SCIENTIFIC OPINION

Scientific Opinion Concerning the Welfare of Animals during Transport[1]

EFSA Panel on Animal Health and Welfare (AHAW)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific opinion replaces the previous version published on 12 January 2011[4].

ABSTRACT

The Scientific Opinion on the welfare of animals during transport reviewed the most recent scientific information concerning the main farm species. New scientific evidence and consequent conclusions and recommendations were arranged following the structure of Annex I of EC Regulation 1/2005[5]. On fitness for transport, recommendations for cattle and poultry were focused on repeated humane handling and careful inspection prior to transport. On the means of transport, use of partitions in horse transport, compulsory fasting of pigs with provision of water at stops, and temperature limits for poultry were major recommendations. Maintaining stability of animal groups was recommended as good practice, with special emphasis on the need to avoid mixing unfamiliar pigs or goats. On watering and feeding intervals, journey times and resting periods, journey duration should not exceed 12 hours for horses and 29 hours for cattle. Horses should be supplied with water one hour before and one hour after transport, and for cattle there should be a 24 hour recovery period with access to food and water. For rabbits, time spent inside the containers during lairage should be considered journey time. Space allowance for horses should be given in terms of kg/m^2 instead of m^2/animal. For cattle and sheep, it is recommended that space allowances should be calculated according to an allometric equation relating size to body weight. Limits for stocking densities of broilers in containers should be related to thermal conditions. On the navigation systems, temperature monitoring systems should be incorporated. Minimum standards should be established regarding data type to be recorded, the

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4 The changes do not affect the overall conclusions of the opinion. To avoid confusion, the original version of the opinion has been removed from the website, but is available on request, as is a version showing all the changes made.

5 Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/97. OJ L 3, 5.1.2005, 1–44

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system and the on-board architecture. Recommendations for further research focused on the thermal limits and regulation for poultry and rabbits, the effects of ventilation on pigs, space allowance for rabbits, newly hatched chicks and pigs, optimal journey times for horses, pigs and calves.

KEY WORDS

Animal welfare, fitness for transport, means of transport, transport practices, watering and feeding, journey times, space allowances.

SUMMARY

Following a request from the European Commission, the Panel on Animal Health and Welfare was asked to deliver a Scientific Opinion on the welfare of animals during transport. An *ad hoc* expert working group was established in response to the request which made use of the information provided by stakeholders during the Technical Meeting held on 13 October 2010. The scientific opinion on the welfare of animals during transport was adopted by the AHAW Panel on 2nd December 2010.

In order to supplement the two previous reports on the welfare of animals during transport (SCAHAW; 2002 and EFSA, 2004), the working group collected the most recent scientific information concerning the main farm species (horses, pigs, sheep, goats, cattle, poultry and rabbits). New scientific evidence and data were then arranged following the structure of Annex I of Regulation 1/2005: fitness for transport; means of transport; transport practices; watering and feeding intervals; journey times and resting periods; additional provisions for long journeys and space allowances. Conclusions and Recommendations have been structured accordingly.

On the <u>fitness for transport,</u> in cattle evidence appears that there should be repeated humane handling during rearing and immediately prior to transport, in order to minimise aversive reactions. In poultry, the type and the age of bird determine its potential for reduced welfare in transport and the presence of metabolic disease and injuries in both broilers and laying hens may be exacerbated by poor transportation conditions and inappropriate handling. Additionally, it is concluded that under current commercial conditions, birds with both old injuries and catching/induced injuries, as well as those with pre/existing pathologies, may be loaded and transported. Therefore, in order to reduce these incidences, careful inspection of both broiler chickens and laying hens prior to transport is recommended.

In the case of the <u>means of transport,</u> new scientific evidence confirms previous conclusions on crate design, floor type, mixing unfamiliar animals, thermal stress and lack of ventilation in rabbits. In horses, it is recommended that to avoid aggression leading to injury, horses (except for mares travelling with their foals) should always be transported in individual stalls or pens, whether by road, rail, air or sea. Equidae find it relatively difficult to maintain their posture during sudden vehicle movements, therefore it is recommended that partitions used between stalls should protect and physically isolate each animal. Pigs should be fasted before transport and water should always be available at the farm, assembly point and lairage. During long transports (over 8 h) water should be provided at rest stops but it is unnecessary to provide water continuously while the vehicle is in motion. In the case of sheep, acceleration, braking, stopping, cornering, gear changing and uneven road surfaces should be avoided and driving quality on long journeys monitored and recorded using accelerometers in the vehicles. In poultry, the main recommendations are that specific thermal limits should be defined for broilers, laying hens and end of lay hens, e.g. the upper limit in a transport container for broilers should be 24-25 ºC assuming a relative humidity of 70% or higher and that a lower limit temperature limit for broilers in containers should be 5 ºC. When transporting poultry for 4 hours or more, vehicles should be equipped with mechanical ventilation systems. On the <u>additional provisions for sea transport</u>, in horses it is recommended that the time spent on a lorry loaded onto a vessel should be considered as journey time. In cattle, ventilation systems in vessels should have the capacity to prevent excessive heat load and electrolyte solutions should be made available on long sea journeys when there is a risk of heat stress.

In relation to the transport practices it is recommended that wherever possible, animals should be kept in stable social groups. Pigs should be loaded onto vehicles in groups no greater than six. Sows and boars should be handled separately and transported in separate compartments. In the case of goats, groups should be kept stable, repeated regrouping should be avoided, and the introduction of new individuals should be monitored closely. Horned and hornless goats should be kept separate. When goats have to be isolated for management purposes, they should be provided with olfactory, vocal, and visual contact with their group members. During transport of rabbits adequate ventilation has to be ensured to maintain the inside crate temperature within a range of 5-20 ºC. Temperature limits for newly hatched chicks during transportation should be introduced.

Recommendations on watering and feeding intervals, journey times and resting periods were drawn. In horses, when untrained horses of uncertain health status are transported for slaughter, the journey time should not normally exceed 12 hours. Horses should have continual access to an unrestricted supply of clean drinking water for a period of one hour before transport and for one hour immediately following transport. In pigs, for journeys exceeding 24 hours, feed should be available every 24 hours at staging points followed by 6 hours rest. Cattle should be offered water during rest periods on journeys of 8 to 29 hours. Adult cattle should not be transported on a journey of longer than 29 hours. After this time there should be a 24 hour recovery period with access to appropriate food and water. In rabbits, time spent inside the containers during lairage should not be considered as a resting period but as journey time. In the case of rabbits transported in containers and kept at arrival for lairage journey time should be defined as commencing when the first animal is loaded into a container and as ending when the last animal is unloaded from a container. For journeys longer than 4 hours for broilers and end of lay hens, vehicles should be equipped with mechanical ventilation and thermal environment should be monitored and controlled.

On the space allowance, conclusions and recommendations are focused on the way of calculation of the spaces depending on the animal type. In the case of horses space allowances should be given in terms of kg/m^2 instead of m^2/animal where animals are likely to differ significantly in weight or body condition. Cattle should be provided with sufficient space to stand without contact with their neighbours and to lie down if the journey is more than 12 hours. Space allowances should be calculated according to an allometric equation relating size to body weight in cattle and sheep. For cattle with horns, space allowance should be 7% higher. Limits for stocking densities of broilers in transport containers should be related to thermal conditions. Numbers should be limited in conditions when external temperatures exceed the proposed acceptable range (e.g. > 22 ºC) and on long journeys.

Animal transport monitoring has been also considered. Navigation systems should incorporate temperature monitoring and warning systems. Common minimum standards should be set up, in particular regarding the data type to be recorded, the system and the on-board architecture. A series of practical clinical measurements and observations, which can provide animal industry professionals and inspector with data to assess the welfare of animals during transport, is also listed.

Recommendations for further research are focused on the thermal limits and thermal regulation for poultry and rabbits and the effects of ventilation in relation to the level of stress of the pigs. The allowed minimum space allowance in rabbits, newly hatched chicks and pigs and the ‘optimal’ journey time in unweaned horses, pigs and calves should be also further studied.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Article 32 of Regulation (EC) No 1/2005 on the protection of animals during transport and related operations foresees that:

"the Commission shall present a report to the European Parliament and to the Council on the impact of this Regulation on the welfare of animals being transported and on the trade flows of live animals within the enlarged Community. In particular, the report shall take into account scientific evidence on welfare needs of animals, and the report on the implementation of the navigation system, as referred to in Annex I, Chapter VI, paragraph 4.3, as well as the socio-economic implications of this Regulation, including regional aspects."

On 11th March 2002, the Scientific Committee on Animal Health and Animal Welfare (SCAHAW) adopted an opinion on the welfare of animals during transport (details for horses, pigs, sheep and cattle). The opinion provides a comprehensive review of the subject, including recommendations on travelling times, resting times and space allowances.

This opinion was the basis of the Commission proposal adopted in 2003 (COM(2003) 425final) for a Council Regulation on the protection of animals during transport. The proposal aimed at replacing the legislation in force at that time, including standards on travelling times, resting times and space allowances.

Based on that Commission proposal, Regulation (EC) No 1/2005 was adopted in December 2004. However, the Council decided to maintain the previous standards on travelling times, resting times and space allowances, which were adopted in 1995 (Directive 95/29/EC[6]) and based on a scientific opinion established in 1992.

Since then EFSA adopted two opinions on animal transport:

- One in March 2004 concerning the welfare of species that were not previously covered by the SCAHAW opinion (i.e. poultry, rabbits, ratites, deer, dogs, cats and other animals, including exotic animals);
- Another one in October 2004 concerning temperature standards.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission therefore considers it opportune to request EFSA to assess the scientific information available on the welfare of animals during transport.

Since an extensive work has been already carried out in the past, the task will be to collect the most recent scientific information concerning the main farm species as follows: horses, pigs, sheep, goats, cattle, poultry and rabbits.

[6] Council Directive 95/29/EC of 29 June 1995 amending Directive 90/628/EEC concerning the protection of animals during transport. OJ L 148, 30.6.1995, p. 52–6.

- In the light of this scientific information, EFSA will assess the risks for the welfare of the transported animals according to the provisions of the present Community legislation (Regulation (EC) No 1/2005).
- EFSA will evaluate outcome-based animal welfare indicators (i.e. indicators of welfare based on the observations of the animals) and their possible use as an alternative to the present requirements. Only indicators which can be used by transporters and veterinary inspectors under commercial conditions should be detailed.
- Risks will be presented according to the following sections of Annex I (Technical Rules) to Regulation (EC) No 1/2005:
    - Fitness for transport;
    - Means of transport;
    - Transport practices;
    - Watering and feeding interval, journey times and resting periods;
    - Additional provisions for long journeys of domestic equidae and domestic animals of bovine, ovine, caprine and porcine species;
    - Space allowances.

  EFSA will in particular indicate the level of risk associated with each area.

ASSESSMENT

1. Introduction

The Scientific Committee on Animal Health and Animal Welfare has previously delivered a Report on the welfare of animals during transport (SCAHAW, 2002). The 2002 Report was the basis of the Commission proposal adopted in 2003 (COM (2003) 425 final) for a Council Regulation on the protection of animals during transport. The proposal aimed at replacing the legislation in force at that time, and set standards for travelling times, resting times and space allowances. Based on that Commission proposal, Regulation (EC) No 1/2005 was adopted in December 2004. However, the Council decided to maintain the previous standards on travelling times, resting times and space allowances, which were adopted in 1995 (Directive 95/29/EC) and were based on a Scientific Opinion established in 1992.

Previous EFSA Opinions on "The Welfare of Animals during Transport" (2004a, 2004b) have been extremely thorough. They describe concepts in animal welfare and approaches to the assessment of animal welfare from observations of behaviour, physiological and biochemical measurements, mortality, injury and carcass characteristics. The impact of transport on susceptibility to and spread of infectious diseases is covered in detail. There are comprehensive reviews of the scientific literature covering all major issues relating to the welfare of animals during transport. These include (*inter alia*) handling and loading procedures, vehicle design and operation, space allowances, journey times and special provisions for rest and refreshment on long journeys.

The opinions and recommendations arising from these earlier EFSA Opinions identify major risk factors and assess their impact. However, they do not use the structured approach to risk assessment now adopted as standard procedure by EFSA, which ranks the importance of different risks on the basis of the severity of the potential hazard and the probability of its occurrence. Moreover, relatively little emphasis was given to the evaluation of animal-based welfare indicators, which can be used by transporters and veterinary inspectors under commercial conditions.

The present mandate requires EFSA to:

- Assess the risks for the welfare of transported animals according to the provisions of the present Community legislation (Regulation (EC) No 1/2005) in the light of the most recent scientific information concerning the main farm species, as follows: horses, pigs, sheep, goats, cattle, poultry and rabbits;
- Evaluate outcome-based animal welfare indicators (i.e. indicators of welfare based on observations of the animals) and their possible use as an alternative to the present requirements. However, only indicators which can be used by transporters and veterinary inspectors under commercial conditions will be detailed;
- Indicate the level of risk for target groups within these species associated with: fitness for transport; means of transport; transport practices, space allowances, watering and feeding interval, journey times and resting periods. In addition, provisions for long journeys of domestic equidae and domestic animals of bovine, ovine, caprine and porcine species will be provided.

The background, as provided by the European Commission, also states: *"the Commission shall present a report to the European Parliament and to the Council on the impact of this Regulation* (Regulation (EC) No. 1/2005) *on the welfare of animals being transported and on the trade flows of live animals within the enlarged Community. In particular, the report shall take into account scientific evidence on welfare needs of animals, and the report on the implementation of the navigation system."*

On the basis of this background and this mandate the following four tasks are set:

1. To collect and review the most recent scientific information concerning the main farm species, as follows: horses, pigs, sheep, goats, cattle, poultry and rabbits.
2. To assess the hazards and risks for the welfare of animals transported according to the provisions of the present Community legislation (Regulation (EC) No 1/2005).
3. To identify and evaluate outcome-based animal welfare indicators, which can be used by transporters and veterinary inspectors under commercial conditions and consider their possible use as an alternative to the present requirements.
4. On the basis of new scientific evidence and this new animal-based approach to monitoring the potential adverse effects of transport-related factors (principally hazards) on animal welfare, to make recommendations relating to transport factors (e.g. travelling and resting times, navigation systems, space allowances, control of the environment within vehicles) and the incorporation of animal-based procedures into monitoring protocols for assessing the welfare of animals before, during and after transport. These hazards include those relating to the occurrence and spread of infectious diseases during and subsequent to transport.

Based on the opinion of the working group of experts, hazards with the highest impact on the welfare of animals during transport were identified and they are listed in Appendix B.

In drafting this Scientific Opinion and in line with EFSA's formal remit, the working group does not take into consideration any ethical, socio-economic, cultural or religious, or human safety or management issues and, whilst fully accepting their importance, bases its opinion solely on the scientific evidence.

2. Review of recent scientific information concerning the welfare during transport of horses, pigs, sheep, goats, cattle, poultry and rabbits

This section reviews the most recent scientific information for each of the named species. Each review begins with a brief summary of the state of the knowledge (and gaps in the knowledge) at the time of the previous reports (SCAHAW, 2002; for horses, pigs, sheep and cattle, and EFSA, 2004a; for poultry and rabbits. N.B. There is no section dealing with goats in either report). New scientific evidence is reviewed under the headings of fitness for transport; means of transport; transport practices, space allowances, watering and feeding intervals, journey times and resting periods; as well as additional provisions for long journeys. Normally, new evidence is defined as that obtained from scientific papers and other appropriate material published after 2002 or 2004 as relevant. A few references prior to these dates, but not previously cited in SCAHAW (2002) or EFSA (2004a), have been included where they are necessary to the interpretation of new evidence relevant to the recommendations contained within EC Regulation 1/2005.

To find relevant published information as per the Terms of Reference of the mandate, bibliographic searches were made for publications appearing after the SCAWAW (2002) or EFSA (2004a) reports. In each case, a list of search key words was created (e.g. equine, horse*, transport*). After the collection of information and data from electronic sources, publications were accepted or not according to their relevance. In addition, a number of evidence-rich reports sent to EFSA by individuals and organisations directly involved with the transport of animals, including veterinarians, non-government organisations and animal welfare charities, have also been considered.

The mandate requires the identification, in the light of new scientific evidence, of the need to reconsider the transport provisions set out in Annex 1 to EC Regulation 1/2005 or, where there is uncertainty, to identify needs for new research. The background to the mandate, as provided by the European Commission, stated that *"Regulation (EC) No 1/2005 was adopted in December 2004. However, the Council decided to maintain the previous standards on travelling times, resting times and space allowances, which were adopted in 1995 (Directive 95/29/EC) and based on a scientific opinion established in 1992"*.

Conclusions and recommendations are based on scientific information arising after the previous SCAHAW Report (2002) and EFSA Opinion (2004a). In the majority of cases, these conclusions and the conclusions of the two previous reports are not in conflict with the provisions set out in EC Regulation 1/2005, so it is not necessary to restate them. Table 1 summarises the issues considered in this Scientific Opinion in relation to the previous SCAHAW Report (2002) and EFSA Scientific Opinion (2004a). It lists the aspects of welfare during transport, as defined in the Terms of Reference, namely: fitness for transport; means of transport; transport practices, space allowances, watering and feeding interval, journey times and resting periods; as well as additional provisions for long journeys. It then identifies those areas where no changes are proposed (the majority); areas where recommendation for review have been made, or for further research in the light of new evidence; and the (few) cases where there were recommendations for review in the Scientific Opinions presented by SCAHAW (2002) or EFSA (2004a) but where these differ from the regulations set out in Annex 1 of EC Regulation 1/2005.

Table 1. Summary of the issues considered in this Scientific Opinion in relation to a previous SCAHAW Report (2002) and EFSA Scientific Opinion (2004a).

	Horses	**Pigs**	**Sheep & Goats**	**Cattle**	**Rabbits**	**Poultry**
Fitness for transport	*		*	*		*
Means of transport						
All means	*					
Additional provisions						
Road and rail						
Ro-Ro ferries	*	*		*		
Air						
Container transport						
Transport practices						
Loading, unloading, handling		*				*
Journey times	*	*				*
Rest periods	*					
Additional provisions – long journeys	*	*				
Watering and feeding intervals		*				
Journey times	*				**	*
Rest periods	*			*		*
Additional provisions – long journeys	*					
Space allowances						
All journeys	*		*	*	**	
Long journeys	*			*		

Blank cell: where there has been no recommendation in relation to Annex 1 of EC Regulation 1/2005;
***** = recommendation for review or further research in the light of new evidence (post SCAHAW, 2002 and EFSA, 2004a);
****** = recommendation for review on the basis of scientific opinion presented in SCAHAW 2002 or EFSA 2004a but not taken into account in Annex 1 of EC Regulation 1/2005. N.B. These are not repeated in the text.

Table 1 enables the reader to readily identify where a need for revision has been recognised. Where no change appears necessary, this is because there is either sufficient scientific evidence in SCAHAW (2002) and EFSA (2004a) to justify existing regulations, or insufficient evidence for revision. In reviewing the new scientific information, the most detailed description has been given to support the justification of those topics where recommendations for revision have been made.

2.1. Horse transport

Horses are commonly transported for racing, breeding, horse shows, deliveries to buyers and veterinary appointments (TRAW, 2009). Welfare issues for horses have been reviewed recently by Minero and Canali (2009). Unlike other farmed species, some horses may be transported many times in their lives (Waran et al., 2002). Eurostat (2009) reported that in 2007 about 600,000 horses were slaughtered in Europe, most of them following long distance transport (> 8 hours) from Poland, Romania and Spain to Italy. Many studies concentrating on the detailed physiological assessment of reactions of horses to transport have been aimed at improving transport conditions for sport, leisure and breeding horses. These animals are generally of high value and are usually transported under optimal conditions at some monetary cost. In addition to these animals there is a very large trade in horses destined for slaughter within the EU. Nevertheless, these animals tend to be of lower value and less is invested in ensuring that conditions of transport are satisfactory. Prior to the last scientific report (SCAHAW, 2002), data regarding the transport of these types of animal were sketchy. However, there are now several survey-based studies of slaughter horses from both the EU and USA which are reported on below. In general, these studies report that the welfare of slaughter horses can be very poor with high rates of disease and injury before transport actually takes place, although even greater levels occur during and after transport.

2.1.1. Fitness for transport

Aggression

Knowles et al. (2010) investigated whether it was possible to determine from pre-transport group behaviour which unbroken ponies, from socialised groups, would be aggressive during transport in penned groups. The approach taken for developing a methodology to predict which ponies would be aggressive during transport was to treat any method as a diagnostic test: sensitivity and specificity tests were employed to identify aggressive ponies and to ensure that unaggressive ponies were not misclassified. However, the study found little correlation between pre-transport and transport aggression and thus provided no diagnostic value in pre-transport behavioural observations.

Injury and Disease

An extensive survey provided data on the physical condition of 1,008 horses arriving at USA slaughter plants in the summer of 1998 (Grandin et al., 1999, 2010). A total of 78 horses (7.7%) had severe welfare problems. Pre-transport conditions of emaciation, fractured limbs, laminitis or weakness were recorded in 1.5% of the horses. The proportion of owner neglect and abuse (6%) occurring prior to transport was significantly greater than the number of injuries (1.8%) that occurred during marketing and transport. Welfare problems in slaughter horses were listed in order of priority and conditions caused by owner abuse or neglect ranked top of this list (Grandin et al., 1999, 2010). Marlin et al. (2011) carried out an extensive survey of over 2,700 slaughter horses transported within the EU. The proportion of horses deemed unfit for transport at the point of origin was 14%. This increased to 37% at the destination. The main symptoms of unfitness were those associated with respiratory disease and these were greatly exacerbated by transport (the "shipping fever" syndrome). New injuries incurred during transport were recorded in 28% of horses.

There are other reports of shipping fever in horses: up to 12% following long-distance road transport and up to 30-40% following air transport (Higgins, 2004). A major risk factor for the development of disease is transportation duration (Oikawa et al., 2005). Other problems include gastrointestinal diseases (reduced water intake affecting gut function, possibly resulting in colic and/or diarrhoea) and predisposition to equine gastric ulcer syndrome (Higgins, 2004).

