compartment and SD compartment.

	PRE pause	HSD/SD	LSD/SD	POST pause	HSD/SD	LSD/SD
Standing posture	<0,10*	=	- 11%	<0,09*	=	- 15%
Perpendicular position	<0,09*	=	- 16%	<0,15*	=	- 22%
Parallel position	NS	=	=	NS	=	=
Changes of position	<0,03*	=	+ 36%	NS	=	=
Loss of balance of standing animals	<0,04*	=	+155%	<0,06*	=	+189%
Free moves of standing animals	<0,09*	=	+166%	<0,02*	=	+100%
Restricted moves of standing animals	NS	=	=	NS	=	=

Table 3. Differences of cattle behaviour in HSD and LSD compartments compared to cattle in SD compartment **: significant differences (p<0.10). NS: no significant difference (Dunnet test, p<0.10).*

No significant differences were observed between HSD compartment and SD compartment.

2.4. No significant effect of available space/group size on biochemical indicators, but a high inter-individual variability of data
No significant effect of the available space was observed on the variation of physiological indicators we dosed. Intra-group variance was high in all compartments, indicating a strong inter-individual variability of doses.

2.5. No drinking and few feeding during the resting time, but no effect of density on the loss of weight
During the resting time of the 3 transports, no cattle was observed drinking, in any compartment. Though drinkers were open, cattle seemed not to look for them or did not identify them. A few hours after the resting time, frequent thirst behaviours were observed, like leaking closed drinkers.
Cattle fed moderately from hay filets: 523 g/animal in LSD compartment, 285 g/animal in SD compartment, 316 g/animal in HSD compartment (p<0.12). No competition behaviour was observed during the resting time related to access to food or water. Some cattle did not even try to feed in front of the hay filets.

The resulting losses of weight during transport were all lower than 10% of body weight. No significant difference was observed in relation with density (p>0.1).

3. Discussion and conclusion

This study, based on 3 experimental transports, realized in the commercial conditions of young cattle export, gave significant evidence that the space allowance has an impact on the behaviour of cattle during the journey. Cattle stocked at 20% higher available space (that is in a small group size) were more mobile than cattle transported at a standard density: they changed more of position and more often, and moved more. At a low density/group size, cattle also stood less (11% less time spent standing), even during the first part of the journey. Nevertheless, in all compartments including the one with the lower available space per animal, cattle laid down and spent more time lying after the resting time.

Knowles et al (1997) and Brule et al (2001) also observed that cattle laid down more at the end of transports, and then hypothesized that this behaviour was related to tiredness. Warris et al (1995) did not observe lying cattle during a 15 hours transport. Lying allow cattle to take rest and, according to sex and breed, cattle can spend between 7 and 13 hours a day lying, by period of 1.5 hour (Phillips, 1993). Anyway, in transport conditions, cattle seem to focus energy on maintaining standing posture and perpendicular position and then favour the standing posture (review in Morrow and Tesch, 2001), which is in agreement with our observations and the correlation we measured between posture and position.
Changes in position were more frequent in the low density/group size compartment during the first part of the journey. Changes in position are a relevant indicator of agitation of cattle and are mostly determined by road conditions and social interactions (Eldridge et al, 1988; Kenny and Tarrant, 1987; Tarrant et al, 1992). Although not presented in this presentation, some agonistic behaviours, like mounting, were almost exclusively observed in the high space allowance compartment, during both the transport and the resting time, and could then explain part of the changes in position and movements of cattle. More over, cattle with more available space showed more losses of balance than these in the standard density compartment, both before and after the resting time. Brule et al (2001) already observed, during several experimental transports, that the losses of balance were more numerous in the 2nd part of the transport, indicating a possible effect of fatigue, as keeping balance requires an effort from the cattle (Broom, 2008). Lying position, earlier and more frequent in the compartment with the higher space allowance would result from stronger difficulties for cattle to keep balance in this compartment, lying posture helping them to avoid losses of balance and/or agonistic interactions. This interpretation must be taken into account to assess the consequences of a decrease of stocking densities: hence cattle spend most transport time standing (Swanson and Morrow-Tesch, 2001) and losses of balance could result in falling and injuries, and in higher muscular fatigue for cattle. As our experimental transports were realized on favourable road conditions (90% time on highway), we can suppose that losses of balance and falls would have been more frequent on secondary roads. Hence, at the beginning of Transport 1, we drove on departmental and national roads for a few hours, the losses of balance in standing animals were 3 time higher.

No significant effect of space allowance/group size was observed on biochemical indicators of muscular fatigue, of dehydration and food deprivation, neither in the stress protein nor in the mean loss of weight. The relevancy of biochemical indicators of stress has been often discussed, and results can be contradictory (review in Grandin, 1997 and Warris, 2007). In our study, a high inter-individual variability was observed which is still to be interpreted.

Weight loss is usually considered as a relevant indicator of the welfare of transported animal. It comes from dehydration and food deprivation (Warris, 2007). Lambooy and Hulsegge (1988) observed a greater weight loss when cattle were transported with a higher space allowance, but we did not confirm their observations.
Cattle did not feed and ate few hay, whatever the space allowance was. This observation must be interpreted with behavioural observations during the resting time (in process) but several interpretations can nevertheless be proposed. In commercial condition of young cattle export trade, cattle are usually fed with hay and granulated food and watered *ad libitum* several hours before loading. It is then possible that the cattle we transported were not yet hungry or thirsty during the resting time. Moreover, Warris (2007) in his review described the limited interest of cattle for drinking during transport, and supposed that this could be explained by some refusal to drink in unknown watering facilities. He also described a preferential interest of cattle in feeding compared to drinking, which our observations confirmed.

We realized our study as close as possible to real commercial conditions. For this reason, it does not allow to distinguish the effects of space allowance from effects of group size. This distinction, and more important the total available space resulting from both, could be important for some behaviours. Moreover, differences between the 1st transport and both others lead to suppose effects of climatic parameters, of breed and sex that should be addressed more precisely and combined with effect of variation in space allowance.

Data collected during this study are still being processed. Nevertheless, first analysis indicate that a 20% increase in the space allowance per animal does not improve young cattle welfare during commercial transport. Some results would even indicate that the increase of available space per animal jeopardizes cattle welfare, with more losses of balance and risk of fallings. Earley et al (2006 and 2007) also observed that the increase of space allowance did not improve significantly calves welfare. Relationship between stocking density, space allowance and group size is now to further investigate. On this account, our study confirms the interest of animal behaviour to study animal welfare during transport to complete other welfare indicators. On a more global scale, the question of space allowance per animal should be addressed from the economical and environmental point of view.

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