According to Oikawa et al. (2005), several factors may contribute to the development of transport-related respiratory disease in horses, such as (1) presence of subclinical respiratory diseases, (2) restraint in the "head-up" posture, (3) stress-related impairment of the immune response, (4) presence of noxious gases and high concentrations of airborne dust and bacteria, (5) length and duration of the journey, and (6) body orientation during transport. The conclusion from this study suggested that rest periods should be as long and as frequent as possible because increased rest duration was associated with a reduction in both transport stress and incidence of pyrexia. Ambient pollution in the vehicle interior was caused particularly by increases in ammonia and airborne dust, suggesting that a clean environment would reduce transport stress and inflammatory reactions.

An extremely comprehensive and recent review of behavioural indicators of pain in horses can be found in Ashley et al. (2005).

2.1.2. Means of transport

2.1.2.1. Provisions for all means of transport

Work by Grandin et al. (1999, 2010) identified fighting as a major cause of injuries. Inter-horse aggression caused carcass bruising (51% of the carcasses) with characteristic patterns of bites or kicks. The authors recommended that management of slaughter horses should consider methods for reducing injuries from fighting during transport, such as the mixing of unfamiliar horses and segregation of mares and geldings in the same manner as applied to stallions.

Horses are social animals but they have a flight or fight instinct. It is safer and less stressful for them to travel in individual compartments. When stocking density increases, horses may not be able to adopt improved balancing strategies because a high density does not allow them any freedom to change their behaviour (Dalla Villa et al, 2009). Researchers travelling in a trailer observed many incidences of aggressive horses repeatedly biting an adjacent horse in an apparent effort to get the horse to move away (Gibbs and Friend, 2000). Knowles et al. (2010) reported that in the UK, experienced commercial transporters had moved away from transporting any equidae in groups, other than unbroken ponies already accustomed to living in a social group, because of high levels of injury and aggression between animals. All animals are now carried only in individual pens. This argument would appear to be strongly supported by the very high levels of injuries reported by Stefancic and Martin (2005) and Grandin et al. (1999, 2010) amongst equidae transported as groups.

Equidae have a high centre of gravity and 60% of their weight may normally be carried on the forelegs. For this reason, transport is likely to be more tiring and equidae are likely to require more room to brace and adjust their position, especially when transport involves many changes in velocity and acceleration, for example during non-motorway road transport. This observation is supported by new evidence from Marlin et al. (2011) who reported a significant increase in injury risk in horses transported in articulated vehicles.

Equidae can have problems with thermoregulation, especially at high temperature. They rely on sweating to thermoregulate at high temperatures and this can be compromised when ventilation within a load is restricted (Davidson and Harris in Waran et al., 2002; Marlin et al., 2011).

2.1.2.2. Additional provisions for transport on roll-on roll-off vessels

If there is sea movement, animals will be required to work to maintain balance and this has been shown to be an extremely important stressor during transport (Giovagnoli et al., 2002).

2.1.3. Transport practices

2.1.3.1. Loading, unloading and handling

Facilities and procedures

<u>Lighting</u>

Horses tend to be reluctant to enter darkened passageways (Phillips et al., 2001). This is probably because they are crepuscular herbivores (Groves, 1989). Although horses have the capability of both nocturnal and diurnal vision (Phillips and Arab, 1998; Holly, 2004), they are specialised for dim-light vision but may have reduced visual detail and response to light of certain (coloured) wavelengths (Timney and Macuda, 2001; Saslow, 2002). Horses have both weak brightness discrimination (Geisbauer et al., 2004) and the ability to close the pupil down to a slit, as a protective mechanism (Saslow, 2002).

Lighting conditions inside and outside a trailer (lit arena and trailer of 5.8 and 8.3 Lux, respectively, dark arena and trailer of 0.025 and 0.035 Lux, respectively) showed little influence on the amount of fear shown by horses being loaded for transportation (Cross et al., 2008). No significant effects were found in the time the horses took to enter the trailer, in the number of steps they took to enter it or in the intensity of the heart rate response. However, there was evidence of negative emotions when they were loaded from a lit arena, particularly when they were entering a dark trailer. In fact, in this situation horses showed significantly more behaviours indicative of reluctance to enter the trailer (lowering of the head and turning away from the ramp). Lowering the head might have facilitated the horses better assessment of the floor and horizon, which in the dark would have been of benefit (Cross et al., 2008). In this position, horses can also use their binocular vision and be able to detect potential threats better with their monocular field by scanning the lateral horizon (Hall et al., 2003). Cross et al. (2008) also found that horses sniffed the ground more often when entering a dark trailer from a lit arena, suggesting that they needed more time for exploration when leaving the open visible arena and entering a dark, enclosed space. This is in agreement with other authors (Marten, 1998; Parelli, 2003), who observed that horses instinctively avoided confined spaces in which they might perceive themselves to be exposed to an increased risk.

Handling

Blindfolding

Blindfolds are routinely used for loading horses that are difficult to control. Parker et al. (2004) contacted a number of national and international horse transport companies to assess the prevalence of blindfolding within the transport industry. They found that, of the six who replied, one reported frequent use of blindfolds, two were vehemently opposed to it, while the remainder were familiar with the practice but used it only as a last resort. The authors carried out a study to assess the effect of blinkering and blindfolding on behaviour and heart rate in three situations: whilst horses were stabled, when being led in a ménage, and when loaded onto a lorry. Blinkering elicited milder effects compared with blindfolding, which was associated with an increase in heart rate in all three situations. Overall, blindfolding appeared to make the horses more nervous and difficult to handle, and did not calm down the horses as hypothesised by some authors. However, the study did not discount the application of this practice for improving welfare and safety when handling particularly fractious horses (Parker et al., 2004).

Previous experience

It is known that habituation to transport can lead to positive effects on horse welfare by reducing the stress response induced by loading. However, only a few studies are available involving horses that are transported regularly and are accustomed to transport, as is the case for sport horses. Transport of horses causes changes in the heart rate (HR). Heart rate variability (HRV) has been widely used as an indicator of the response of the autonomic nervous system to stress in farm animals (von Borell et al., 2007) and horses (Visser et al., 2002). HRV is driven by the antagonistic oscillatory influences of the components of the nervous system, thus a decrease in HRV reflects a shift towards more sympathetic dominance, while an increase indicates a shift towards parasympathetic dominance (von Borell et al., 2007). The effect of long-distance transport (2-days outbound road transport over 1,370 km and 2-days return transport 8 days later) was studied on equitation horses accustomed to transport (Schmidt et al., 2010a). Salivary cortisol increased 30 minutes before loading and after transport, but the response tended to decrease with each day of transport, whereas concentrations of faecal cortisol metabolites increased on the second day of both outbound and return transports and reached a maximum the following day. The onset of each transport was associated with a transient increase of sympathetic tone and reduction of parasympathetic activity, as shown by a transient rise in HRV (Schmidt et al., 2010a). Increased cortisol release and changes in HR and HRV indicated a stress response to transport most pronounced on the first day of both the outbound and the return journey (Schmidt et al., 2010a).

Horses transported for the first time showed a greater increase of HR compared with those with previous experience after 2-5 h transport (Andronie et al., 2009). However, HR decreased between loading and unloading time indicating animals adapted relatively quickly to the new conditions (Andronie et al., 2009). Plasma cortisol levels increased despite the fact that horses had previously been exposed to transport, but the increase was lower in horses accustomed to transport than those unaccustomed, and it varied according to journey duration. Similarly, serum lactic acid levels were decreased in horses accustomed to transport compared with those not, indicating some accustomisation in these animals (Andronie et al., 2009).

Separation

Confinement

The effect of confinement and grouping of horses during transport has been a source of debate from the point of view of animal welfare. Garey et al. (2009) studied the effects of loose groups *vs.* individual stalls during transport in yearling horses with no prior transport experience. Increases in cortisol and dehydroepiandrosterone (DHEA) indicated that transportation was a significant stressor for horses, but no significant differences were found between horses being transported in a loose group compared with those in individual stalls. However, differences were found between trials conducted under different housing conditions, because changing the horses from group housing to individual stalls resulted in significant increases in pre-transport concentrations of those stress-related compounds (Garey et al., 2009).

Isolation

Isolation is a source of stress in herd species such as the horse, resulting in a number of physiological (increased heart rate, hyperventilation and sweating) and behavioural responses (attempting to join other horses, erratic movements, pawing, turning round and vocalisation). These responses are also seen in non-transport situations (Strand et al., 2002). The consequences of transporting horses alone for 30 minutes were investigated and compared with travelling with a single companion or with a mirror acting as surrogate companionship (Kay and Hall, 2009). Horses travelling with a live companion spent significantly less time vocalising, head-turning, head-tossing and pawing, and more time eating than those travelling alone. Increases in HR and rectal temperature (Tr) and decreases in rear-pinna temperatures (Tp) were also reduced. Similarly, horses travelling with a mirror spent less time head-turning, vocalising, head-tossing and more time eating. The surrogate companionship of the mirror also reduced increases in Tr and decreases in Tp compared with horses travelling alone. The only significant difference between travelling with a companion and a mirror was that time spent turning the head round was less with a companion (Kay and Hall, 2009). The provision of surrogate companionship was found to be preferable to travelling alone, suggesting that the mirror mimics visual contact (but not aural or odour contact) with conspecifics thereby minimising the sense of social isolation. In addition, it may provide environmental distraction or additional visual stimuli. Isolation during transportation in a trailer was found to suppress feeding behaviour, and correlation analysis confirmed there was an inverse relationship between eating behaviour and other anxiety related behaviour. Kay and Hall (2009) recommended that horses are transported with a live companion, but if none are available, surrogate companionship, such as a mirror, is preferable to travelling alone.

In a study of socialised, unbroken ponies Knowles et al. (2010) found marked behavioural indicators of stress in ponies transported in a single pen and separated from, but adjacent to, their social group. The degree of stress was increased in younger animals. The authors therefore recommended that this type of animal should ideally not be individually penned during transport, especially if they are immature.

Group Size

Knowles et al. (2010), in an investigation of transport conditions for unbroken ponies, found increased levels of aggression (aggressive acts per pony) with ponies transported in groups of eight compared with transportation in groups of four.

2.1.4. During transport

2.1.4.1. Water and feeding interval, journey times and resting periods

Water

Since weight losses during transport have been primarily attributed to dehydration, this is a concern for slaughter horses undergoing long journeys. Gibbs and Friend (2000) found that horses readily consumed water from wall-mounted water troughs when onboard a trailer, but these devices were not suitable in commercial practice. Recently, Iacono et al. (2007a) tested the use of a practical, onboard watering system for use during long-distance transport. Stocking density (from 205 to 318 kg/m^2) did not appear to influence drinking behaviour in the watered compartments, while there was evidence that drinking was partly dependent on the temperature during shipment. When transported during hotter shipments (mean 30 ºC) all the horses that drank (88% of the subjects) took their initial drink within the first 20 minutes of the first watering session, whereas drinking did not take place until after 21-60 minutes in 75% of the horses in cooler shipments. These findings indicated that an hour was an adequate time for a water stop to allow most of the horses to drink at least once. There was a trend for horses even with access to water to lose weight, indicating that they did not consume a large amount of water under these conditions of transport (Iacono et al., 2007a). It should be noted that these results may not be pertinent to EU conditions, as horses are required to be individually penned during long distance transport.

Iacono et al. (2007b) also found that mean water intake was only 3.8, 1.9 and 2.8 litres for journeys of 16, 23 and 28 h, respectively. It has been shown that depending on the conditions under which a horse is exercised, total water intake by a 500 kg horse could reach more than 90 litres per day (National Research Council, 2007). Taking this into account, equine intake of the levels found during transport would have minimal impact on transport-induced dehydration. Such a low mean intake could be due to horses consuming only small volumes due to disruption of normal behaviour.

Observations on slaughter horses observed when transported within the EU indicated that the provision of water was consistently inadequate and that horses frequently displayed behaviour indicative of extreme dehydration and thirst (Marlin et al., 2011).

Journey time

Horses transported for 26 h by commercial horse haulers, returning to base every six hours, showed increased levels of cortisol levels after the first 24 h transport (Guay et al., 2009). Similarly, horses transported by commercial haulers with single stall capacity showed cortisol concentration increases in response to a 6, 12, 18, and 24 h transport period, compared with baseline values, and a return to baseline values 24 h after transport (Guay et al., 2009). Respiration rate also increased after 6 and 12 h transport periods, suggesting that horses were experiencing stress and/or heat stress, but it was similar to baseline values after 18 and 24 h transport periods. HR was reduced in horses after 18 and 24 h of transport compared with baseline values. Transport did not influence rectal temperatures of horses regardless of transport duration (Guay et al., 2009). A deeper insight into the response to transport stress in the horse could be useful to identify warning signs in subjects prone to developing post-transportation respiratory diseases and other debilitating conditions in horses (Guay et al., 2009).

During prolonged road transportation, changes in HR and HRV have been measured in five 2-year-old thoroughbreds in order to assess these measurements as a sensitive index of autonomic stimulation (Ohmura et al., 2006). Diurnal rhythms of HR and other electrocardiogram (ECG) parameters showed significant changes or disruption during transport. The authors concluded that HR is influenced by different sympathovagal mechanisms during stall rest, compared with the period during road transportation, and that HRV may be a sensitive indicator of stress in transported horses.

β-endorphin, adrenocorticotrophic hormone (ACTH) and cortisol responses of 42 thoroughbred and crossbred stallions, before and after road transport to breeding stations over distances of 100, 200 and 300 km, were studied by Fazio et al. (2008). All horses had previous trailering experience. The response of animals to transport stress was influenced by the different distances and/or duration. β-endorphin levels increased immediately during the earlier phase of transportation (100 km), but decreased during the subsequent phases (for distances of 200 and 300 km). Increases in ACTH and in cortisol levels after transport confirmed these as important hormones in mediating endocrine responses under conditions of physical or psychological stress in horses (Fazio et al., 2008).

The influence of journey times has been further assessed in horses by means of non-invasive techniques. Salivary cortisol, faecal cortisol metabolites, HR and HRV in horses with no experience of transport were measured during short (1 and 3.5 h) and medium (8 h) road transport (Schmidt et al., 2010b). The degree of change was related to the duration of transport. A marked increase in salivary cortisol concentration was observed mainly towards the end of transport, decreasing to baseline values rapidly after unloading. HR increased during transport and remained high until horses were unloaded and returned to their stables. These finding are in contrast with previous studies (Schmidt et al., 1994; Ohmura et al., 2006) where HR initially increased but then decreased during transport, suggesting that loading may be the most stressful part of the procedure (Shanahan, 2003). According to Schmidt et al. (2010b) the persistently higher HR recorded during transport might be due to the relatively short transport time and to the horses' lack of previous transport experience. Increased HRV indicated a reduction in vagal tone during transport. Since horses in this study did not receive water and feed during transport, the experimental stress might be considered a combined action of transport and of temporary feed and water withdrawal.

Tischner et al. (2005) studied levels of catecholamines and cortisol in pony mares and their foals subjected to 20 minutes of road transport nine days after parturition. They noted that the increase in concentration of all variables following transport was three times higher in the mares than in the foals. Transport studies of other farmed species have suggested that very young animals are unable to mount the usual stress response to transport seen in older animals.

Pinchbeck et al. (2004) found that journey time to the track was a key risk factor for a fall in UK hurdle and steeplechase racing. They found an increasing risk of a fall to a maximum at 7 hours. The risk was decreased for journey times greater than approximately 7 hours, however, the majority of horses transported for greater than 7 hours were rested overnight at the track before the race.

Oikawa and Jones (2000) demonstrated a rising percentage of pyrexic horses as the duration of a journey by road increased. They found that a dramatic rise in the percentage of pyrexic animals began after 18 hours of transport. This rose to approximately 44% of horses by 38 h of transport with the first horses beginning to show effects at only 10 hours.

Marlin et al. (2011) provided data from observations of 2,790 animals at two assembly centres in Romania which showed that horses destined for slaughter within the EU were often in a poor state of health before transport, were generally transported under poor conditions, and suffered numerous abuses of their welfare.

On a study undertaken on horses transported long distances for slaughter in Europe (Westen and White, 2010), it was found that all shipments contained horses showing clinical signs of disease. The number of animals affected per shipment ranged from 83-100%, with 95% having some form of acute injury and 99% showed evidence of sweating. Behavioural data collected on a restricted number of animals (18 horses) selected at random showed that 83% of them were weight shifting and 94% had an abnormal stance, suggesting a problem such as pain or discomfort.

Resting time

Few studies have been carried out on the effect of a rest-stop during a long road journey. However, the activity of unrestrained slaughter horses during a one hour stop, with and without water provided, was quantified to determine if horses obtain meaningful rest (Friend et al., 2006). Five shipments of horses at high, medium, and low density (averaging 397 ± 6.5 kg/m^2, 348 ± 5.2 kg/m^2, and 221 ± 7.6

kg/m^2, respectively) were transported for 16 to 20 hours. Although there were several exceptions, watered groups tended to have more movement at the beginning of a stop (up to 25 min) as the horses tried to access onboard watering systems. The increase in locomotor activity was more pronounced in high-density groups, in addition to display of aggressive behaviours. However, this may have been due to the greater need for these horses to manoeuvre in order to obtain access to the water. There was also a slight tendency for animals at medium and low densities to become more active after 55 minutes of onboard rest, perhaps indicating they had received some rest (Friend et al., 2006).

Including a 12-h stop for rest and feeding in the middle of two 12-h periods of transport led to some immunophysiological benefits in horses compared with travelling continuously for 24 hours (Stull et al., 2008). Transport significantly increased the horses' plasma cortisol concentrations, neutrophil counts and neutrophil:lymphocyte ratios, and decreased the numbers of all the lymphocyte subpopulation cell types. However, whereas the pattern of endocrine and haematological changes was similar in both treatments, there were differences in the transport-related decline of some lymphocyte subpopulations, suggesting that a 12-hour stop allowed the subpopulations to recover towards resting levels (Stull et al., 2008).

Werner and Gallo (2008) studied the physiological response of cull racehorses transported in groups of two to four for approximately 1 hour and then held for 18 to 21 hours in lairage. Plasma lactate, glucose, creatine kinase, cortisol and packed cell volume increased after loading, transport and unloading, returning approximately to pre-transport levels by the end of the lairage period.

2.1.5. Additional provisions for long journeys

2.1.5.1. Water supply for transport by road, rail or sea containers

Different densities (ranging from 205 to 318 kg/m^2) did not appear to influence drinking behaviour in horses offered a practical, onboard watering system for use during long-distance transport (Iacono et al., 2007a). However, drinking appeared to be partly dependent upon the environmental temperature during shipment. When transported during a hotter shipment (mean 30 ºC), all the horses that drank (88% of the subjects) took their initial drink within the first 20 minutes of the first watering session, whereas drinking did not take place until after 21-60 minutes in 75% of the horses in the cooler shipments. The findings indicated that an hour is an adequate time for a water stop to allow most of the horses to drink at least once. There was a trend for horses, even with access to water, to lose weight, after journeys lasting 18/20 hours, indicating that they did not consume a large amount of water under the studied conditions of transport (Iacono et al., 2007a).

Calabrese and Friend (2009) studied groups of 5 to 7 horses transported for 18 to 20 hours at 'low' to 'moderate' stocking densities (216.5 and 345.5 kg/m^2, respectively). They found that movement caused by disruptive animals was highest when the truck was in motion and reduced during rest stops. Movement during rest stops was stimulated by the availability of water from water bowls inserted during the rest stop.

2.1.5.2. Ventilation for means of transport by road and temperature monitoring

Purswell et al. (2006) in an investigation of ventilation rates within a four-horse trailer concluded that no combination of vent set-up and road speed provided adequate ventilation compared with that recommended for stabled horses. They suggested that the most cost-effective method of increasing air exchange was to design trailers with greater window and vent areas.

2.1.6. Space allowances (transport by rail, by road, by air, by sea, densities)

Balance preservation

Loss of balance can be a source of injuries and behavioural problems in horses. Waran et al. (2002) state that "*Equidae's need for adequate space when travelling is different to that of other species, for example pigs, which have a much lower centre of gravity. Equidae's centre of mass is high off the ground because they are long legged relative to their body mass and they carry 60% of their bodyweight over the forelegs*".

If a horse does become recumbent during transport a much greater width is required than when standing, as a horse puts its hind legs to one side because they are unable to fold them underneath the animal. When standing, the nose to tail length of a horse may be 150% of that when recumbent. There is the risk that horses can be trodden on or kept down after lying down because of lack of space and the close proximity of neighbouring animals, so the problem is increased with increasing stocking density. Horses respond to forces during transportation, activating different balance adjusting mechanisms, defined as passive, yielding, or reactive sway (Roberts, 1990). Overall, the main reactive responses involve attempting several steps in different directions in association with foot repositioning movements, as well as moving the head more upright or forward in relation to acceleration and the direction in which they are facing (Stull, 1997). When facing forward, severe braking may force horses to take forward steps to preserve their balance, and this brings their head closer to the bulkhead, with the possibility of collision.

In the horse, effort spent maintaining balance during transport, in relation to human driving attributes, was evaluated by comparing electromyographic (EMG) data collected during a 26 minute journey with those obtained when animals were resting (Giovagnoli et al., 2002). Data were correlated with the HR recorded at the same time and observable behaviours related to other muscular activities (e.g. searching, scratching). Erratic driving caused continual postural adjustments in order to maintain balance and this affected HR. Results showed that the horse's efforts to maintain balance during transport should be considered one of the main contributors to transport stress, either as a direct factor (e.g. rhabdomyolysis, injuries, etc.) or as an indirect one (e.g. shipping fever through immune suppression) (Giovagnoli et al., 2002).

Knowles et al. (2010) observed that ponies transported by road in single pens at 90º to the direction of travel tended to brace themselves by placing their rump in a forward corner of the pen.

Space Allowance

Knowles et al. (2010), in an investigation of transport conditions for unbroken ponies, found increased aggression with increased stocking density within groups of 4 and 8 ponies transported by road for 1 hour. The number of slips, falls and collisions with other ponies and the pen sides increased with increasing stocking density, as did levels of plasma cortisol and creatine kinase (CK). The authors concluded that for groups of small (mostly under 300 kg and of approximate average weight 200 kg) unbroken ponies, a maximum acceptable stocking density was 200 kg/m^2. Knowles and Warriss (2009) pointed out the problem of defining acceptable stocking densities for groups of animals containing individuals of widely heterogeneous size and weight.

Iacono et al. (2007b) studied horses transported in groups for long distance (9 and 20 h) and observed no significant differences in aggression, cortisol, plasma chemistry profile, dehydration and weight loss in relation to animal density or provision of water. Individual horses, rather than the influence of high density, were found to be the major cause of aggressive behaviour. However, high density increased the tendency for horses to go down during shipments, which could result in injury or death. High versus low densities in terms of occurrence of aggression has been debated for horse transport (Friend, 2001; Stull and Rodiek, 2002). High density would be likely to prevent severe injuries from kicking by avoiding direct, aggressive kicks but would prevent horses that were being bitten from escaping. In contrast, at a lower density, the less dominant horses presumably would be able to get away from aggressive horses, but full-contact kicking would be facilitated. However, no published data support the concern about an increase in kicking at lower densities (Iacono et al., 2007b).

The discrepancy between the work of Knowles et al. (2010) and Iacono et al. (2007b) as to the effect of stocking density on aggression and physiology may be due to the type of animals being transported and conditions of transport. Knowles et al. (2010) were working with unbroken ponies (average weight approximately 200 kg) from established social groups, mostly from free roaming herds, whilst Iacono et al. (2007b) studied collected, cull horses within the USA with a weight range of 248 to 680 kg. Additionally, it is difficult to make a comparison between the stocking densities used in the two studies as a given stocking density, in figures of kg/m^2, has different physical consequences for animals of different weights. Furthermore, the transport time for the ponies was only 1 hour, whilst for the cull horses it was from 18 to 20 hours.

Horses can also be injured if a compartment is underloaded, since with too much space animals may lie down and be stepped on by other horses (Grandin et al., 1999, 2010). However, at too high a stocking density, animals that become recumbent may not have sufficient room to regain their feet. Additionally, there will be an upper limit to the desirable amount of free space, since, during emergency braking or other rapid changes in velocity, an animal would not have too far to move before it encounters a physical object. With too much space it could fall or collide with the pen wall or another animal at an injurious speed (Knowles et al., 2010).

In a study of the effects of stocking density during transport on welfare that used groups of ponies of heterogeneous weight, Knowles et al. (2010) found little relationship between space allowance (m^2/animal) and measures related to welfare, although they found a strong relationship between stocking density (kg/m^2) and measures related to welfare. This suggests that space allowances within the regulations should be defined in terms of m^2/kg rather than m^2/animal, especially when animals are not of similar weights.

According to Westen et al. (2010), because of the large heterogeneity of transported horses, space allowances should be based on the length and width of individual animals rather than fixed figures for the total population. These authors discussed the pros and cons of three different approaches for attempting to define the specific requirements of the space allowance for horses and ponies during transport: 1) minimum floor area, 2) space allowance per animal on body mass or wither height, 3) amount of space between the horse and the compartment walls (partitions and sides of the vehicle). Westen et al. (2010) concluded that providing a fixed space allowance that covers "adult" horses is entirely inappropriate, whereas an alternative acceptable approach would be to specify that individual compartments must be at least X cm wider and Y cm longer than the horse when standing in a natural posture.

2.2. Pig transport

In order to find relevant published information in agreement with the Terms of Reference of the mandate, a bibliographic search was performed. For the search, a list of key words was created (welfare, pig, transport) and the year of publication was set to be after 2003. After the collection of information and data from electronic sources, publications were accepted or not according to previously established criteria. After the first screening, a total of 50 references were obtained dealing exclusively with welfare aspects of the transport of pigs. Of these, 40 references passed the screening process according to the acceptance criteria and were used for the development of the following section of the Scientific Opinion. In addition, as well as papers between 2003 and 2010, two papers from November and December 2002 were included as they were not considered in the previous report.

2.2.1. Fitness for transport

Studies on pigs during the suckling and fattening periods suggest that enrichment of the preweaning environment may have a positive effect on the coping behaviour of preweaned pigs. This was considered to have prevented an increase in salivary cortisol concentrations after transport and resulted in a decrease in meat pH 45 minutes post-mortem, at an age of 6 months (Chaloupková et al., 2007). However, Bärlocher et al. (2008) reported that the impact of housing systems on postmortem parameters such as pH was relatively low. This was generally confirmed in a comparison between outdoor and conventionally raised pigs (Gade, 2008), although conventionally raised pigs may have been more stressed by mixing pigs pre-slaughter than outdoor pigs were. Fàbrega et al. (2002) determined the halothane genotype of pigs that died during transport. Affected animals showed severe signs of dyspnoea, cyanosis and hyperthermia and may have developed rigor in the muscles before death occurred. They assumed that elimination of the halothane gene, which is associated with porcine stress syndrome (PSS), would substantially reduce pre-slaughter mortality. Gade et al. (2007) emphasised that total pre-slaughter mortality was reduced eight-fold in Danish slaughter pigs during the period of halothane gene removal from the pig population. Jackowiak et al. (2006) evaluated the effectiveness of on-farm antemortem inspection in Australian pig farms. From their field study it was concluded that producer inspections may have benefits for animal welfare and chain efficiency.

In a survey of 739 journeys to 37 slaughterhouses in five EU countries, the risk for mortality and injuries was evaluated (Averós et al., 2008). Mortality risk increased with average air temperature and was highest when pigs were not fasted. Depending on the distance to the slaughterhouse or transport station, feeding should be stopped the night before transport, but water should be available. Fàbrega et al. (2002) found a relationship between a high percentage of dark, firm, and dry (DFD) meat and a lower welfare index (based on a questionnaire). They suggested that meat quality parameters such as DFD could be used at the abattoir to perform a "snapshot" welfare evaluation.

2.2.2. Means of transport

2.2.2.1. Provisions for all means of transport

At present, transport by train is not common, because the pigs have to be transported to a station and reloaded thereby increasing the adverse effects of loading and lengthening some journeys. However, conditions by train can be very good. The use of aircraft is limited to breeding pigs, because it is expensive. The most common means of transport for pigs is by road vehicle even though it is generally found that truck transport is worse for the animals than rail, sea or air transport. Pigs are usually transported in large trucks that may hold over 200 animals in 3 tiers with a compartment height of 90 cm. In the EU, most of these trucks are equipped with a loading lift.

2.2.2.2. Additional provisions for transport on roll-on roll-off vessels

Transport by ship is seldom feasible. However, it is sometimes used in a roll-on roll-off situation, where the truck with pigs is placed on a ferry to transport them to an island. The truck is placed on the upper deck to guarantee ventilation.

2.2.2.3. Additional provisions for transport by air

According to the IATA Regulation (IATA, 2010)[7], the loading should not exceed 175 kg/m^2, although transporters load them at 125 to 150 kg/m^2. The wooden pens have a height of 1.55 m and are used in 2 layers. There are 3 drinking nipples connected to a tank filled with water. The bedding is sawdust with some pellets for food. Full flow ventilation is common for pigs of a live weight of 40

7 IATA (Air Transport Association), 2010 Livestock Transportation General Guidelines. (2010), http://www.iata.org/whatwedo/cargo/live_animals/Pages/index.aspx

kg at a temperature of 12 ºC. In these circumstances, pigs lie close together, and are kept in complete darkness.

The regulations for air transport are set up and controlled by the transporters themselves (IATA Live Animal Resolution 620, 2010), and EC Regulation 1/2005 adopted these regulations. It was found that the Regulation was based upon the version of 2004. However, this version is not available anymore and the version of 2010 seems to have been adopted for the Regulation, and includes the respective changes.

2.2.3. Transport practices

2.2.3.1. Loading, unloading and handling

Facilities and procedures

The loading procedures, facility design and the presence of unfamiliar animals can cause alarm. Also, the way in which pigs are reared can affect the ease of handling during loading. Pigs reared on slatted floors were more difficult to load onto vehicles, showing more balking compared with pigs reared on solid floors, due to the novelty of the floor type (Nanni Costa et al., 2007). The use of an electrical goad increased heart rate over a short time interval, by up to 200 bpm, partly because the pigs were running to avoid the electric shock (Küchenmeister et al., 2005). Averós et al. (2008) reported in their survey on mortality of pigs in five EU countries that the risk of mortality increased as the average time taken to load them decreased. Driessen et al. (2008) studied the influence of olfactory substances on heart rate and lying behavior of pigs during transport simulation. Vibration was found to increase heart rate and ventricular ectopic beats. No dose-dependent synthetic pheromone effects were found and lying time was not affected. Driving style was shown to have an effect on different stress variables (Peeters et al., 2008). Increased accelerations were associated with a higher proportion of pigs standing during the journey. The heart rate variability (HRV) measurement revealed that lateral acceleration was an important stressor for pigs.

Handling

Hemsworth et al. (2002) demonstrated the effects of fear of humans and pre-slaughter handling on the welfare and meat quality of pigs. Fear of humans and the time taken by the pig to move along the final route to the stunning area were positively correlated with the number of highly negative interactions received by the pig prior to slaughter. Moving groups of 5 or 6 pigs at a time was the optimum number for loading. Loading 170 pigs onto a truck took the same amount of time using groups of 5 or 6 pigs compared with groups of 10, and the latter resulted in elevated heart rates (Lewis and McGlone, 2007). The mixing of unfamiliar pigs at loading can increase both transport deaths and carcass damage (Gosalvez et al., 2006).

Loading distances of 47 to 67 m increased the incidence of pigs displaying open-mouth breathing and skin discoloration compared to short distances (< 24 m) (Ritter et al., 2008).

Both aggressive handling and driving pigs long distances during loading adversely affected rectal temperature and blood-acid balance (Ritter et al., 2009). Averós et al. (2007) determined serum stress parameters in pigs transported to slaughter under Mediterranean conditions in different seasons. They found that stress reactions were largely determined by season (higher stress levels during winter) and genetics (depending on the halothane gene). Dalla Costa et al. (2007) evaluated the effect of pig lairage time (3, 5, 7 and 9 h) and position in the truck under summer and winter conditions. It was concluded that increasing lairage time > 3 h had no effect on heart frequency, glucose, creatine phosphokinase (CPK) levels and salivary cortisol but affected blood lactate levels, which increased after 5 and 7 h.

Entire males are more aggressive than females and castrates, which also applies to play fighting behaviour in young pigs. Both aggression/fighting and sexual behaviour/mounting which take place in groups with male pigs, compromise animal welfare as well as meat and carcass quality through skin damage and leg injuries. Recently, agonistic behaviour at feeding and sexual (mounting) behaviour was found to be significantly higher in entire male groups, and mixed male and female groups compared to females alone (Giersing et al., 2006). Current industry standards and codes of practice have attempted to alleviate the problems associated with shipping cull boars for slaughter by requiring individual segregation and detusking, but welfare concerns remain. Cull boars being transported in groups should to be detusked prior to mixing and, if not, must be transported in individual compartments (Hook et al., 2010).

2.2.4. Water and feeding intervals, journey times and resting periods

In considering the duration of the transport, it has been shown that not only long (8 h), but also short (1 h) journeys can affect the welfare of the animals with increased mortalities and pathological findings (Werner et al., 2007). Acute phase proteins were increased and cortisol was unchanged in postmortem samples after long-duration transport (6 h transport and 14 h lairage). The impacts of transport distance and season on losses during transport were recorded between 1997 and 2004 in the Czech Republic (Vecerek et al., 2006). Mota-Rojas et al. (2006) studied the effects of mid-summer transport on pre- and post-slaughter performance and pork quality in Mexico. Based on meat quality and transport losses they concluded that transport should not take more than 16 h in order to improve carcass quality and animal welfare. Assessment of mortality risk factors revealed that average temperature is more important than the duration of the journey (Averós et al., 2008).

Increasing transport duration from 6 to 12 and 24 h increased fatigue in weaned piglets, but was also associated with some indicators of habituation, such as sitting and establishment of dominance hierarchy. Increasing transport duration increased drinking post-transport and blood haematocrit, indicative of rising levels of dehydration and thirst (Lewis and McGlone, 2007). Averós et al. (2009) compared the physiological stress response of piglets during short (0.6 h) and long (8.3 h) commercial transport from rearing to growing-finishing farms. Overall measurements did not show a clear picture of which of the 2 durations was more detrimental for piglet welfare and suggested a different stress response in piglets in comparison to that of older pigs.

Recently, several studies of journey times have focused on the effect on mortality. Werner et al. (2007), analysing data from large slaughter companies in Germany, showed that both long (8 h) and short transports (1 h) increased mortality rates for animals being transported and that the percentages of pathological findings (e.g. circulatory problems, fractures) were more of a problem after short journeys. On the basis of data collected on 2.7 million pigs slaughtered in the USA, Sutherland et al. (2009) found that the effect of journey time on the percentage of dead pigs increased for journeys lasting more than 30 minutes and decreased for journeys lasting between 5 and 11 hours. In a survey of 37 abattoirs in five EU countries, Averós et al. (2008) showed that the risk of mortality in relation to journey duration increased when the pigs had not been fasted before the journey, but that duration had little effect when the pigs had been fasted, even for journeys lasting 24 hours. Kephart et al. (2010) did not observe any association with journey length in deliveries characterised by low mortality rate (0.06%).

Conversely, Ritter et al. (2006) reported that total time from loading to unloading was positively correlated with transport losses (dead and non-ambulatory pigs). Examining records collected through 10 years of pig slaughtering in the Czech Republic, Malena et al. (2007) found that mortality in fattened pigs increased with distance travelled (0.07% for journeys under 50 km, 0.32% for journeys over 300 km), without specifying the conditions during transport.

If pigs miss one or more meals in a 24 h period, they do not compensate for this missed feed intake. When the feed withdrawal is more than 24 h in finishing pigs it is likely to result in catabolism of body stores. Liver glycogen was shown to be completely depleted after 12 and 18 hours food deprivation at the slaughterhouse. Live weight loss was approximately 0.21% per hour. Since pigs do not show clinical signs of starvation within 24 h there is no sense of urgency to reduce the incidence

(Richert and Brumm, 2005). Weight loss attributable to withdrawal of food and water over long journeys represents an economic loss. The range of weight loss in pigs, even in short-term transport, is between 4-6% (Lambooij, 2007). In a study carried out in the Mexican summer, Mota-Rojas et al. (2006) found that weight loss during transport for 8, l6 and 24 h was 2.7%, 4.3% and 6.8%, respectively. However, eight hours of lairage allowed the pigs to regain some weight due to rehydration. Chai et al. (2010) considered the effects of three different transport times (40 min, 3 h and 5 h) on some blood constituents and meat quality. The pigs from the 3 h transport group had significantly lower lactate, red blood cells (RBC), haemoglobin (HGB) and haematocrit (HCT) compared with the shorter or longer time transport groups. Drip loss was found to decrease according to the increase of journey time.

Newly weaned and breeding pigs are also transported for considerable durations but they have not received much research attention. In a recent review, Lewis (2008) stated that the transport of early weaned piglets for up to 24 h is not more detrimental than early weaning with respect to early feed consumption, as both transported and control piglets lose similar body reserves and recover at the same time. However, increasing transport duration from 6 to 12 and 24 h increased fatigue (Lewis and Berry, 2006). Increasing transport duration was also associated with increased drinking post-transport and higher haematocrits, indicative of rising levels of dehydration and thirst. Recently, Averós et al. (2009) compared short (0.6 h) and long (8.3 h) commercial transport of weaned piglets from a rearing to a growing–finishing farm. Cortisol concentrations did not show significant changes in either short or long journeys. According to CPK and lactate dehydrogenase (LDH) activities, some physical fatigue was detected at the end of transport, particularly on the short journeys. However, they were unclear which of the two durations was more detrimental for piglet welfare and suggested that weaners may show different stress responses to transport from those seen in older pigs.

These observations suggest that it is not possible to recommend an "optimal" journey time. Moreover, recent findings confirm that the journey time "*per se*" is unlikely to be a risk factor but it becomes a risk when other aspects related to transport, such as animal fitness, fasting, vehicle design, driving style, stocking density, weather condition, ventilation, etc., are neglected.

2.2.5. Additional provisions for long journeys

2.2.5.1. All long journeys (roof, floor and bedding, feed, partitions)

Animals must be able to stand in their natural position and all must be able to lie down at the same time. For animals that may stand during the journey, the roof must be well above the heads of all animals when they are standing with their heads up in a natural position. This height will ensure adequate freedom of movement and ventilation and will depend on the species and breed concerned (see Regulation (EC) 1/2005). Guardia et al. (2009) evaluated the risk of skin damage due to pre-slaughter conditions. Skin damage increased with on-farm fasting time, loading time and carcass weight. In winter transports the risk decreased with higher space allowance in the lorry.

Dalla Costa et al. (2007) reported no effect on skin bruises at slaughter and on pork quality when comparing a single deck with a double deck. Guardia et al. (2004) found that a rubber floor, when compared with aluminium flooring, reduced the incidence of pale, soft, exudative (PSE) meat.

2.2.5.2. Ventilation for means of transport by road and temperature monitoring

Stocking rates, ventilation and noise have been emphasised in a review on the welfare of cattle, sheep and pigs in lairage (Weeks, 2008). Levels of vocalisation in pigs were considered as potential indices of animal welfare.

Dewey et al. (2009) recorded that the air temperature in truck compartments holding slaughter pigs increased by 0.99 ºC as the environmental temperature increased by 1 ºC, and by 0.1 ºC as the relative humidity increased by 1%. Compartment temperature decreased 0.06 ºC for each increase in driving speed of 10 km/h and increased by 7 ºC with an increase in pig density from 1 to 2.6 pigs/m^2.

A combination of sprinklers and fans can be activated to cool pigs when the temperature within the vehicle is too high (Haley et al., 2008). Fan-assisted ventilation is feasible for vehicles transporting pigs provided that it has adequate capacity, although this is not always the case. Warriss et al. (2006) reported that fan-assisted ventilation was less effective than natural ventilation in allowing the animals to thermoregulate. Pigs had a slightly higher body temperature but did not show changes in their blood composition or muscle glycogen levels (Warriss et al., 2006).

Temperatures within the vehicles are reflected in the ear and rectal temperatures and in the behaviour of piglets. During summer, the temperatures meet the comfort zone (24 to 34 ºC) but not during autumn and winter (Lewis et al., 2005; Lewis and Berry, 2006). A decrease in temperature in the compartment was followed by a decrease of the ear and rectal temperatures in the final 12 h of transport. However, both temperatures remained within normal limits (Lewis et al., 2005). In autumn and winter, resting (lying) frequency is observed to be low initially but increases substantially after 12 h transport. Lying frequency is spread more throughout the transport during summer (Lewis and Berry, 2006). Blood temperature of slaughter pigs measured during exsanguination in the slaughterhouse can be used to monitor changes in the body temperature. Although the measurement would be carried out after the animals may have been subjected to other stresses at the abattoir, it can be used to identify combinations of conditions, handling, mixing, environment and transport (Brown et al., 2007).

2.2.6. Space allowances (transport by rail, by road, by air, by sea, densities)

Floor space can have a major effect on transport losses. Ritter et al. (2006, 2007) concluded that floor space per pig on the trailer and transport conditions can affect pig welfare and transport losses. Losses were minimised at a floor space of 0.462 m^2/pig or greater. Restricted floor space (0.39 m^2/pig) increased creatinine kinase values but not rectal temperature, blood acid-base balance and glycolytic potential (Ritter et al., 2009). However, transport floor space did not affect the percentage of non-ambulatory, injured pigs at the slaughterhouse.

Sutherland et al. (2009) compared space allowances of 0.06 m^2/pig and 0.05 m^2/pig for weaned pigs (5 kg) transported for 60 min during summer. Neutrophil and lymphocyte ratios were increased and lying behaviour was reduced in pigs at 0.05 m^2/pig. Chai et al. (2010) concluded from a study with different transport durations and stocking densities that the most adequate pre-slaughter transport time was 3 h for medium stocking density (less than 275 kg/m^2) under Chinese transport conditions. Petherick and Phillips (2009) suggested space allowances based on allometric equations (Table 2). As a minimum, the space allowance per head determined from the equation area (m^2) $=k0.027xW^{2/3}$ would appear to allow simultaneous lying. However, the authors admit that there are insufficient data to determine whether this allowance would provide sufficient space to adequately access feed and drink onboard a vehicle/vessel. They further point out that these suggestions require validation under different thermal and vehicle/vessel stability conditions. A previous EFSA Opinion (EFSA, 2005) referred to equations for on-farm space allowances for sternal lying (k=0.019) and full recumbent lying (k=0.046), respectively. A k-value of 0.036 is estimated to represent the average floor requirement for lying pigs during the finishing pase under thermoneutral conditions (Ekkel et al., 2003).

Table 2. Area occupied by standing and sternal lying pigs at different live weights (LW) based on the allometric equation of Petherick and Phillips (2009).

Live weight (kg)	Area (m^2) = $0.027 x LW^{2/3}$
10	0.1234
20	0.1950
30	0.2548
40	0.3081
50	0.3570
60	0.4027
70	0.4458
80	0.4869
90	0.5262
100	0.5641
110	0.6007
120	0.6362
130	0.6708
140	0.7044
150	0.7372
160	0.7693
170	0.8007
180	0.8315
190	0.8617

2.3. Sheep transport

In order to find relevant published information in agreement with the Terms of Reference of the mandate a bibliographic search was performed. For the search, a list of key words was created (sheep, lamb*, welfare, stress, transport*) and the year of publication was set up to be after 2003. After the collection of information and data from electronic sources, publications were accepted or not according to previously established criteria. After the first screening, a total of 43 references were obtained dealing exclusively with the welfare aspects of the transport of sheep. Of these, 28 references passed the screening process according to the acceptance criteria and were used for the development of the following section of the Scientific Opinion. Several relevant, pre-2003 papers, which were not referenced in the previous scientific review (SCAHAW, 2002), are also included.

2.3.1. Fitness for transport

Breed, age and previous experience of handling situations affect the magnitude of the stress reaction during transport. Sowinska et al. (2006) reported higher blood cortisol concentration in the Ile de France breed than in Pomeranian lambs, and higher concentrations in 50-day-old lambs than in 100-day-old ones irrespective of the breed. The increased cortisol levels in younger lambs were influenced by the weaning effect.

2.3.1.1. Provisions for all means of transport

Although sheep might show less obvious signs of distress during road transport than other species of farm animals, it is likely to be an aversive experience, since it has been shown to induce similar plasma cortisol and plasma adrenaline responses to other known psychological stressors, such as isolation (Cockram, 2007) and stress-induced hyperthermia, manifested by an increase in core body temperature (Ingram et al., 2002, Lowe et al., 2002). However, plasma cortisol concentration declines within a few hours and was often near to, or at, pre-treatment values by the end of a 24 h journey, although these values will also be influenced by other factors such as the experience and condition of the animals, the driving events during the journey, and the duration of transport. Cockram (2007) concluded that there is not yet sufficient evidence to specify maximum journey times and more emphasis should be placed on the quality of the journey rather than focusing exclusively on duration.

Sheep spend most of a 7 h journey standing rather than lying down, but the amount of lying behaviour increases with journey duration (Cockram et al., 2004). Sheep always endeavour to stand in a vehicle in such a way that they brace themselves to minimise the chance of being thrown around, and avoid making contact with other individuals. They do not lean on other individuals and are significantly disturbed by too much movement or too high a stocking density. Therefore, driving events, such as acceleration, braking, stopping, cornering, gear changes and uneven road surfaces can have a major influence on welfare by affecting the risk of injury and by disturbing the ability of the animals to rest and ruminate during the journey. Cockram et al. (2004) concluded that about 80% of the losses of balance during transport could have been caused by driving events, although only about 22% were actually followed by a loss of balance. Sheep not subjected to frequent disturbance, and with optimal conditions, such as a low stocking density and adequate litter, as well as uninterrupted sections of a journey on a motorway (where there are few driving events), can provide an environment in which sheep lie down and ruminate, thus substantially reducing plasma cortisol concentrations. Training and education for drivers to promote careful driving would improve the welfare of animals in transport (Cockram et al., 2004).

During transport, there is no obvious preferred orientation adopted by sheep in relation to the direction of travel (Cockram et al., 2004). However, fewer sheep spent the greatest percentage of their standing time during a journey orientated towards the direction of travel than either across or backwards to the direction of travel. The significance of this is unclear.

2.3.1.2. Additional provisions for transport by sea

Small ruminants are mainly transported by sea between Australia, New Zealand and the Middle East countries. Most of the intra-EU transport from islands, such as from Sardinia to mainland Italy or from the UK to mainland Europe, involves the use of roll-on roll-off truck transport. Australia conducted industry-funded research on the ventilation efficiency of ships and its importance in reducing the effects of heat stress on sheep (MAFF, 2003). This work has enabled the introduction of a scientifically-based risk assessment model to manage the problems of heat stress and salmonellosis in consignments travelling during Australia’s winter months. It was important in achieving improved outcomes for livestock exports to the Middle East in 2003, a year when there was only one occasion on which sheep mortality reached 2%; the level required to be reported to the Australian Maritime Safety Authority (AMSA). The industry proposed to extend this work to consider other risk factors, such as those associated with the preparation and transport of livestock, including watering and feeding regimes, culling practices and transport and handling approaches. Overall sheep mortalities declined from 1.34% in 1999 to 0.79% for the year to September 2003.

2.3.2. Transport practices

2.3.2.1. Loading, unloading and handling

De la Fuente et al. (2010) found higher plasma cortisol and LDH in unweaned lambs at the conclusion of 30 min transport compared with 5 h. This suggests that loading and initial transport caused a significant stress response in suckling lambs that was reduced over the time course of the journey. Lambs transported over the shorter duration did not have sufficient time to habituate to the transport conditions.

Blackwood and Hurst (2001) reported 25% bruising in sheep before slaughter. The main causes of bruising were one sheep riding up onto the one in front when drovers push them to move faster than they can go, and reluctant sheep being pulled (by drovers) by the wool to move them forward.

2.3.2.2. Space allowances

There are two opposing views about the space required for sheep in transport. One is that they should be transported at low space (high stocking density) so that they can avoid slipping or falling by bracing themselves against each other. The other is that they should be transported at high space (low stocking density) to avoid slipping or falling by adopting an independent, wide stance to brace against the motion of the vehicle (Jones et al., 2010). Currently, the legislative limits for space allowance given to sheep in transport are defined for sheep weighing less than or greater than 55 kg and whether they are fleeced or shorn (Regulation 1/2005). Furthermore, for small lambs, an area of under 0.2 m^2 per animal may be provided. These recommendations might be considered inadequate (Broom and Fraser, 2007; Petherick and Phillips, 2009) as they take no account of variation in animal weight and can lead to unfeasibly large numbers of permissible sheep per pen, and this is not practical, (Jones et al., 2010). Allometric equations of the form $A = kW^{2/3}$, where A is the area in m^2, k is a constant and W is the liveweight in kg, can be used to estimate the volume of space an animal occupies as a function of its mass, whilst varying k can estimate space according to whether the animal is standing or lying (Petherick and Phillips, 2009). The value of the constant k will be determined by the spacing strategy of the sheep, whether they want to lie in transport, and whether all animals need to lie at the same time (Petherick and Phillips, 2009). Jones et al. (2010) examined space requirements by measuring the incidences of loss of balance, slipping and falling at different space allowances during transport and the extent to which sheep show evidence of choosing to brace themselves against each other or to stand independently. Four categories of sheep, shorn and fleeced ewes and lambs, were transported at five space allowances on standard journeys of 6 h. Minimum space allowance was taken from the legislation, whilst medium-low, medium-high and high allowances were calculated from the allometric equation $A = kW^{2/3}$ (W: average liveweight/pen and k: empirical constant) where k values were 0.021, 0.026, and 0.037, respectively. Fleeced animals were given an additional 25% space. A control group providing more than 1 m^2/animal was also included. Results showed that sheep transported at control and high spacing suffered fewer losses of balance and slips than sheep transported at low and medium-low spacing, especially on roads with rough ride characteristics. Rates of falling were highest for shorn sheep in the low and medium-low spacing, where sheep were also forced to the floor by their pen-mates and unable to stand immediately. Sheep transported at control and high spacing were seen to stand close to, but not touching their pen-mates, bracing themselves against the motion of the vehicle by spreading their feet, not by leaning on their pen-mates. They were also seen to lie in transport at higher space allowances. The results suggest that if given sufficient space, sheep stand close to but not touching their pen-mates in transport and are able to support themselves against the motion of the vehicle better if loosely packed than tightly packed. The space provided by minimum legislation and calculations with a k value of 0.021 are unacceptable, as they do not allow the sheep to adopt their preferred spacing strategy and, therefore, lead to more losses of balance, slips and falls. Calculation of the space required from allometric equations is proven appropriate. The recommended empirical coefficient (and space allowances) for journeys of 6 h with a mix of road types is: (i) shorn ewes, k = 0.026 (0.44 m^2 for 67 kg), (ii) fleeced ewes and lambs, k = 0.033 (0.56 m^2 for 65 kg, 0.4 m^2 for 40.5 kg), and (iii) shorn lambs, k = 0.029 (0.3 m^2 for 32.5 kg).

However, the effect of transport on suckling lambs could be different from that described in older lambs and sheep, even for short journeys. Ibañez et al. (2002) analysed the effect on suckling lambs of two stocking densities (high, 8 lambs/m^2; low, 4 lambs/m^2) during short transport (30 min, 40 km). At a body weight of 10 kg these correspond to k values of 0.026 and 0.052, respectively. The number of lambs standing was lower at low density, whilst the number observed walking was greater at low than at high density. This may be because at the higher space allowance the lambs were more likely to adjust to movements of the lorry by moving to retain their balance. The higher LDH activity observed at the lower stocking density may reflect the greater amount of exercise of these lambs.

2.3.3. During transport

2.3.3.1. Water and feeding interval, journey times and resting periods

Water deprivation presents a more acute hazard to welfare than food deprivation, given the risk of dehydration. Krawczel et al. (2007) determined the efficacy of rest stops recommended for improving welfare in lambs transported in low density (0.23 m^2 per lamb). They compared continuous transport for 22 h, transport for 22 h with the prescribed rest stops (transported for 8 h, unloaded and rested for 6 h, transported for 8 h, unloaded and rested for 24 h, transported for 6 h; for a total journey duration of 52 h), and remaining in the home pen. Food deprivation in the continuously transported lambs was reflected by a decrease in bodyweight and plasma concentrations of glucose, and an increase in plasma concentrations of blood urea nitrogen, creatinine, and total bilirubin relative to rested or control lambs. The loss in bodyweight for the lambs transported continuously for 22 h was still significant 8 d after transport. Cockram (2007) also reported mobilisation of body energy reserves in response to an energy deficiency after 24 h fasting. Access to food and water during the intermittent rest periods was sufficient to prevent rested lambs from experiencing the same decrease in bodyweight as the lambs during continuous transport, and eliminated the physiological indicators of food deprivation (Krawczel et al., 2007).

Although the continuously transported lambs also experienced water deprivation and a peak temperature of 39.6 ºC during transport, results of electrolyte analysis did not indicate that they were dehydrated at the conclusion of 22 h transport. When water was offered from buckets to lambs after 14 h of continuous transport (Krawczel et al., 2007), the majority were not interested in the water offered on the trailer, and the few that showed interest appeared only to investigate the novelty of the bucket rather than consume appreciable amounts of water. Furthermore, when access to food and water was provided during the rest stops or after their final unloading, sheep still went to water only when grain was provided during rest stops or after their final unloading (Krawczel et al., 2007). However, it is recognised that many sheep will not drink water from unfamiliar sources in novel environments. Furthermore, ingestion of dry feed after transport can contribute to dehydration. Thus after a period without access to feed and water during transport, sheep must be allowed sufficient time to drink after eating and before a subsequent journey is undertaken.

Sheep raised in arid environments are extremely well adapted to minimise water loss in urine and faeces (Krawczel et al., 2007). However, under conditions of heat stress, their main mechanism of thermoregulation is through evaporative heat loss by thermal panting. This will inevitably increase the risk and the rate of dehydration. Jacob et al. (2006) measured urine specific gravity (SG) as an indicator of hydration status and observed that up to 50% of lambs slaughtered in two Australian abattoirs over one year had urine SG values indicative of some dehydration. The SG values were higher in unweaned than weaned lambs; highest in August and lowest in January. In other studies of food and water deprivation during transport, measurements of haemoconcentration [e.g. total protein, packed cell volume (PCV), osmolality] were elevated following water deprivation, thus indicating some level of dehydration (Fisher et al., 2008). However, the level of dehydration was not classed as being of clinical concern as many of the other measurements were still within normal physiological ranges (Ferguson and Warner, 2008).

Factors associated with transportation, in addition to water deprivation and high ambient temperatures, for example, adrenocorticoid stress responses (Parker et al., 2003), might increase the risk of dehydration in transported sheep. Furthermore, if an animal did experience dehydration during a journey, it might be less able to respond effectively to other environmental challenges, such as an increase in environmental temperature during a journey. Alamer and Al-hozab (2004) observed reduced sweating in sheep kept for up to 3 days without water at an air temperature of 36 ºC and 22%

relative humidity. Although water deprivation did not affect the increase in respiration rate (an important part of the mechanism by which sheep lose heat from evaporation), sheep that had been deprived of water had higher rectal temperatures (about 40 ºC) compared with sheep with access to water (39.3 ºC). However, Lowe et al. (2002) were not able to demonstrate an additional effect of water deprivation for 12 h on the rectal temperature and respiration rate of sheep exposed to an air temperature of 33 ºC and relative humidity of 85–100%.

Journey times

Sarozkan et al. (2009) determined the weight loss and subsequent recovery rate of yearling lambs transported for 3, 6, 9 and 19 h (transported 9 h+1 h resting+9 h) at a density of 0.35 m^2/lamb. Results revealed that the lambs transported for 3, 6 and 9 h lost more live weight than untransported lambs, the losses increasing with increasing journey duration. Fisher et al. (2010) determined the responses of healthy sheep to road transport under good conditions for 12, 30 or 48 h without access to feed and water on board the vehicle. Increasing transport duration resulted in lower body weight and increased haemoconcentration at arrival, but these effects did not exceed clinically normal ranges for any transport duration, and sheep generally recovered to pre-transport values within 72 h of arrival. Much of these changes in bodyweight were likely to be related to losses and recovery in gut fill. There was no consistent effect of transport on blood urea nitrogen and β- hydroxybutyrate concentration, suggesting that the transport-associated feed withdrawal periods did not result in significant protein catabolism and sheep were not clinically compromised. There were no effects of transport duration on plasma cortisol concentrations and fatigue levels, assessed through animal lying times during the first 18 h after arrival. These reports demonstrated that healthy adult sheep, transported under good conditions can tolerate transport durations and associated feed and water withdrawal periods of up to 48 h, without undue compromise to their welfare. Cockram (2007) suggested that if care is taken only to select animals fit for transport, the environmental conditions (including driving style, road conditions, vehicle design and operation, space allowance, thermal conditions and ventilation), and the pre- and post-transport handling of the animals are optimal, it may be possible to transport certain types of animals over long distances without major welfare problems.

After the initial psychological stressors of loading and the transport environment, increasing transport duration may challenge welfare through fatigue. Cockram et al. (1997) showed that lying behaviour increased during a 24 h road journey. It is uncertain, however, whether an increase in lying behaviour represents an adaptation to the environmental conditions (‘rest’ as a coping mechanism) or ‘exhaustion’ (failure to cope).

Tadich et al. (2009) studied the effect of weaning followed by a 48 h transport to slaughter that included road transport and a sea-ferry crossing in a three-deck truck with a space allowance of 0.2 m^2/lamb without access to food and water. The handling procedures related to rounding up and weaning before transporting the lambs induced high initial plasma concentrations of cortisol, packed cell volume, glucose, lactate and activity of creatine kinase (CK). Lactate and CK decreased after transport for 48 h, whereas cortisol increased. The plasma concentrations of haptoglobin and β-hydroxybutyrate increased immediately after rounding up and weaning, reflecting the effects of the long-term stress and fasting. Lairage for 10 h after transport decreased the concentrations of cortisol, glucose, CK and lactate, but not packed cell volume attributable to dehydration and sympathetic-adrenal stimulation, or haptoglobin. Plasma β-hydroxybutyrate increased in concentration throughout the study, reaching the highest values after resting and at bleeding. This is an indicator of prolonged lack of food, or indeed starvation, in which functional body tissue is being utilised. The lambs used in the study were in a transition period from suckling to full ruminants, therefore during the fasting period to which they were submitted they would have needed to mobilise body fat reserves earlier than adult sheep that had a greater reserve of fermentable energy from the rumen. It was concluded that the commercial procedures of weaning and prolonged transport immediately after in lambs destined for slaughter are stressful and exhaust body reserves.

Resting periods

Krawczel et al. (2007) concluded that off-trailer rest stops with feed and water during long distance transport at high ambient temperatures eliminated signs of food deprivation and maintained bodyweight but did not alleviate transport stress and evidence of immunosuppression. Furthermore, off-loading can increase the stress associated with handling, loading, unloading, and possibly social changes, after exposing the animals to another novel environment. Krawczel et al. (2008) observed how lambs utilised two consecutive rest periods in novel environments with access to food and water that occurred during 22 h of transport. They concluded that lying down had a greater priority than eating during the second (24 h) rest period.

In an effort to promote standardisation and improve product quality in lamb production, some new intermediate steps have been introduced prior to slaughter, including cooperative classification centres (Miranda de la Lama et al., 2009). Lambs are taken from their original farm to classification centres, where they are classified and kept for hours or days before continuing to the slaughterhouse, depending on whether new lambs need to be fattened to the required commercial slaughter live weight. As a result, animals often require two journeys before arriving at the abattoir instead of one, with variable stay times at the classification centres. In the classification centres the lambs are regrouped after classification according to live weight. The average weight of lambs on arrival was 19.6 kg, and on departure 25.6 kg, with a staying time of about 21 days (minimum 1 week, maximum 4 weeks). More than 20% of the lambs arrived at the classification centres at the required slaughter live weight so were on-transported a second time to the abattoir (i.e. no fattening stage at the classification centre was necessary). There are several commercial and productive reasons why classification centres are useful in the lamb meat production chain, including simplification of farm management, scarcity of specialised man power and product standardisation. These developments can increase efficiency but increase the incidence of pre-slaughter stress, affecting their physiological state, even under optimal commercial conditions (Miranda de la Lama et al., 2010). Adding classification centres in the logistic chain forces the need for two journeys, normally with several loading stops, that increase journey times and the number of times lambs are handled, mixed and exposed to a novel environment. The stay times in the classification centres and the season have a significant effect on the stress variables. All lambs taken to the classification centres had increased adrenocortical activity, compared with similar animals sampled at the farm. Cortisol levels were higher in lambs which underwent two journeys in the same day, suggesting that classification and transport could be an acute stress. On the other hand, the authors also reported higher levels of cortisol, lactate and white blood cells levels with the increasing stay times. In winter, cortisol and glucose levels were higher, while in summer CK was highest. However, it is unclear whether the increased levels of cortisol were due to handling stress or just a metabolic adaptation to the cold environment. In winter, lambs may have increased levels of cortisol due to their increased metabolic rate to produce heat (to maintain their body temperature within the thermo-neutral range). In any case, the biological cost of this response is aggravated by the action of other stressors associated with new housing and management conditions.

2.3.4. Additional provisions for long journeys

2.3.4.1. Ventilation for means of transport by road and temperature monitoring

For a given stocking density and vehicle design, the temperature-humidity index (THI) inside the transport vehicle generally increases when vehicles are stationary in proportion to the duration of the stop. Fisher et al. (2005) reported that during journeys in summer, the stationary periods and the increase of external climatic temperature (>25 ºC) could induce thermal stress and be detrimental to the welfare of sheep. In this study, during stationary periods, 34% of THI readings exceeded 75, and, on average, the THI increased by 0.16 for every minute of a stationary period. Therefore, lack of air flow is a critical factor and sheep transport vehicles should not be placed where air flow is absent or minimal, and duration of stops should be minimised where possible.

2.4. Goat transport

In order to find relevant published information in agreement with the Terms of Reference of the mandate a bibliographic search was performed. For the search, a list of key words was created (goat* kid, welfare, stress, transport*) and the year of publication was set up to be after 2003. After the collection of information and data from electronic sources, publications were accepted or not according to previously established criteria. After the first screening, a total of 41 references were obtained dealing exclusively with the welfare aspects of the transport of goats. From these, 33 references passed the screening process according to the acceptance criteria and were used for the development of the following section of the Scientific Opinion. Several relevant, pre-2003 papers, which were not referenced in the previous scientific review (SCAHAW, 2002) are also included. SCAHAW (2002) and EFSA (2004a) contained no specific recommendations concerning the welfare of goats during transport. Therefore, in the case of goats, all evidence is new evidence.

2.4.1. Fitness for transport

Transportation can cause emotional and physical stress in goats that can affect their welfare (Richardson, 2002). Handling, loading and unloading, noise, vibration, and social disruption can cause significant changes in the stress responses of goats, such as increased plasma concentrations of cortisol, glucose, and non-esterified fatty acids (NEFA). The magnitudes of cortisol and glucose responses to stressor treatment are greater in older goats (Kannan et al., 2003). Behaviours such as freezing, vocalisation, kicking, struggling and escape attempts observed in goats during transportation are behavioural indicators of discomfort (Kannan et al., 2002).

2.4.2. Additional provisions for transport by sea

Goats are transported by sea from Australia to the Middle East Countries on journeys exceeding 10 days, mainly at the time of the Hajj. More and Brightling (2003) reported average mortality rates of 1.4%. Animals at greatest risk were adults (>40 Kg) and those unaccustomed to being fed in a managed production system. They recommended that only younger animals between 22-40 kg that had been reared in a managed production system should be considered fit for travel.

2.4.3. Transport practices

2.4.3.1. Loading, unloading and handling

Goat behaviour is quite different from sheep behaviour. Goat herds establish a stable and linear hierarchic order (Barroso at al., 2000), maintained by agonistic and affiliative social interactions among individuals. Mixing unknown animals alters this social hierarchy and can lead to an increase in agonistic behaviour (Addison and Baker, 1982; Andersen et al., 2007), expressed as aggression with contact, such as biting, bumping, or aggression without contact, leading to threat displays, chases, and escapes (Alvarez et al., 2007). However, aggressive interactions have been shown to decrease dramatically 24 h after mixing (Alley and Fordham, 1994). In intensive goat production systems, the levels of aggression are higher than they are in semi-intensive or extensive farming systems, probably because of differences in the amount of space available, which is more limited indoors (Barroso et al., 2000). Therefore, the establishment of a new hierarchical order in the new environment experienced during transportation aggravates behavioural patterns and can increase aggressive behaviour in dominant goats, leading to more attacks and possible injuries (Ayo et al., 2006).

The establishment of the dominance order in goats is influenced by individual characteristics, such as aggressiveness, age, size, body weight, breed, sex, parentage, experience, the presence of horns and horn length (Miranda de la Lama et al., 2010). In situations where individual distances are reduced and goats have fewer opportunities to perform butting activity, biting became a more frequent means for goats to maintain individual distances (Tolu and Savas, 2007).

During handling, goats are more reactive than sheep, because they are more aggressive (i.e. when they are attacked, goats tend to face the attacker, but sheep usually flee) and they exhibit more exploratory behaviours, whereas sheep are more fearful and shy (Miranda de la Lama, 2010). When goats are reared under an extensive management system, with little or no contact with the stockman, the behaviours that constituted a threat to the goats (slips, falls, and jumps) were significantly higher during handling than loading (Minka and Ayo, 2007). Overall, the result of the behavioural events per goat and time taken to unload each goat showed that the unloading procedure is less stressful than handling or loading (Minka and Ayo, 2007).

Early contact with humans and gentling treatments can improve the human–animal relationship and result in tamer animals that exhibit less fear and, therefore, are easier to handle during transport. Boivin and Braadstad (1996) observed that gentled kids were calmer, more easily approached by humans and, when isolated, were less frightened than were non-gentled kids.

2.4.3.2. During transport

Short-term transport can cause noticeable changes in stress responses and muscle metabolism in goats. Kannan et al. (2003) studied the effects of 2 h transport on physiological responses and meat quality in young (6 to 12 months old) and old (24 to 30 months old) goats. The goats subjected to transportation had higher plasma cortisol, glucose and non-esterified fatty acid concentrations than goats that remained unstressed in holding pens. The cortisol concentrations increased markedly within 1 h of beginning transportation, and remained at a higher level until completion of the journey. Kannan et al. (2000) reported that plasma glucose concentrations remained elevated for about 3 h in Spanish goats after 2 h transportation, whereas Nwe et al. (1996) observed a similar trend in Japanese native goats after 6 h transportation.

There are two kinds of transportation stress factors. The “short-acting” factors tend to have emotional effects on animals and the “long-acting” factors have physical effects and may accumulate over time (Richardson, 2002). Rapid and short-acting factors include fear due to unfamiliar surroundings, unstable footing on the floor of the moving vehicle and being deprived of sure footing on the ground.

The response of goats to being transported starts at the beginning of the ride with a startle reaction to the novelty of ‘being moved’. The way the vehicle is driven has a significant effect on the goats’ stability and balance, and may cause goats to fall down. Braking and cornering cause 75% of falls, and crossing bumps and acceleration account for the other 25% (Richardson, 2002). A rough start causes hormones and blood components to fluctuate and may increase heart rates up to twice the normal rate (Richardson, 2002). Controlling the reaction of the goats at the start of transportation will reduce the stress response.

Das et al. (2001) observed the standing orientation and behaviour among a group of meat type goats during transport by road. During a 50 min road journey, the most frequent standing orientation adopted by goats was parallel to the truck's direction of travel (24 min) followed by diagonal orientation (12 min) and perpendicular orientation (9 min). The goats were not observed to orientate themselves opposite to the truck's direction of travel. During transportation, goats changed their orientation frequently, apparently to maintain balance, suggesting they are restless.

Slow and long-acting factors include noise, vibration, forceful contact with the vehicle and/or other animals, lack of exercise, prolonged standing, insufficient consumption of water and feed and environmental temperature and humidity (Richardson, 2002). The noise level in livestock trailers is often high and, in the study by Richardson (2002), it had a greater effect on releasing stress related hormones than motion.

In goats, the thermoneutral zone is 12–24 ºC (Nikitchenko et al., 1988) and the upper limit of heat tolerance 35 to 40 ºC (Appleman and Delouche, 1958). Transportation during thermally stressful hot-dry seasons may overtax their homeostatic control mechanisms (Igono et al., 1982; Minka and Ayo, 2007), and may have longer-term negative effects on health status and productivity (Ayo et al., 2006). Overcrowding will exacerbate heat stress. Animals standing with their necks extended and with open-mouthed breathing indicate severe heat stress.

Kadim et al. (2006) investigated the effects of short duration transport stressors at high ambient temperatures on physiological parameters in three breeds of Omani goats. Transported goats had significantly higher plasma cortisol, adrenaline, nor-adrenaline, and dopamine levels than controls in holding pens with feed and water provided *ad libitum*.

Heat shock proteins (HSP) have been proven to play a key role in protecting stressed cells and organisms, and preventing or reversing disorders caused by stress (Barbe et al., 1988). Feed restriction (Zulkifli et al., 2001) and confinement in crates (Zulkifli et al., 2009) may also elicit an HSP response. Zulkifli et al. (2010) concluded that transporting goats for 3 h in a hot, humid tropical climate altered HSP 70 expression. However, further studies are warranted to investigate whether the increase in HSP 70 actually protects the goats against the adverse effects of road transportation.

Ayo et al., (2006) suggested that the oral administration of ascorbic acid (AA) at a dose of 100 mg/kg body weight prior to transportation may reduce the amount of stress induced in animals during road transportation procedures. Ascorbic acid is known to inhibit cortisol release and is also a powerful antioxidant that scavenges reactive oxygen species (ROS) generated in the body due to stress (Balz, 2003). Treating goats with AA prior to transportation significantly reduced dehydration, haemoconcentration and body weight loss (Minka and Ayo, 2007).

Separation during transport

Goats are very gregarious, prefer to stay close together, and individuals are rarely seen apart from the group (Ross and Berg, 1956). Transport in isolation from other goats induced large increases in cortisol, glucose and non-esterified fatty acids (Duvaux-Ponter et al., 2003; Kannan et al., 2002). There was a greater elevation of cortisol concentrations when goats were not able to maintain visual contact with other animals and the longer they remained in isolation, the greater the emotional stress (Richardson, 2002). Alarm vocalisations are indicators of social isolation (Boivin and Braadstad, 1996), and consist of high-pitched sneezes, which are often accompanied by visual signals, such as stamping (Houpt, 2005). When isolated on trucks, goats do more rearing and vocalising than when isolated in their home pens (Richardson, 2002).

2.4.4. Water and feeding interval, journey times and resting periods

Water

The new environment during pre-slaughter holding and social isolation may be a stronger stressor than feed and water deprivation for goats (Richardson, 2002). Several studies have confirmed differences between sheep and goats in their water consumption and water conservation capacities. Mutton Merino lambs had a 49% higher water intake per kg mass gain than Boer goats (Ferreira et al., 2002). Higher water turnover rates were also found in sheep compared with goats kept under tropical conditions in Nigeria (Aganga et al., 1989). The lower water turnover rates in goats suggest that goats are better adapted to withstand dehydration than sheep under dry climatic conditions (Silanikove, 2000). Goats very rarely drink water during the pre-slaughter holding period. However, withholding of feed coupled with dehydration can cause live-weight shrinkage as high as 10% in the summer (Richardson, 2002).

Food

Prolonged feed deprivation may alter plasma triiodothyronine (T3) and tetraiodothyronine (T4) concentrations and elevate plasma urea nitrogen and NEFA levels in goats (Kannan et al., 2003).

Resting

Kannan et al. (2000) studied the live weight loss and stress responses in goats at two different stocking densities (0.18 v. 0.37 m^2/animal) during 2.5 h transportation and 18 h holding without food. Cortisol values began decreasing within 3 h of transportation, irrespective of the stocking density. However, leukocyte profiles suggested that transportation stress may have had a prolonged effect that could affect their immunocompetence. Postmortem examinations have indicated that goats become susceptible to respiratory infections after prolonged journeys under adverse weather conditions. Therefore, prolonged holding of animals, especially in hot weather, could increase stress in animals due to extended feed deprivation.

2.5. Cattle transport

2.5.1. Fitness for transport

The broad criteria that cover fitness for transport are adequately defined in Annex I of Council Regulation (EC) 1/2005. Stresses associated with mixing, handling and loading are likely to be influenced by the quality of previous experience and careful handling. Davidson and Beede (2009) demonstrated the merits of preconditioning cattle to the potential stresses of handling and transport.

2.5.2. Transport by sea

The impact of sea transport on animal welfare has been documented by Barnes et al. (2008). Norris and Norman (2004) and Norris et al. (2003) identified heat stress as a major cause of poor welfare and increased mortality of cattle transported by sea. More (2002) reported 28.5% mortality in unacclimatised cattle transported by sea from Australia to Saudi Arabia and presented convincing evidence to conclude that this could be attributed almost entirely to heat stress. A heat stress risk management model (Stacey, 2003) was developed, which used all available data on ships, weather conditions, voyages, and animal factors such as heat stress thresholds for different classes of animals and stocking rate to determine and, therefore, reduce the risk of a heat stress incident. The heat stress threshold was determined as the prevailing wet bulb temperature at which the animal’s core body temperature was 0.5 ºC above normal. The provision of electrolytes for the animals was advocated as a measure to reduce adverse effects in heat-stressed cattle.

Additional provisions for transport on roll-on roll-off vessels

Earley et al. (2007) studied the effects of transporting 40 young cattle (heifers, average weight 245 kg) by truck from Ireland to France on a roll-on roll-off ferry at a stocking density of 0.93 m^2/animal and then by road for 9 h to a French lairage, by comparison with 20 that remained in Ireland as controls. Transported heifers lost 6.2% of their live weight while control heifers lost 2.1%. During the sea crossing (22 h) from Ireland to France, heifers spent 39% of the time lying. Neutrophil numbers were greater ($p<0.05$) at day 6 in heifers remaining on the transporter (in France) than in those that were unloaded. Using this limited range of measurements, no substantial difference was found between unloaded animals and those that stayed on the transporter during the rest period.

As reported in SCAHAW (2002), heat stress can present a major threat to cattle welfare that can, in extreme cases, result in mortality in transport. The problem is exacerbated when the vehicles are stationary for prolonged periods within the hold of roll-on roll-off vessels. Provision of forced fan ventilation can prevent exposure to excessive heat and humidity on both moving and stationary vehicles.

2.5.3. Transport by air

A limited number of livestock, usually only those of high value, are transported by air (Knowles and Warriss, 2000). Recommendations for the transportation of live animals by air are detailed in the

IATA Livestock Transportation General Guidelines (IATA, 2010) that address both safety and animal welfare, and they are updated annually to take account of the latest research findings. Australian Standards for the Export of Livestock (2008), version 2.2 December 2008 Standard 6 Air Transport of Livestock, includes the following criteria as elements that will contribute to ongoing fitness for travel on long journeys by air:

- Treatment for internal and external parasites;
- Allowing the animals to mix before loading to establish social hierarchy;
- Allowing the animals to become accustomed to being handled and to close confinement;
- Allowing animals to become accustomed to the type of feed.

2.5.4. Transport practices by road

2.5.4.1. Loading, unloading and handling

During the assembly of cattle prior to transport, animals will be exposed to numerous stimuli that could potentially prove stressful: unfamiliar cattle, confinement in yards, novel food delivered in a novel way, and novel-tasting water delivered in novel ways (Petherick and Philips, 2005).

Adverse factors can combine to make the journey very stressful to the animals (Hartung et al., 2003; Minka and Ayo, 2007). In many transports, the physiological (e.g. heart rate) and biochemical (e.g. cortisol) indicators do not exceed normal clinical limits, except during loading and unloading (Hartung et al., 2003). One indicator of welfare problems during handling, loading and unloading is the quality of the skin and meat after slaughter. The biochemical changes in muscle, especially in glycogen metabolism, are affected by the responses of the animals to various handling conditions before slaughter. Cattle in such extreme conditions are prone to develop dark, firm and dry (DFD) meat when insufficiently supplied with energy (glycogen). However, under usual transport conditions this is relatively rare (Hartung et al., 2009).

Tied cattle are more stressed by transport, than cattle from group housing, which show lower NEFA and ß-hydroxybutyrate levels indicating lower energy consumption (Holleben et al., 2003).

2.5.4.2. Vehicle driving quality

As discussed by SCAHAW (2002) and Broom (2008), careful driving, especially on bends and corners on route, and during acceleration and braking, have a substantial effect on the welfare of cattle. If driving standards are not good, animals may be thrown around or have difficulty maintaining their balance and spurious results can be obtained in comparisons of the effects of stocking densities on welfare.

2.5.4.3. Separation and space allowances

Separation

Isolation of cattle induces struggling, vocalisation, increased heart rate and plasma cortisol levels (Færevik et al., 2006). The presence and sight of conspecifics are found to moderate the behavioural reaction of cattle to separation. The calming effect of familiar animals should be taken into consideration during transport, handling and regrouping of cattle. Vocalisation can be a useful indicator of impaired welfare for both experimental and practical purposes (Watts and Stookey, 2000).

Group partitions

Lapworth (2008) found that many journeys for cattle were of more than 500 km. On long journeys, for example 2,000 km, when cattle were allowed to rest once or twice on the vehicle and were given access to feed and water, the optimal design for partitioning involved pens running the full width of the vehicle i.e. two cattle pens per deck in a 12.2 m long trailer. These provided more room for movement and to escape aggressive actions. In addition, this arrangement allowed faster loading and unloading and animals could settle better on trucks. When vehicles were partitioned at appropriate lengths and driving was good, the cattle were not caused injury or unnecessary suffering by being thrown about by the motion of the vehicle. Appropriate partitions are of rigid construction, strong enough to withstand animal weight and constructed and positioned so as not to interfere with ventilation.

Space allowance

There has been considerable debate as to whether cattle should be transported at a relatively high stocking density, so as to minimise movement and prevent falls while the vehicle is in motion, or given sufficient space to enable them to lie down and stand up without the risk, or fear, of injury (Petherick and Phillips, 2009; Mounaix et al., 2011, in press). The behavioural response of cattle to overcrowding in transport is well documented as individual animals may become recumbent on the vehicle floor and struggle or be unable to regain their footing. The situation was described as going down underfoot or involuntary recumbency (Whiting, 2000). Cattle avoid contact with other individuals when they can and maintain their balance better when not touching other individuals (Cockram, 2007; Broom, 2008). Several authors have explored the possibility that giving cattle more space than required by the regulations may increase the risk of injury (Eldridge and Winfield, 1988; Tarrant and Grandin, 2000; Mounaix et al., 2011, in press). However, these results are equivocal and did not take account of driving quality. Where driving quality is good, animals at higher stocking densities are neither more nor less likely to fall or to be injured. Tarrant et al. (1992) found that the frequencies of falls and bruising and the plasma concentrations of cortisol and creatine kinase all increased with stocking density. Problems caused by losses of balance associated with braking are less critical for very young calves, which lie down for most of the journey. Brüser-Pieper (2006) transported 517 Holstein Friesian fattening bulls (average weight 545 kg) in 15 journeys over approximately 1,350 km from the north of Germany to Trieste in Italy between July and October at stocking rates of 1.41 m^2/animal (according to EC Regulation 1/2005). The transport times varied between 22 and 27 hours. None of the animals showed any sign of clinical disease. Directly after unloading, biochemical indicators such as cortisol, NEFA and haematocrit stayed within normal clinical levels, while the energy consumption increased as indicated by a slight increase of glucose and hydroxybutyrate (energy mobilisation from reserves). Creatine kinase increased significantly after transport from below 200 to 800 IU/L. This increase continued in the resting pens to 1,500 IU/L by rank fighting and unrest. The space allowance given in EC Regulation 1/2005 seems to be sufficient for slaughter bulls. Nevertheless, for breeding animals like pregnant heifers, lower stocking densities are used in practice (pers. observation J. Hartung).

During three transports in August, temperatures above 30 ºC were reached for a few hours, and for short periods of time the temperature-humidity index (THI) reached 79 and slightly higher without causing visible suffering of the animals. Critical situations occurred when the vehicle was standing in the sun at high ambient temperatures. The THI statistically influenced the levels of cortisol, triiodothyronine (T3), glucose, potassium, haematocrit, magnesium, creatine kinase, but not NEFA and β–hydroxybutyrate. Forced ventilation can help to reduce the heat load at high ambient temperatures.

Methods for calculating space allowance for transported animals are discussed in the SCAHAW and EFSA reports on the welfare of animals during transport and by Broom (2003, 2007 and 2008). When specifying space allowances for cattle in transport one can make use of the allometric equation:

$$A(m^2)=kW(kg)^{2/3}$$

This equation relates body volume (A) to mass (W). The UK Farm Animal Welfare Council (FAWC, 1991) proposed the equation $A=0.021W^{2/3}$ m^2 to define minimum acceptable floor space for cattle. For cattle of 400 kg this calculates a space requirement of 1.16 m^2/animal.

Petherick and Phillips (2009) have applied two allometric equations (area = $kW^{2/3}$ and linear dimension = $kW^{1/3}$) to interpret findings from research on the welfare of livestock during transportation where space requirements have been expressed per unit of body weight (kg). Values of k for cattle standing and lying become 0.019 and 0.027, respectively. Table 3 compares values from EC Regulation 1/2005 for space requirements for cattle travelling by road or rail at liveweights from 50-500 kg with those obtained from the allometric equations of FAWC (1991) and Petherick and Phillips (2009) for standing and lying cattle.

Table 3. A comparison of recommended space allowances for cattle of different weights.

Category	Weight (kg)	Reg. 1/2005	FAWC	Petherick Standing	Petherick Lying
Small calves	50	0.30-0.40	0.29	0.25	0.36
Medium sized calves	110	0.40-0.70	0.46	0.42	0.60
Heavy calves	200	0.70-0.95	0.73	0.63	0.90
Medium sized cattle	325	0.95-1.30	0.97	0.88	1.25
Heavy cattle	550	1.30-1.60	1.33	1.21	1.71
Very heavy cattle	>700	>1.60	>1.54	>1.40	>2.00

In general, the lower and upper limits of the range of space allowances specified by EC Regulation 1/2005 are similar to the values derived by Petherick and Phillips (2009) for standing and lying cattle, respectively.

The loading densities for cattle in the present regulation are adequate for the animals when driving. However, cattle with horns may require more space than polled animals, so floor space allowances are usually increased by about 5 to 7% for horned animals. Bruising is reduced if horned cattle have 10% more space. Lower total loading densities improve air quality and facilitate ventilation. A proper ventilation system makes the transport more independent of climatic variances (Hartung et al., 2003). Vehicles with fan ventilation allow drivers to react to problems of high temperatures.

For cattle that tend to stand during the journey, the ceiling must be set well above all the animals when they are standing with their heads up in a natural position. This headroom will ensure adequate freedom of movement (SCAHAW, 2002). However, it remains unclear from this statement, how the height of the animal is determined. One definition of the height of cattle is taken as the height at the withers (CIGR, 2004). The range in height can vary between animals. Therefore, it is recommended to determine the height of the deck or compartment as the space between the withers of the tallest animal per deck (Lambooij et al., 2010). Holleben et al. (2003) observed more and severe bruising when the space above the animals was small (i.e. 10 cm). It was concluded that during transport and lairage the ceiling should be fixed higher than 20 cm above the withers (Holleben et al., 2003; Lambooij et al., 2010).

2.5.4.4. Water and feeding interval, journey times and resting periods

Cattle, being heavy animals, avoid lying down when they may be injured by banging against the hard floor during transport and especially to avoid the danger of being trampled by the other animals.

However, prolonged standing on a moving vehicle is tiring, so after 12-16 hours they begin to lie down anyway. Mortality of adult cattle during transport increases with the length of the journey (Malena et al., 2007). The vast majority of cattle are bruised during loading and transport. Jarvis et al. (1995) reported bruises on 97% of the carcasses at two slaughterhouses. Hartung et al. (2007) reported significant heat stress during long road transport of cattle in the summer, especially during stops.

There have been several recent studies on the effects of long journeys on physiological and behavioural indicators of cattle welfare. Gupta et al. (2007) investigated the effect of 12 h transport by road of mature bulls that had been housed prior to transport at different space allowances (1.2, 2.7, 4.2 m^2/bull) on slatted floors for 97 days. Effects of loading bulls on a transporter, transporting for 12 h and subsequently unloading included body-weight loss, neutrophilia, eosinophilia, lymphopaenia and increased packed cell volume, red blood cell and haemoglobin levels. While transport increased cortisol and suppressed indicators of the immune response in the short-term, these changes were within normal physiological ranges, suggesting that 12 h road transport had no significant adverse effect on welfare over this period. Furthermore, transport of bulls housed at increased space allowance (4.2 m^2/bull) resulted in a greater cortisol response, albeit still within a normal physiological range. The effect of transport for up to 0, 6, 9, 12, 18 and 24 hours followed by 24 hours recovery on live weight, physiological and haematological responses of bulls was investigated by Earley and O'Riordan (2006a; 2006b). Bulls travelling for 6, 9, 12, 18 and 24 h lost 4.7, 4.5, 5.7, 6.6 and 7.5% live weight compared with the baseline. During the 24 h recovery period, live weight was restored to pre-transport levels. Lymphocyte percentages were lower and neutrophil percentages were higher in all transported animals. Blood protein and creatine kinase concentrations were higher in the bulls following transport for 18 and 24 h and returned to the baseline within 24 h. Live weight, physiological and haematological responses returned to pre-transport levels within 24 h after bulls had access to feed and water (Earley et al., 2007). These studies are based on a limited range of measurements but show that the animals studied could recover from adverse effects of transport that increase from 6-24 hours. Fatigue would increase during this time, especially if the animals could not lie down. Marahrens et al. (2003) found that bulls kept in lairage after a journey of 25-29 hours still had elevated creatine kinase 24 hours after the transport, mainly as a consequence of aggressive behaviour in the lairage. Heifers, treated in the same way, did not show aggressive behaviour but showed continuing increases in NEFA, indicating continuing attempts to recover from fatigue, in the 24 hours after transport. As reported in SCAHAW (2002), after longer journeys recovery is possible but is more prolonged and more likely to be associated with increased disease incidence.

As reported by SCAHAW (2002), if cattle are allowed to feed and drink on a vehicle, as well as rest, the space required is given by the formula $A=0.0315W^{2/3}m^2$.

Relative to many ruminants, cattle have a limited ability to concentrate urine so are particularly sensitive to water restriction. Many factors may influence the amount of water required by cattle but it depends especially on the temperature, relative humidity, development stage and production (e.g. lactation). Mature cattle drink at least every 12 hours if they can. Lactating animals drink much more often. Water requirements will increase on hot days. Mature animals normally eat several times during 24 hours and young animals eat more often CARC (2004). Many animals chose not to drink during the rest stop. However, they may have been disturbed by the conditions and body fluid concentrations may have been abnormal.

2.6. Rabbit transport

2.6.1. Fitness for transport

Reports on the welfare of rabbits in transport relate only to the transport of animals for slaughter. No data are available on the impact of transport practices on some rabbit categories (i.e. newly borne animals, lactating females, fat animals, caged and unfit rabbits). However, extrapolation from other species would indicate these categories may be more vulnerable to adverse effects related to the risk factors involved.

2.6.2. Means of transport

2.6.2.1. Provisions for all means of transport

Previous reports (EFSA, 2004a, 2004b) have indicated the size and height of the crates, type of floor, mixing unfamiliar animals, thermal stress and lack of ventilation as the most important hazard factors involved in transport stress on rabbits. New scientific evidence confirms these conclusions, and also investigates their impact in relation to other factors, such as the position of the crates within the truck (Vignola et al., 2008; Liste et al., 2008).

2.6.2.2. Additional provisions for transport in containers

2.6.3. Transport practices

Under commercial conditions, rabbits are often transported in stackable crates placed on vehicles in multi-floor crate stands with a total loading capacity ranging from 1,500 to 6,000 rabbits (TRAW, 2009). Crates can be of such a size that a person can lift one of them, or be modular units that need to be lifted with a forklift vehicle (Verga et al., 2009). In commercial situations, standard rabbit crate dimensions are as follows: 100-110×50-60×22-30 cm (length×width×height) (Verga et al., 2009).

2.6.3.1. Loading, unloading and handling

The human-animal relationship plays a key role in handling commercial rabbits, due to their shyness and diffidence towards man (Trocino and Xiccato, 2006). Rabbits that have been scared by humans, or have not been handled, try to flee and may injure themselves (Lidfors et al., 2007). Positive interactions with humans at an early stage in life could reduce their level of fearfulness. It has been shown that, in an apparent sensitive period, even minimal human contact is effective in reducing avoidance of the caretaker, and thus handling might be a useful tool to reduce stress and improve welfare even under intensive farming conditions (Csatadi et al., 2005). It has also been shown that handled rabbits approached humans significantly sooner than non-handled ones (Csatadi et al., 2007) and that frequent handling of young rabbits not only changes their behaviour in terms of reducing fear of humans but also positively influences the growth rate and reduces the mortality rate (Jezierski and Konecka, 1996).

Handling and the method of loading can affect carcass quality. Rabbit loading is commonly carried out in one of two ways: individually loading rabbits in transport containers filled on the farms, or collecting and placing, even throwing, animals into containers fixed on a truck. Rabbits on the upper truck levels are often subjected to a greater number of falls than on lower levels (TRAW, 2009). The type of floor for transport crates is still debated. Under housing conditions, growing rabbits prefer a plastic net floor to a wire mesh floor (Princz et al., 2007). Plastic has low thermal conductivity, therefore it may give a sensation of warmth so rabbits prefer staying on plastic (rabbits choose plastic floors if they can). In addition, plastic could reduce footpad injuries, although rabbits could chew plastic floors (TRAW, 2009). However, solid floors may impede ventilation and are more difficult to wash at the abattoir after slaughtering compared with those made of wire (Verga et al., 2009).

The effects of the loading method and crate position on the trucks on some stress indicators in commercial rabbits transported to the slaughterhouse were studied (Vignola et al., 2008). In July, a total of 192 animals were transported on 100 min journeys to the slaughterhouse. Animals were equally distributed at random in top front (TF) and bottom front (BF) crates, and top rear (TR) and bottom rear (BR) crates in order to evaluate the effects of crate position. Rabbits were loaded either in a smooth way (taken from the farm crates, placed in a wide trolley and carried gently into the transport cage) or in a rough way (rabbits from four crates were carried all together in the same trolley and loaded hurriedly). The TR crates showed the highest mean temperature and the lowest relative humidity while the other cages on the truck differed only in humidity. Rabbits transported in TR crates showed a significant increase of total protein level, as a possible consequence of dehydration. Loading methods or crate position in the truck did not significantly affect weight losses during transport. Corticosterone significantly increased only during transport using the rough loading method. Neutrophilia and lymphocytopaenia were significant for all rabbits, independent of the treatment received. No differences in PCV were found among groups. Aspartate transaminase (AST) and CK activities significantly increased in all the animals.

Rough rabbit handling has been reported to increase pre-slaughter mortality and main carcass defects, such as haemorrhages, bruises and broken bones (Verga et al., 2009). Mazzone et al. (2010) investigated the impact of handling during loading on rabbit meat quality. A total of 192 rabbits were loaded onto the truck smoothly (each subject carefully placed into the transport crate) and 192 rabbits were loaded roughly (loading was hurried and carelessly executed by the transport operator, throwing each animal into the crates fixed on the truck) and then transported (100 minutes, 12 animal per cage, 57.7 kg/m^2) to the abattoir. Transport, independently of loading method, significantly increased neutrophilia, lymphocytopaenia, serum AST, alanine transaminase (ALT) and CK activities, and serum corticosterone concentration. PCV after transport did not differ from values detected at the farm, in agreement with Liste et al. (2008), who did not find any variation of PCV in commercial rabbits transported to the abattoir. No adverse effects of loading method on carcass traits and meat quality were highlighted. On the basis of these findings, Vignola et al. (2008) and Mazzone et al. (2010) concluded that the stress parameters analysed were more influenced by transport (including handling) rather than by the different loading methods or crate position in the truck. Liste et al. (2006) also found that different loading methods did not exert significant differences on the stress indicators. They concluded that, in order to have an effect on meat quality, the threshold for stress was probably higher than the threshold needed to have an effect on welfare indicators.

Buil et al. (2004), in a survey of Spanish rabbit abattoirs, found that handling procedures differed widely among farms, especially regarding cage size (ranging from 1,430 cm² to 10,000 cm²). Loading facilities were adequate but only a few haulers had received specific training courses. All the farms used a cage system for transport, and 68% of the cages were of a medium sized type (from 0.35 m^2 to 0.5 m^2), with an animal density of 349.2 ± 83 cm^2/rabbit. The mean height of the cages was 30.7 ± 9.3 cm. Average time before unloading was short (4.5 ± 13.8 minutes) but lairage time before slaughter was usually longer than one hour and varied widely between abattoirs (ranging from 0 to 420 minutes). Loading time ranged from 10 to 240 minutes, but the most frequent was 90 minutes (33%). Animals were normally unloaded in the morning (55%) and this procedure lasted an average of 23 ± 15 min. Nearly all the abattoirs (85%) unloaded the cages in groups or cage stands. The unloading point was covered and protected from the wind in about 80% of the abattoirs. The holding area was also covered and half of the areas surveyed had an air ventilation system.

2.6.3.2. Facilities and procedures

It is well known that transport involves several potentially stressful factors: climatic factors such as temperature or humidity; physical factors such as noise and vibration; and emotional factors such as unfamiliar environment or social regrouping. The effects of four potential transport-related stressors (heat [HS], cold [CS], noise [NS] and mixing with unfamiliar animals [MS]) on certain physiological and meat quality parameters of rabbits were studied (de la Fuente et al., 2007). The rabbits were exposed to each potential stressor for 4.5 h prior to slaughter. HS groups showed the highest plasma concentrations of cortisol, lactate and glucose and greater PCV and osmolarity, and the meat exhibited a low initial pH following lactic acid accumulation (de la Fuente et al., 2007). The high plasma lactate concentration in HS rabbits could be due to an accumulation of lactic acid induced by excessive panting. Panting is an inefficient mode of heat loss when the environmental temperature is in excess of 30 ºC and, if it is prolonged, a metabolic alkalosis can occur due to CO_2 deficit (Fayez et al., 1994). CS and NS exposed rabbits also showed physiological responses to the potential stressor, although to a lesser degree than rabbits exposed to HS (de la Fuente et al., 2007). Cold stressed rabbits showed increased levels of CK and a higher PCV, as well as decreased muscle glycogen concentration. NS exposed rabbits showed muscular damage, as demonstrated by increased CK and LDH activities in the blood and a high final pH in meat. Mixing unfamiliar rabbits (MS) led to higher CK activity, lower lactate and glucose concentration and a slight increase of the meat pH. In summary, rabbits exposed to heat were the most affected of all three groups, although cold, noise and mixing with unfamiliar rabbits also had a detrimental effect on physiological and meat quality parameters (de la Fuente et al., 2007).

2.6.4. Water and feeding interval, journey times and resting periods

Water and food withdrawal – Lairage

In most commercial abattoirs rabbits are unloaded from the lorry and kept in their crates in a protected area to await slaughter. Thus lairage represents an additional period of deprivation of water and/or food. Usually, this area is placed outside, and very few abattoirs provide an enclosed area equipped with forced ventilation and water-misting sprays as a control strategy for adverse environmental conditions (Verga et al., 2009). In a survey of Spanish rabbit abattoirs (Buil et al., 2004), it was found that, before slaughter, there is a short lairage time where animals are kept in the same multi-floor transport cages in a holding area near the stunning facilities. About 80% of the abattoirs surveyed used multi-floor cages during this period with an average capacity at the holding area for 250 (range 45 to 1,500). Only one abattoir used specific lairage cages for the rabbits in the hold area (i.e., rabbits were transferred from the transport cage to a lairage cage). The average waiting time before slaughter was 110 ± 113 min (range 0 to 420 minutes). Only one abattoir had facilities to provide food and water to the animals under long holding periods (Buil et al., 2004).

There is much evidence that antemortem harvesting, transport and lairage of rabbits at the abattoir are stressful, as indicated by physiological and biochemical changes occurring during these phases (de la Fuente et al., 2004; María et al., 2006; Liste et al., 2006). However, it has also been suggested that lairaging rabbits at abattoirs under favourable conditions may lessen the effect of transportation on animal welfare and meat quality properties (Cavani and Petracci 2004; María et al., 2006). A study was conducted to determine the effects of journey duration (1 versus 3 h) and lairage time at the abattoir (0 versus 5 h) on rabbit meat quality traits (Petracci et al., 2009). Rabbits transported for 3 h produced meat with significantly higher pH values, which was darker and had less yellow colour, as well as lower losses during cooking, than those transported for 1 h. Moreover, animals laired for 5 h yielded meat with more yellow colour, cooking losses and higher shear values than rabbits not laired before slaughtering, revealing that lairaging before slaughter at the abattoir can only partially contribute to lessening the effect of transportation on rabbit meat quality properties (Petracci et al., 2009).

Liste et al. (2009) studied the effects of duration of lairage time before slaughtering (2 v. 8 h) and position in a multi-floor cage rolling stand (MFRS) truck, on welfare and on instrumental and sensorial meat quality of hybrid commercial rabbits. Stocking density in the truck during lairage was 360 cm^2/animal. Lairage time and position on the MFRS had significant effects on blood stress indicators (haematocrit, glucose, lactate, CPK and corticosterone). On the other hand, lairage time only had a slight effect on meat quality traits (pH24, water holding capacity (WHC), colour, raw and cooked texture, sensory quality). The extent of haematomas (bruising) on the carcass was significantly higher in the short lairage group (0.82% ± 0.11) than in the long lairage group (0.48% ± 0.11). This higher incidence of bruising may have been due to contusions or gripping produced during pre-slaughter handling of more stressed and, consequently, more reactive rabbits (Liste et al., 2009). Lairage duration of 6–8 hours was recommended as an adaptation period required before slaughter to allow animals to recover from the stress of transport and, presumably, improve meat quality (Liste et al., 2009).

The level of stress required to decrease meat quality substantially is greater than the level required to affect plasma stress indicators. On this basis, the lack of an effect on meat quality does not necessarily imply that animal welfare is optimal during the slaughter process. Slight negative effects on rabbit meat quality probably are an indicator of serious welfare problems during pre-slaughter handling, including lairage (Liste et al., 2009). These conclusions contrast with findings of a survey on the effect of pre-slaughter conditions on commercial rabbits, in which short lairage (<150 minutes) was less compromising for welfare than medium and long lairage (150-240 minutes and >240 minutes, respectively; Petracci et al., 2008).

Preslaughter conditions have been shown to affect mortality rate, live weight loss, carcass yield and quality grades, especially the proportion of carcasses downgraded because of quality defects (Cavani and Petracci, 2004). Petracci et al. (2008) conducted a survey on 831 herds of rabbits in a commercial chain to determine the effect of the season, journey (short: <220 minutes; medium: 220-320 minutes; long: >320 minutes) and lairage (short, medium, long, from 150 up to >250 minutes) on mortality, live weight loss, slaughter yield and carcass quality. The overall average mortality rate and live weight loss were found to be 0.082% and 3.39%, respectively. Herds subjected to short lairage exhibited a significantly lower mortality rate (0.065% vs. 0.075% vs. 0.105%) and higher carcass yield (57.8% vs 57.4% vs 57.1%) when compared with medium and long lairage times. As for carcass evaluation, overall average incidence of downgraded and condemned carcasses was 0.40% and 0.46%, respectively, while the bruised carcass level was 2.22%. In a previous study by Cavani and Petracci (2004), the authors found that the areas most frequently bruised were the legs, thoracic muscles and the internal part of the loin region. These bruises were mostly not detectable in the live rabbit and only become visible when the skin was removed after slaughter. Petracci et al. (2008) concluded that lairage time was one of the main critical points affecting mortality rate, slaughtering yields and carcass quality.

Fasting time during transport is important because it affects animal welfare, but it also affects carcass yield (live weight losses), carcass contamination, and product safety and quality (Verga et al., 2009). Cavani and Petracci (2004) determined that rabbits lose 3-6% of body weight during the first 12 hours of fasting, which increases to about 8-12% at 36-48 hours. According to Lambertini et al. (2006), weight loss recorded in the first 4-6 hours in transported rabbits with food removal is mainly due to emptying of the gut, so carcass yield is not negatively influenced. For longer periods (after 6 hours fasting), Trocino et al. (2003) found that there was also a loss in moisture and nutrients from body tissues, which can impair carcass yield. De la Fuente et al. (2004) found that rabbits exposed to both fasting and transport lost more live weight compared with those that merely fasted, although in a later study (de la Fuente et al., 2007) involving animals fasted for 4.5 hours, no significant differences in live weight losses were seen between all four treatments.

In conclusion, the critical points during the transport process that emerged from a survey performed in Spain (Buil et al., 2004) were waiting time at the farm before loading, loading, ventilation and temperature during transport (cage position), loading stops, unloading, holding time before slaughter, environmental conditions during holding and time between stunning and bleeding. Another critical aspect was that many slaughterhouses took the rabbits to be slaughtered directly from the transport cage tower. Towers are moved from the waiting area and located very close to the stunning point, so most rabbits can see, hear and smell the animals which are slaughtered at a close distance. This point could represent an additional stressor to the rabbits because there is some evidence that animals can smell pheromones associated with the slaughter stress, which could increase fear before slaughter (Buil et al., 2004). Furthermore, fear and distress could be communicated among rabbits kept in the same room (Beynen, 1992).

This new scientific evidence does not confirm that “rabbits can reasonably withstand food and water deprivation for 24 hours without significant adverse effects on bodyweight and carcass quality” as previously stated (EFSA, 2004).

Journey time - position on the truck

It is well known that rabbit transport is characterised by multiple collection points, which implies that animals may wait for an indeterminate time in the containers either at the farms, on the means of transport and at the slaughterhouse holding area (Buil et al., 2004). In addition, at present, long journeys are increasing because of the reducing numbers of rabbit abattoirs (Buil et al., 2004).

In Italy, commercial rabbits are usually transported to the abattoir using a commercial lorry, which has two or three axles and a loading capacity ranging from 1,500 to 6,000 rabbits (Verga et al., 2009). In a recent survey conducted in Spain, the average transport time was 154 minutes (range: 20 to 600 minutes) corresponding to 137.5 km (range: 25 to 500 km) (Buil et al., 2004). Similar journey durations were also registered in Italy (Petracci et al., 2008). In the survey conducted by Petracci et al., (2008), short journeys (<220 minutes) exhibited lower mortality rate compared to medium (220-320 minutes) and long journeys (>320 minutes) (0.053% vs. 0.080% vs. 0.113%, respectively), as well as lower live weight loss (2.43% vs. 3.47% vs. 4.26%), and higher slaughter yield (58.0% vs. 57.3% vs. 57.0%). Long journeys also showed a higher incidence of bruised carcasses, but did not influence downgraded and condemned carcass rates. In conclusion, long journeys, in addition to lairage, were shown by the survey to be the main critical points, and these impaired the mortality rate, slaughtering yields and carcass quality (Petracci et al., 2008).

Lambertini et al. (2006) assessed the impact of transport time (1, 2 and 4 hours) on commercial rabbits. They found that live weight losses increased from 1.6 to 3.3% following journeys that lasted 1–4 hours, but they were about 2% when rabbits were transported for 2 hours. Live weight loss was caused by urine and faecal losses but also by a reduction of carcass weight during transport. In addition, higher pH values (6.01) and lower pH decreases were reported when rabbits were subjected to longer journeys (4 hours) compared with the 2 hour ones. In addition, the longest journeys were associated with a more purple-red meat, darker, and firmer when raw, and less cooking loss compared with the shortest journeys.

The effect of journey duration, position on the truck and high temperature on some physiological indicators of stress and meat quality in commercial rabbits has been investigated (Liste et al., 2006). In the summer, 78 rabbits were subjected to either long (7 hours) or short (1 hour) journeys to the abattoir. There was a trend for the levels of corticosterone, glucose, lactate, and CK to be slightly higher after long journeys compared with short journeys, but the differences were not significant. Short and long journeys did not affect pH24 and WHC values, whereas journey time had a significant effect on meat tenderness and compression values (measurements of the tenderness of raw meat).

The effect of transport time (1 hour vs. 7 hours) on the stress response of rabbits was further studied in relation to season (summer and winter) and the position on the truck (top, middle and bottom layers; Liste et al., 2006, 2008, 2009). Corticosterone and CK levels were highest after 1 hour transport compared with 7 hours. However, pH24, which is considered one of the main parameters of welfare measurements, was not affected by transport time or position on the truck. The pH24 values were within normal ranges for all treatments but slightly higher for animals transported in winter. Position on the truck, in particular the lower one, also affected the physiological response to stress in rabbits. Corticosterone concentrations were highest on the bottom layer, and lowest on the top. Rabbits on the bottom and middle layers had significantly higher levels of CK and lactate, indicative of higher muscular activity. On the contrary, position on the truck did not influence the measurements of meat quality (Liste et al., 2008).

In a similar study (María et al., 2008), position on the truck did not affect measurements of meat quality, both in relation to journey time and seasons. Conversely, journey time had a significant effect on meat texture parameters, as measured by compression, but did not affect average pH24, WHC, shear force or toughness. In general, transport time had much less of an effect on meat quality compared with season, and higher responses were recorded in summer than in winter.

Season and thermal stress

Exposure to high ambient temperature induces rabbits to try to balance the excessive heat load by using different means to dissipate, as much as possible, their latent heat. Optimal climatic conditions for rabbits would be: air temperature 13 to 20 ºC (average 15 ºC), relative humidity 55 to 65% (average 60%) (Marai and Rashwan, 2004). Adult rabbits exposed to ambient temperatures below 10 ºC curl up to minimise their total body surface area exposed and lower their ear temperature, and the ear pinnae are folded to avoid internal surface contact with air. At the same time, they drag the ear to bring it closer to the body (Marai and Rashwan, 2004). Since rabbits do not sweat, at temperatures above 25-30 ºC they stretch out to lose as much heat as possible by radiation and convection, raise their ear temperature, stretch the ear pinnae and spread them far from the body to expose the surface to the surroundings in order to increase heat dissipation. Above 35 ºC, rabbits can no longer regulate their internal temperature and heat prostration sets in, while at 40 ºC, considerable panting and salivation have been shown to occur (Lebas et al., 1986 in Marai and Rashwan, 2004). The average lethal ambient temperature is 42.8 ºC. The comfort limits for rabbits (Marai et al., 2002) are defined as: temperature-humidity index (THI)<27.8 ºC, absence of heat stress; 27.8 to 28.9 ºC, moderate heat stress; 28.9 to 30 ºC, severe heat stress; THI>30 ºC, very severe heat stress.

Although thermal stress is a crucial factor in transport stress in rabbits, few studies have focused on this research area. Recently, Liste et al. (2006) found that, in the presence of high temperatures, the position on the transport truck had a greater influence on rabbit welfare than the duration of the journey. In another study (Liste et al., 2008), it was observed that winter temperatures increased corticosterone, while summer temperatures increased CK.

The season proved to have a significant effect on pH24, WHC, all colour parameters, shear force and toughness (María et al., 2008). In general, season had a greater impact on meat quality parameters compared with journey time, with higher impacts recorded in summer than in winter. These findings are in contrast to those obtained in the survey of Petracci et al. (2008). During winter, lower live weight losses (3.12%) were recorded, whereas carcass yield (57.9%) was higher during summer. Carcass bruising had a higher incidence during summer in respect to other seasons, while downgraded carcass prevalence was higher in autumn. Petracci et al. (2008) concluded that season played only a minor role, probably due to the fact that transport was mainly conducted during the night and early morning, thereby moderating the effect of high summer temperatures.

De la Fuente et al. (2004) has demonstrated that some rabbits may experience heat stress during transport in summer. This included signs of severe heat distress, elevated blood cortisol, lactate and glucose, CK and LDH, and greater dehydration and osmolarity. The same authors observed that winter transport increased muscle activity, as evidenced by the lower liver and muscle glycogen concentration. This implies some degree of cold stress. Mazzone et al. (2009) investigated the effect of microclimatic conditions within the vehicle on the welfare of rabbits during transport to the slaughterhouse. The top rear position on the truck was characterised by the highest T°C and, particularly in summer, by the lowest relative humidity. In winter, BF showed the lowest T°C and the highest relative humidity. However, these differences in microclimate had no effect on stress parameters, although rabbits transported in summer were more stressed than in winter (Mazzone et al., 2009).

In a survey of Spanish rabbit transport procedures (Buil et al., 2004), it was found that the roof and walls of the trucks were made of different materials, but normally aluminium (23.8%) and steel (14.3%). Only half of the trucks had an insulated roof and in 76.2% of the lorries the environmental conditions during transport were not controlled, and they lacked artificial light, mechanical ventilation or temperature control.

2.6.5. Space allowances (transport by rail, by road, by air, by sea, densities)

Commercial rabbits marketed for slaughter are generally transported in crates. Swallow et al. (2005) suggested a minimum stocking density for transporting laboratory rabbits in filtered crates ranging from 0.2 m^2 to 0.16 m^2 for rabbits weighing more than 1 kg and, in unfiltered crates, ranging from 0.1

m^2 to 0.08 m^2 for rabbits weighing more than 2.5 kg. Swallow et al. (2005) also suggested that container height should be restricted for laboratory rabbits to prevent back injury caused by kicking out. However, since rabbits do not sweat, at environmentally high temperatures space is needed to allow rabbits to assume appropriate postures, such as sitting up or lying flat with ears extended in both of these positions to expose the surface to the surroundings in order to increase heat dissipation (Marai and Rashwan, 2004). In addition, rabbits tend to sit upright as a control "security and safety" behaviour (Lidfors et al., 2007).

In practice, crate dimensions for transporting commercial rabbits can vary. Standard measurements are reported to be 100-110×50-60×22-30 cm (length×width×height) (Verga et al., 2009). The number of rabbits loaded into crates also varies according to animal weight and environmental conditions: 14-16 animals/crate for rabbits weighing 2.0-2.7 kg and 12-14 animals/crate for rabbits weighing 2.8-3.2 kg, resulting in a commercial stocking density varying from 0.03 to 0.05 m^2/rabbit (Verga et al., 2009).

Notwithstanding these indications, little new scientific information has been added to previous knowledge. Lambertini et al. (2006) investigated the effect of journey time (1, 2 or 4 hours) and stocking density (high and low, 75.5 or 49.0 kg/m^2, respectively) during transport on carcass and meat quality of 450 rabbits (75.5 kg/m^2 is that normally used when transferring rabbits from the farm to the slaughterhouse in Italy). These authors found that longer journeys significantly reduced live weight, whereas there were no significant effects of animal density in the transport cages on weight losses and slaughter data, and there was also no significant interaction between transport time and stocking density (Lambertini et al., 2006). These findings are consistent with those previously obtained by de la Fuente et al. (2004) who worked with even lower densities (high and low, 53.6 or 37.0 kg/m^2, respectively) comparing summer and winter journeys. These authors found that season significantly influenced the loss of live weight during transport, with lower live weight loss in rabbits transported in summer than in winter. Plasma concentrations of cortisol, lactate and glucose, CK and LDH activity, and osmolarity, as well as liver and muscle glycogen concentrations were higher in rabbits transported in summer than in winter, suggesting that the welfare of the rabbits was more at risk during transport in hot weather. Stocking density had no effect on the analysed parameters. The authors hypothesised that the lack of differences between the two stocking densities may have been because differences between them were insufficient to improve rabbit welfare, suggesting that other factors, such as the height of the cage or the number of layers might be more significant (de la Fuente et al., 2004).

2.7. Poultry transport

In order to find relevant published information in agreement with the Terms of Reference of the mandate, a bibliographic search was performed. For the search, a list of search key words was created (i.e. poultry, chicken, broiler, hen, chick, transport, stress, welfare, journey, regulation, legislation) and the year of publication was set up to be after 2004. After the collection of information and data from electronic sources, publications were accepted or not according to previously established criteria. After the first screening, a total of 199 references were obtained dealing exclusively with the welfare aspects of the transport of all relevant species. From these, 94 references passed the screening process according to the acceptance criteria and were used for the development of the following section of the Scientific Opinion (search protocols undertaken in accordance with EFSA, 2010b).

The current section is focused upon chickens, although the principles, conclusions and recommendations may be pertinent to the transportation of turkeys, ducks, guinea fowls and other game birds.

It is necessary to examine the transportation of poultry in terms of the primary species involved in commercial production and to include due consideration of the ages or stages of development at which birds may be transported. Thus, the main categories may be considered to be:

- Broiler chickens to slaughter,
- Day old chicks from hatchery to farm (or other sites),
- Breeding birds to farms,
- Pullets to farm,
- Laying hens to slaughter.

2.7.1. Fitness for transport

Concerns about the fitness for transport of poultry may be considered in relation to the types/ages of the birds involved. One-day-old chicks, whilst vulnerable to a number of stressors in transport, are routinely screened at hatching and during placement in transport containers. Little or no information is available to indicate the extent of any problems that occur.

The transport of end of lay hens to processing plants for slaughter constitutes a particular cause for concern in relation to bird welfare. The hens have relatively little economic value and thus there is little financial incentive to encourage careful handling and good welfare in transport. In addition, the number of processing plants able or willing to handle spent layers is much fewer than those dealing with broilers and, as a consequence, journeys between farm and slaughter may be of much longer duration than for meat type birds. In addition, the pre-transport handling of layers (depopulation) may result in extensive skeletal trauma mainly due to the fragility of the bones resulting from the altered calcium economy associated with prolonged egg (shell) production. Pre-transport injury, fractures and dislocations will result in painful conditions and the effects will be exacerbated by transportation. It should be noted that hens with unhealed old bone breaks, or fresh breaks, cannot legally be transported under current EU legislation because transportation would inevitably lead to greater potential pain and suffering, therefore making the birds "unfit for transport". In addition, poor feathering and depressed metabolism due to prolonged feed withdrawal and transport times may make spent layers excessively vulnerable to the effects of cold and or wet conditions, particularly at high air speeds. Paradoxically, over enthusiastic "protection" of loads of spent layers by means of tarpaulins on the trailers, particularly when vehicles are stationary for prolonged periods and during warm weather may result in mortalities and reduced welfare from heat stress.

Laying hens are often very poorly feathered at the point of transfer to the slaughter-house and may be subject to "physiological fatigue". These problems may be exacerbated by prolonged pre-transport feed withdrawal. These factors will compromise their ability to thermoregulate in the face of thermal challenges, particularly if ambient temperatures are low. The reduction in peripheral insulation will make the birds more vulnerable to forced convective cooling and to the detrimental effects of wetting on "open vehicles" at low ambient temperatures. Webster et al. (1993) and Weeks et al. (1997) using physical models of chickens to estimate the heat exchange of poultry in transport have reported that even for well feathered birds the conditions necessary for thermal comfort are rarely achieved on passively ventilated vehicles. That work also confirmed the large difference in the "thermal comfort zone" between well-feathered broilers or pullets (10-15 ºC) and poorly feathered spent layers (22-28 ºC) in passively ventilated open vehicles. A further implication of the findings was the heterogeneous distribution of ventilation within the bio-load and the risk of localised over ventilation in such circumstances (mean air speed within the transport crate between 0.9-2.4 ms^{-1} and a maximum air speed of 6.0 ms^{-1}). From these findings and those relating to the thermal micro-environments on broiler transporters and the associated ventilation regimes, a number of recommendations concerning the carriage of spent layers may be presented for improved procedures, practices and vehicle operation.

Modern, rapidly growing strains of meat poultry exhibit an elevated incidence of spontaneous or idiopathic myopathy and an increased susceptibility to stress induced myopathy (Mitchell, 1999; Sandercock et al., 2006). These pathologies are attributable to alterations in intracellular calcium homeostasis (Sandercock and Mitchell 2003; Sandercock et al., 2006) and consequent changes in sarcolemmal integrity and may result from excessive myofibre hypertrophy and inadequate development of support tissues and vascular supply (MacRae et al., 2006, 2007). These myopathies may have, in turn, a range of implications for both product quality and bird welfare (Mitchell, 1999). Rapidly growing lines of birds may exhibit a reduced thermoregulatory capacity compared to their genetic predecessors and may thus be more susceptible to heat stress in transport and to consequent problems, including muscle damage, acid-base disturbances and reduced meat quality (Sandercock et al., 2006). Genetic selection for improved growth rate and feed conversion efficiency may be associated with altered mitochondrial function (Bottje et al., 2006) and changes in the production of reactive oxygen species (ROS). In this context acute heat stress has been demonstrated to increase superoxide free radical production in chicken skeletal muscle (Mujahid et al., 2005). This process is mediated by altered mitochondrial function and down-regulation of “uncoupling protein content” (Mujahid et al., 2006, 2007). This mechanism may be responsible for the transport stress and heat stress induced muscle damage and for the changes in muscle and meat quality observed in broilers. Thus, derangements of antemortem muscle cell metabolism and alterations in sarcolemmal integrity and tissue structure associated with oxidative damage and myopathy may have profound implications for meat quality and the incidence of specific conditions such as pale, soft and exudative (PSE) like meat. Also, it may be suggested that muscle dysfunction may lead to problems of altered locomotor capability and therefore behavioural changes and reduced welfare. This situation may be further compounded if the observed myopathies are accompanied by muscle discomfort or pain.

2.7.2. Means of transport

2.7.2.1. Provisions for all means of transport

The effects of pre-slaughter conditions on stress and welfare of farm animals in general have been reviewed by Terlouw et al. (2007). The physiological responses of food animals to road transportation stress have been reviewed by Minka and Ayo (2009). Doktor and Poltowicz (2009) have reported associations between meat quality and a range of “stress” indicators in birds transported for 2.5 hours. The effects of handling and transport upon broiler meat quality have been extensively reviewed by Petracci et al. (2010).

Pullets and laying hens

Pullets at the point of transfer to the layer farm are generally well feathered and are fully fed and hydrated and free from metabolic disease. Their thermoregulatory capacity may be regarded as optimal and thus they may be able to withstand greater excursions in ambient thermal conditions than either day old chicks or end of lay hens. The physiological response models derived for broilers are thus likely to be applicable and the recommendations for the acceptable thermal envelope will be as described for the meat birds (Mitchell and Kettlewell, 2004a, b). It might be proposed that, for such high value birds, mechanical ventilation ensuring homogeneous distributions of temperature and humidity within the bio-load and improved control of the ‘on-board’ thermal micro-environment and higher standards of welfare in transport may prove cost effective.

Spent laying hens present a number of major challenges concerning welfare during transportation. Spent layers are reported to exhibit increased fear following transportation as assessed by measurement of tonic immobility responses (Mitchell and Kettlewell, 2004b). These birds did not appear to habituate to transport stress on journeys lasting up to 5 hours. The well recognised, high incidence of bone fractures in birds at the end of lay (Mitchell and Kettlewell, 2004b) also constitutes a major cause for concern during transportation. It is clear that birds with new fractures associated with depopulation or old unhealed fractures are "unfit for transportation” and as such should not be taken to the slaughter house. Any pain or suffering associated with such injuries will be exacerbated by the accelerations, vibrations and impacts imposed during the journey. It may be suggested that motion and vibration represent significant stressors in the absence of any injury as hens exhibit aversion to specific frequencies typical of those encountered on commercial transport vehicles (Mitchell and Kettlewell, 2004b).

Broiler chickens – meat birds

All poultry species and major breeds employed in the main intensive production systems are transported at least twice during their lifetimes, over distances that may range from a few kilometres, to journeys with durations of many hours. Most journeys are by road (e.g. from hatchery to production site or from farm to processing plant) but some birds may also be transported by air or sea. All modes of transport involve the placement of birds or chicks into transport containers that are subsequently loaded on to vehicles, aircraft or vessels for translocation to their intermediate or final destinations. All the procedures and practices involved in transportation and the micro-environments prevailing in containers and vehicles may impose varying degrees of stress upon the birds which will result in compromise of their welfare status, health and productive efficiency depending upon the magnitude of the challenges imposed. Various components of the topic have previously been discussed and reviewed (e.g. Mitchell, 2006a, 2006b; 2008; 2009; Mitchell and Kettlewell, 2004a, 2004b; 2008a, 2008b) but it is timely to incorporate existing knowledge of the physical aspects of transport practices and environments with complementary knowledge of the physiology and behaviour of the major types of poultry that are transported. It is also relevant to assess how these breeds and species of birds are equipped to respond to 'transportation stress' and how these characteristics have been influenced by genetic selection for production traits. In transport, birds may be exposed to a variety of potential stressors including the thermal demands of the transport microenvironment, acceleration, vibration, motion, impacts, fasting, withdrawal of water, social disruption and noise (Mitchell, 2006a; 2008, 2009; Mitchell and Kettlewell, 2004a, 2004b; 2008a, 2008b). Each of these factors and their various combinations may impose stress upon the birds, but it is well recognised that thermal challenges and in particular heat stress constitute the major threat to animal well being and productivity (Mitchell, 2006b; 2008; 2009; Mitchell and Kettlewell 2008 a, 2008b).

Heat and cold exposure - vehicle microclimate

The work reported by MacCaluim et al. (2003) employing behavioural studies involving the assessment of aversion, suggested that thermal stress in transport constituted the major threat to the birds' well being. Ritz et al. (2005) emphasised the difficulty in relating mortality in transport or dead on arrival (DOA) figures with thermal stress in specific components of the pre-slaughter period including transportation. That study claimed that whilst elevated temperatures in transport could pose some risk, the main causes of DOA, identified by postmortem examination, were pre-existing disease and trauma caused by catching and handling.

A study by Warriss et al. (2005), of almost 60 x 106 birds killed in a commercial processing plant, established a significant relationship between maximum daily temperature and mortality in transport or DOA. Overall mortality was 0.126%. The DOA figure for maximum daily temperature values between 1 and 17 ºC was approximately 0.10% and this value increased from 0.13% at temperatures of >17 ºC but <20 ºC, and to 0.66% at temperatures between 23-27 ºC, thus indicating the upper limits and ranges that might be proposed for the safe transportation of broiler chickens. These values are very similar to those proposed by Mitchell and Kettlewell (2008b) from an experimental modelling approach.

The temperature at the point of loading or crating of broilers and subsequently in transport exerts a profound effect upon mortality and meat quality (Aksit et al., 2006). Temperatures of 34 ºC resulted in increased heterophil-lymphocyte ratios in broiler birds. Meat was lighter (L* value) and pH was lower. Low temperatures during winter transport of broiler chickens in Canada are also associated with increased losses and poor welfare. The issue has been addressed by the design of improved ventilation systems to ensure adequate removal of heat and water loads accumulating inside closed vehicles but with heating of incoming very cold air to avoid "internal fogging and wetting" of the birds (Cochran et al., 2006). This elegant solution may be applied in European conditions where very low ambient temperatures in winter apply and ventilation regimes on commercial transport vehicles are inadequate. On the other hand, elevated temperatures in transport in summer conditions in Canada are also associated with increased mortality (DOA – 0.35%), an effect exacerbated by high stocking densities on the truck (Drain et al., 2007; Whiting et al., 2007). Exposure of broilers to a temperature of 35 ºC and 85% relative humidity for periods between 30 and 129 minutes resulted in increasing and profound heat stress, hyperthermia and metabolic derangements in slaughter weight broiler chickens (da Silva et al., 2007). Barbosa et al. (2009) have characterised the microclimate on boiler transport trucks in Brazil using the enthalpy thermal comfort index and by examining the incidence of dead on arrivals (DOA). In summer conditions, the afternoon periods of transport constituted the greatest risk of stress and the heterogeneous distribution of heat loads on the vehicle resulted in a greater risk of losses occurring towards the middle and rear of the truck. Exposure to such conditions was also associated with an increased incidence of PSE meat at slaughter and the problem was attributed to poor ventilation towards the rear of the vehicles (Simoes et al., 2009). Ambient temperatures of 0 ºC or below during transportation caused cold stress sufficient to reduce deep body temperature in broilers, increased the incidence of dark, firm and dry (DFD) breast meat but decreased the incidence of PSE (Dadgar et al., 2010). Mitchell and Kettlewell (2008b) have described the use of physiological stress modelling to provide thermal comfort zones for livestock. In the case of broilers, they recommended an upper temperature limit of 24 ºC for on-board temperature and proposed engineering solutions and ventilation regimes that can achieve this. The imposition of thermal loads upon the birds in transport will result in moderate to severe thermal stress and consequent reduced welfare, increased mortality due to either heat or cold stress and induced pathology, including muscle damage and associated changes in product quality (Mitchell, 2006a; 2008; 2009; Mitchell and Kettlewell 2004a, 2008a, 2008b).

The effects of any hostile "on-board" thermal conditions will become more severe with increasing journey length. Mortality has long been a concern in relation to poultry transportation (Mitchell, 2006a; 2008; Mitchell and Kettlewell 2004a, 2004b, 2008a, 2008b) and continues to be an episodic issue in all countries where meat birds are produced. Thus, Warriss et al. (2005) have described a highly significant relationship between mortality of broilers in transport (DOAs) or in lairage and the maximum daily ambient temperature. It was proposed that at external temperatures greater than 17 ºC measures might be required to ameliorate the damaging effects of transport on bird welfare. In contrast, Ritz et al. (2005) have reported that elevated temperature during hot weather poses a greater pre-slaughter risk of mortality to broilers during loading and lairage at the slaughterhouse than on the vehicle if it is constantly moving. The authors acknowledge, however, that it is difficult to attribute DOAs precisely to a specific part of the process of handing and transport. Under commercial conditions it is difficult to establish the causes of mortality in transport and there are few studies available that have provided such information. Nevertheless, Hunter et al. (1997) examined the distribution of mortalities on commercial broiler transport vehicles and reported a significant link between the on-board thermal micro-environment and DOA values. Mortality was highest in those parts of the vehicle where temperatures and humidities were greatest or where the ingress of cold air and water resulted in cold stress. Postmortem examination indicated that an underlying level of randomly distributed DOAs on the vehicles encountered on journeys at all times of the year could be attributed to existing pathologies and/or catching injuries. Elevations in DOA values above this baseline were almost entirely the result of thermal stress (95%) and were concentrated in specific vehicle locations. Nijdam et al. (2006) reported that 89.4% of dead birds (DOA) exhibited macroscopic pathological lesions. Infectious disease states were the main cause of lesions (64.9%) followed by heart and circulation disorders (42.4%) and trauma (25%). However, conditions of transport, especially thermal conditions, were not discussed in detail. Birds affected by any of these pathologies may be more susceptible to thermal stress and thus may succumb when exposed to thermal conditions that would not adversely affect healthy birds. Thus, it may be assumed that levels of mortality or DOA vary widely depending upon many factors including season, geographical location, journey length, size of bird, stocking density, health status, vehicle design and slaughterhouse design and practice. Broiler DOA figures (annual averages) may vary from around 0.15% (Mitchell, 2006a) to values as high as 0.25% (Vecerek et al., 2006), 0.35% (Bianchi et al., 2005; Petracci et al., 2006) and 0.46% (Nijdam et al., 2004). Average values of DOA tend to be elevated in the summer months in many studies and a model developed by Nijdam et al. (2004) indicates that temperature multiplied by journey duration is an important determinant of DOA, as are transport time *per se* and lairage time. Journey length has long been recognised as an important factor in broiler DOA (Warriss et al., 1990). More recent studies (Vecerek et al., 2006) indicate that short journeys (up to 50 km in length) are associated with relatively low mortalities (0.15%) but for journeys of 300 km or greater the value increases to 0.86%. The mean value for all journeys in that study was 0.25%, thus emphasising the requirement to optimise transport conditions on longer journeys to reduce mortality, losses and to improve welfare. In periods of high ambient temperatures in summer, high episodic mortalities may occur where DOA figures may exceed 1-2% and, occasionally, even larger numbers of birds may be lost, reaching many hundreds of birds on a few journeys (personal communications – industry). Another important consequence of transportation of broilers, which is exacerbated by elevated thermal load, is weight loss through increased demand for evaporative heat loss (Mitchell, 2006b; 2008; Mitchell and Kettlewell, 2004a, 2008a, 2008b). This reduces product delivery weight and significant dehydration will compromise bird welfare and affect product quality.

Since thermal challenges represent a major risk to both the welfare of birds in transport and to meat quality, understanding the thermal micro-environment on commercial transport vehicles is essential to the development of appropriate strategies to control that environment and to reduce the risk of thermal stress in transport. The internal thermal microenvironment in poultry transport containers is the product of the inlet air temperature and humidity, airflow rate and the heat and moisture production of the birds (Mitchell, 2006b). The passive ventilation regimes of most commercial broiler transport vehicles result in low rates and heterogeneous distribution of airflow within the bio-load. Studies have characterised the pressure profiles over the surface of, and within, commercial broiler vehicles (Mitchell, 2008; Mitchell and Kettlewell, 2004a, 2008a, 2008b). It is these pressures that drive passive ventilation within the vehicle. A central feature is the tendency for air to move in the same direction as the motion of the vehicle: thus air tends to enter at the rear and move forward over the birds exiting towards the front. This pattern accounts for the distribution of temperatures and humidities observed on commercial vehicles, the existence of the "thermal core", the ingress of water spray and bird wetting and the pattern of DOAs and thermal stress found within the load (Mitchell, 2006a; 2008; Mitchell and Kettlewell, 2008a, 2008b). When vehicles are stationary there is no external force driving the ventilation, thus heat and moisture removal is then dependent upon free convection. Problems of heat stress may be markedly exacerbated even on open or semi-open vehicles, particularly when stationary in hot and humid weather conditions. Any practical solution to these problems must involve modification and improvement of the ventilation regime. The degree of physiological stress imposed upon slaughter weight broilers by a range of temperature and humidity combinations has been determined in transport simulation studies and the development of physiological stress models based upon apparent equivalent temperature or AET (Mitchell, 2006b; Mitchell and Kettlewell, 2004a). This approach allowed definition of thermal comfort zones, optimum transport conditions, and acceptable limits for temperature and humidity for broilers in transport crates under commercial transport conditions. It was suggested that the ‘in-crate’ dry bulb temperature should be maintained below 23-24 °C and preferably around the controlled house temperature of 20-21 ºC. Introduction of mechanical ventilation systems would facilitate control of the on-board thermal environments of poultry transport vehicles within the prescribed range. This can be achieved with knowledge of the thermal loads encountered in transport vehicles and the heat production of the birds in transport. The only published data on heat production based on field measurements rather than predictive models for poultry under transport conditions is that for broiler chickens (Mitchell, 2006b; 2008; Mitchell and Kettlewell, 2008a, 2008b). Recent studies (Knezacek, 2010) have reported extreme examples of the detrimental effects of inadequate ventilation on commercial broiler transport. During transportation in Canada in external conditions where air temperatures were as low as -30 ºC, inadequate ventilation and air distribution within the load led to localised hot spots and cold spots. Temperature lifts in the thermal core of as much as 60 °C led to hyperthermia, heat stress and increased mortality despite some broiler birds on the same journeys experiencing conditions that might potentially cause cold stress. Controlling the thermal environment within prescribed limits in transport (and indeed during catching and lairage) is a major step towards reducing losses and improving the welfare of meat type birds. Failure to adopt these measures is partly attributable to the costs of implementation and operation of the systems. It is proposed that thermal environments for spent layers in transport should conform to those recommended for broiler birds from physiological response modelling. These acceptable ranges and limits for temperature and humidity can be applied, assuming space allowances of 160 cm^2/bird, or greater, and journey times of less than 12 hours. For spent layers, particular attention should be paid to the thermal conditions, and cold and wetting should be avoided wherever possible. Journey durations should be kept to an absolute minimum and holding the loads stationary should be avoided. The use of mechanically ventilated vehicles for transportation of spent layers is recommended, despite the obvious financial implications. Alternatives to transport of hens to slaughter, such as 'on-site' slaughter and disposal, should be considered to improve the overall welfare of the production process.

Muscle pathology in broiler chickens – effects of transport

The genetic selection of broiler chickens for improved production traits has resulted in both growth-associated and stress-induced muscle pathologies or myopathies (MacRae et al., 2006; 2007; Sandercock and Mitchell, 2003; Sandercock et al., 2006; 2009), which are coupled to an impaired thermoregulatory capacity and consequent susceptibility to thermal stress in transport. These are important factors, since the risk of hyperthermia in commercial lines of birds is increased compared to their genetic predecessors and thus the induction of pathology and distress may be greater in the current broiler strains. The thermal limits that might be imposed for the transportation of poultry must include recognition of these issues and future breeding programmes for slower growing lines may reduce the risks associated with current transport practices and environments. The importance of these muscle pathologies on the general health and welfare of broiler chickens and possible interactions with stress (e.g. transportation) has been recognised in a recent EFSA Technical Report (Lefebvre et al, 2010).

Noise

Transportation procedures may involve exposure to elevated levels of noise which may constitute a stressor. Chloupek et al. (2009) have assessed the physiological and behavioural responses to noise in broiler chicken and concluded that exposure to levels of 100 dB for 10 minutes or greater constitutes a significant stress and should be avoided.

2.7.2.2. Additional provisions for transport by air

Air transport of chicks

This topic has been reviewed by Mitchell and Kettlewell (2004b) and Mitchell (2009). For all long distance transport of chicks it has been recommended that water and feed should be available in transport to reduce mortality and maintain welfare and productivity (Mitchell, 2009). Water may be provided in the form of commercial hydration gels (e.g. Aqua-Jel® or Pacific Oasis®), which are cut in to slices and placed in each container. As with road chick transporters, further research is required to characterise the prevailing on-board environments, the nature of the ventilation regimes and the consequences for the birds. Only in this way can strategies for improved conditions and welfare be developed.

2.7.3. Transport practices

2.7.3.1. Space allowances

The principles that might be employed in the calculation of space allowances for livestock discussed by Petherick and Phillips (2009) did not give specific consideration to poultry. In broiler chickens, a space allowance of 115 $cm^2\ kg^{-1}$ body weight in transport was not associated with any significant signs of stress but a further reduction to 105 $cm^2\ kg^{-1}$ resulted in an elevated heterophil-lymphocyte ratio (Bedanova et al., 2006). Delezie et al. (2007) have reported that crating or stocking density in transport is a major factor leading to stress and mortality in transport and may have a more marked effect upon the birds than pre-slaughter fasting or transport *per se*. The mortality rate on journeys up to 8 hours duration in Hungary (Miklos, 2008) was higher (0.60%) for space allowances of less than 207 $cm^2\ kg^{-1}$ than for higher allowances (0.49%).

The space allowances recommended for poultry in transport by EC Regulation 1/2005 are as follows:

Newly hatched or day old chicks	21-25 cm^2/chick
Other poultry	
<1.6 kg	180-200 $cm^2\ kg^{-1}$
1.6 – 3.0 kg	160 $cm^2\ kg^{-1}$
3.0 – 5.0 kg	115 $cm^2\ kg^{-1}$
>5.0 kg	105 $cm^2\ kg^{-1}$

There is no new evidence to suggest that current recommendations for poultry stocking densities should be altered.

2.7.3.2. During transport

Transportation of chicks

It is important to recognise the unique nature of the transportation of newly hatched chicks. No other species is transported in the immediate post-natal period. Chicks possess energy and water reserves in the form of the yolk sac and the chicks are sustained by this for a period after hatch. The frequently used term day-old chick may be misleading in relation to commercial chick transport, since some chicks may have been in a hatcher for some time after hatching and may thus be transported when approximately 24 hours old. Any debate on journey times for newly hatched chicks may thus focus upon the duration of the period in which the chick can be reasonably sustained by yolk sac reserves with no undue effect upon welfare or health. There have been a number of scientific publications relating to this topic and it was from this literature that the original definition of a maximum journey time for newly hatched chicks of “24 hours as long as this is completed within 72 hours of hatching” was developed. The current concerns arise from an assertion that modern genetic lines of broiler chickens with high metabolic rates may deplete yolk sac reserves more quickly than their genetic predecessors and thus may be at greater risk of detrimental consequences. The most recent publication that might provide additional information for this debate is Malik et al. (2010). In this study, yolk sac reserve utilisation was compared in fast and slow growing lines of chicks. The chicks were fasted and water withheld for the first 48 hours post-hatch or with early provision of food and water. The study concluded that rapidly growing chicks do utilise yolk substrates more efficiently and quickly in the 48 hours after hatch. However, as the slow growing line studied represented laying hen chicks, it is not possible to extrapolate the findings in the context of yolk sac reserve utilisation in relation to transport. It may be proposed, however, that this topic requires further investigation.

Commercial breeders and producers have long recognised the necessity to maintain an appropriate thermal environment for chicks in transport (Mitchell, 2004; 2009; Mitchell and Kettlewell, 2004b). The conditions employed have been largely defined by empirical means and based upon minimisation of mortality rates during and following transport, and efficient productivity during the subsequent rapid growth phase. In current practice, the recommended temperature for chick transport is 24-26 ºC (Ross Breeders, 1996; Weeks and Nichol, 2000). Advice from breeder companies includes a recommendation for controlled humidity (75% at 24 ºC). In many transported animals, the physiological challenges presented by the thermal conditions are compounded by extended periods without access to food or water. It has long been thought that the one-day old chick may be partially protected from such stresses by the presence of energy and water reserves in the yolk sac. Older studies proposed that yolk stores in the newly hatched chick constituted 18% of total body weight and contained approximately 2 g of lipid and 2.5 mL of water. In the absence of excessive thermoregulatory demands this represents energy and water supplies sufficient for 3 days without further provision of food and water (Mitchell, 2009). More recent studies have indicated that in modern day old chicks, high metabolic rate and rapid utilisation of resources in the first 24 hours post-hatch, coupled to delays in transport and placement, result in poorer performance and health status throughout flock life (Mitchell, 2009). Major causes of in-transport and post-transport mortality and morbidity are dehydration and under-nutrition (Mitchell, 2009). A suggested strategy to reduce metabolic depletion during extended transport is through exploitation of the reduction in metabolic rate in crated chicks in the dark (Mitchell, 2009). Neonatal chicks do not possess fully developed, effective homeothermic mechanisms (Mitchell, 2009) and consequently are vulnerable to the detrimental effects of thermal loads, fatigue and dehydration. Both body temperature and metabolic rate increase immediately after hatching. However, body temperature remains labile during exposure to sub-optimal thermal environments (Mitchell, 2009). Thus, if the transportation environments are unduly hot or cold then immaturity of thermoregulatory homeostasis, including inadequacy of lipid mobilisation or efficient evaporative heat loss during thermal polypnea may result in stressful or life threatening hypothermia or hyperthermia. In addition, the accelerated rates of utilisation of energy and water reserves may result in premature dehydration. Freeman (1984), quoted in Mitchell (2009), has estimated that reserves may be completely exhausted in as little as 8-10 hours at a temperature of 40 °C.

An effective strategy to optimise survival, productivity and welfare of the newly hatched chick in transport would be to match the thermal characteristics of the microenvironment to the biological requirements of the birds. Laboratory based modelling studies (Mitchell, 2009) have employed physiological stress modelling, measurement of metabolic rate and the concept of AET to determine optimum transport thermal environments for one-day old chicks. All measurements were performed on chicks in commercial transport containers in calorimeter chambers housed in controlled climate rooms. Temperatures of 20-35 ºC accompanied by relative humidities (RH) of 50-65% and durations of exposure from 3-12 hours were employed. Metabolic heat production ranged from 7.8 to 8.7 W/kg. An optimal temperature-humidity range of 24.5-25.0 ºC and 63-60% RH for the transport of chicks at commercial stocking density was identified on the basis of minimal change in body temperature and minimal alterations in basal metabolic rate, hydration state, electrolyte balance, body weight loss and plasma metabolite concentrations. These physiologically ideal conditions are very similar to those currently employed by commercial breeders and producers. The studies also provided evidence that if the thermal micro-environment is appropriately controlled then journey durations of at least 12 hours are acceptable. Further work on behavioural identification of the preferred thermal conditions for chicks in commercial transport simulations will provide additional refinement and support to these strategies. Transportation of one-day old chicks, for 18 hours at 25 ºC reduced the rate of subsequent growth to 45 days of age and some sustained biochemical changes compared with un-transported chicks (Pijarska et al., 2006). On the other hand Valros et al. (2008) observed no indications of lasting effects of simulated transport upon behavioural development. A recent HSUS report (HSUS, 2009) concluded that "extreme temperature ranges encountered during the transportation of one-day old chicks may be detrimental to welfare. At excessively high or low temperatures chicks may die within one hour. Reduced ventilation may also adversely affect chicks. While good ventilation can reduce the effects of high temperatures it is not clear in commercial practice if stacking configurations and spaces are adequate to promote proper ventilation." It was concluded that optimal conditions for chick transport were 24-25 ºC at an RH of 65% and a space allowance of 21-25 cm^2/chick, in accordance with previously published data, and that journeys should last for no longer than 48 hours from hatching. The temperature in the containers should be 31 ºC. The problem of inadequate ventilation of chick boxes was emphasised and it was proposed that current container densities and ventilation should be reviewed and further research undertaken to define optimal practice. It was also proposed that further work upon feeding and watering requirements of chicks in transport are required. Current industry practice and experience also suggest that due to disparities in the design and structures of chick boxes employed in different member states it may be necessary to revise stocking densities and space allowances for chicks during road transport. This is based upon reports of chilling of chicks and decreased performance and increased mortalities in chicks transported at the upper end of the current space allowance range. This is attributed to the relatively low total metabolic heat production of chicks in each compartment of chick boxes with increased heights and therefore volumes. This must be a concern in relation to chick welfare but more published data are required to support new recommendations. The industry proposes to call for a reduction in the minimum space allowance from 21 to 19 cm^2/chick in order to avoid chilling in well ventilated boxes or stacks in transport. It is unclear if increasing the recommended vehicle temperature and/or humidity would achieve the same objective and, therefore, further research is required in this area.

Mortality and physical damage

There appear to be no accurate scientifically-based surveys yielding estimates of mortality or physical damage during road transportation of one-day old chicks. Commercial practice often involves calculation of three day and seven or eight day mortality, reasoning that these values (total losses from birds transported) integrate the overall effects of the transport process and placement. On this basis mortalities of 1.0-1.7% have been reported (Mitchell, 2009). There are no studies correlating such losses with road transport conditions and events.

Vehicle and transport container ventilation

The ultimate determinants of the localised on-board vehicle (chick transporter) microenvironment are the prevailing climatic conditions, the addition of heat and water vapour to the load space from all sources including the bio-load (chicks), ventilation rate and distribution. All these issues have been extensively addressed in relation to the transport of broiler chickens at slaughter age (Mitchell 2006a; 2008; Mitchell and Kettlewell, 2008a, 2008b) but the corresponding characteristics of chick transporters have received less detailed study. Mitchell and Kettlewell (2004b) and Mitchell (2009) have reviewed work examining the ventilation characteristics of commercial chick transporters using full-scale experimental determination of ventilation patterns and their prediction by computational fluid dynamics (CFD). Their findings included the observation that the presence of the load of stacked chick boxes had a channelling effect upon the air flow through the load space with significant amounts of air by-passing the chick boxes and being re-circulated. The implications of this ventilation regime for air flow in the chick containers was seen in the temperature distributions, with peak temperatures occurring in the front central boxes and cooler air by-passing the load. In addition, cooler air entered from beneath the vehicle in the fully loaded configuration and reduced flow through the load as well as potentially introducing exhaust fumes in to the load space. It must be concluded that more research is needed to address these issues.

2.7.4. Water and feeding interval, journey times and resting periods

Journey length / duration

Criteria and potential reasons for the definition of maximum journey times for livestock travelling to slaughter have been proposed and discussed by Cockram (2007). This subject, however, has received little attention in relation to the transportation of poultry. Transport-related mortality in broilers is related to journey length, which has been shown to be 0.15% on journeys of up to 50 km and 0.9% on journeys of over 300 km under the conditions prevailing in the Czech Republic over the year, with further increases in losses during the summer months due to the increases in temperature during transport (Vecerek et al., 2006). Longer transport times for broilers may be associated with decreases in meat quality variables, which is an effect exacerbated by longer holding times at the processing plant (Bianchi et al., 2006). Elevated mortalities in transported broilers are associated with longer journeys (300 km) compared to shorter journeys (<50 km), with the incidences being 1.6% and 0.6%, respectively, under European conditions in a study over 4 calendar years (Voslarova et al., 2007a, 2007b). In these studies higher mortality rates were observed in the winter season. Longer journey times and pre-slaughter heat stress result in an increased incidence of lower ultimate pH in muscle and paler meat from broiler birds (Ali et al., 2008). Mortality rate on longer journeys of up to 8 hours duration in Hungary (Miklos, 2008) was higher (0.74%) than on shorter journeys (0.40%). Under warmer conditions, the DOA was lower (0.46%) than under cold winter conditions (0.73%). Ali et al. (2008) have reported that problems of paler meat were associated with longer transport times and the incidence of low ultimate pH post-transport was exacerbated by exposure to elevated temperatures in the pre-slaughter period.

Body weight loss in response to transportation has been described by Nijdam et al. (2005a, 2005b). Broiler chickens transported for 3 hours after feed withdrawal for 10 hours lost body weight at a rate of 0.42% per hour, whereas birds that had *ad libitum* access to food prior to transportation exhibited a rate of only 0.30% per hour. Weight loss of broiler chickens in transport has been found to be related to journey duration (Karaman, 2009). Birds of lower initial body weight (<2.0 kg) lost 47, 64 and 106 g of body weight on journeys of 1, 2 and 3 hours duration, respectively. For heavier birds (>2.5 kg), the corresponding figures were 93, 139 and 141 g, respectively. Wojcik et al. (2009) reported that the plasma and muscle concentrations of calcium, magnesium, sodium, potassium, phosphorus and iron alter reciprocally in response to transport and, whilst serum concentrations increased on the journeys of 100 km length, on longer journeys of 200 km a decrease was induced.

A comparison of journey times of between 30 and 180 minutes under Brazilian summer conditions (Oba et al., 2009) and lairage periods of 0-180 minutes indicated that the incidence of PSE was higher on short journeys and short holding periods, whereas the DOA rate was highest on longer journeys and longer lairage. Zhang et al. (2009) have proposed that extended recovery periods after transport for up to 3 hours may allow for a beneficial lowering of plasma corticosterone, decreased muscle glycolysis and improved broiler meat quality.

Recent evidence suggests that longer transport times for broilers are associated with the increased production of the superoxide radical in skeletal muscle, increased lipid peroxidation and changes in expression of avian uncoupling protein, responses that mediate subsequent changes in meat quality and losses (Zhang et al., 2010). Some of these effects may be mitigated by appropriate “resting” times at the processing plant. In a contrasting study, Yue et al. (2010) reported no marked effect of transportation of Chinese Lignan chickens for up to 3 hours on meat quality variables and no beneficial effect of subsequent resting for up to 3 hours, which tended to further reduce muscle glycogen content and reduce blood glucose.

3. Identification of animal-based indicators of welfare during transport for their possible use as an alternative to the present legislative requirements.

The mandate requires to “to identify and evaluate outcome-based animal welfare indicators which can be used by transporters and veterinary inspectors under commercial conditions and consider their possible use as an alternative to the present requirements”. An approach to risk/benefit assessment in animal welfare that is focused on defined welfare outcomes, rather than driven by input factors, is consistent with current thinking as exemplified by the Welfare Quality® project (Botreau et al., 2007). It is self-evident that decisions related to the acceptability of transport strategies or individual journeys, which are based on direct assessment of the physical and mental welfare of the animals, are more likely to be beneficial to the animals (and acceptable to society) than those solely based on strict formulaic input measurements of, for instance, vehicle dimensions and journey lengths. Moreover, a strategy for risk management that is defined by the desired outcome (indicators of welfare) is methodologically more likely to achieve a closer approximation to the desired outcome than a strategy solely defined by inputs (e.g. vehicle dimensions and journey lengths), since the effects of all inputs carry a degree of uncertainty and, as inputs proceed to outcomes through a succession of steps, the level of uncertainty is liable to increase.

There are, however, several conceptual and practical problems associated with the use of animal-based assessments of welfare outcomes as a basis for legislation, codes of practice, or professional advice.

Any assessment of animal welfare based on observations of appearance, attitude and behaviour is likely to carry an element of subjectivity, especially when observers are required to "score" the intensity of an adverse (or beneficial) effect. It is necessary therefore to select animal-based indicators and a scoring system that will produce consistent results when used in practice by trained observers.

Observation and recording of animal welfare indicators by transporters and veterinary inspectors under practical conditions should be designed to minimise disturbance both to the animals and to the process. Further disturbance to the animals at an already stressful time (e.g. unloading) may exacerbate the stress. Moreover, any procedure that requires restraint of individual animals (e.g. measurement of heart rate) would confound any assessment of the stress of the journey with the stress of the procedure itself.

The welfare of farm animals managed by humans, whether on the farm, in transport, in lairage, or at the point of slaughter depends on the quality of the environment, the provision of suitable resources, and the competence and compassion of those in direct contact with the animals. Any welfare assurance scheme based on effective monitoring and control has to take these concepts into account. Moreover, in the specific context of monitoring animal transport, any system solely dependent on the measurement of welfare outcomes can be criticised on the basis that it is reactive rather than proactive: it relies on evidence of malpractice rather than seeking to prevent it through better attention to the provision of good husbandry. It should be recommended that monitoring protocols for the assessment of the impact of transport practices on animal welfare should incorporate both robust measures of welfare outcomes and sound evidence of good practice.

Animal-based welfare indicators suitable for use in practice need to be not only unambiguous and measurable without disturbance to the animals but they also have to be reasonably consistent with, and therefore justified by, more scientific measurements of physiology, biochemistry, neurobiology and behaviour. It is also necessary to relate these observations, which are no more than signs of (adverse) effects, to measurable changes in the parameters of welfare itself (e.g. pain, fear, exhaustion, thermal stress).

Table 4 presents a series of observations that can be incorporated into protocols for use by transporters and veterinary inspectors at the points of departure and destination, in conjunction with appropriate "input" measures relating, for instance, to the design and condition of vehicles, provisions for feed, water and bedding. The observations are categorised according to the adverse effects (e.g. thermal stress, dehydration, pain, fear) of which they are the visible signs. They should, in most cases, be sufficient to provide inspectors with the necessary evidence to:

- identify individuals at the point of departure that are unfit to travel;
- provide evidence of adverse effects of the journey on some or all of the animals observed at their destination.

The table also presents a series of relatively practical clinical measurements and diagnostic procedures that may be carried out on selected individuals to confirm or reinforce conclusions derived simply from observation of the group. It may be necessary, for example, to demonstrate an elevated body temperature and/or abnormal respiratory sounds in order to justify the decision to declare an individual animal unfit for transport. In the event that a veterinary inspector considered that the adverse effects of heat stress and dehydration were severe enough to justify criminal proceedings from observation of a group of animals at their destination, then it would become expedient to reinforce these observations with more tangible evidence (e.g. elevated body temperature, haematocrit, abnormal blood pH and pCO_2).

The most important and practical of these welfare indicators are listed below. Since the categorisation of adverse effects is the same for all species of concern, and many of the indicators are similar, these have been condensed into a single table. However, veterinarians and other inspectors should not consider them as exhaustive. More comprehensive tables for each of the species of concern are given in Appendix A.

Table 4. Summary of observations and clinical measurements that can be used as practical indicators of adverse effects of hazards associated with transport on animal welfare.

Adverse effect	Observations	Clinical measurements
Heat stress	Thermal panting, drooling Extreme thirst Prostration, collapse, mortality Sweating (horses, cattle) Gaping, gular flutter (poultry)	Body temperature
Cold stress	Shivering, huddling Piloerection of feather erection Skin colour (pig, poultry) Prostration, collapse, mortality	Body temperature
Dehydration	Extreme thirst 'Skin-pinch test' Prostration, collapse, mortality	Haematocrit Blood pCO_2, pH Urine colour, specific gravity
Exhaustion	Apathy, reluctance to move Prostration, collapse, mortality	Dark, firm dry carcasses Blood enzymes (CK, CPK)
Disease	Prostration, collapse, mortality Nasal, ocular discharge Abnormal respiration Diarrhoea, blood in faeces Vomiting (motion sickness in pigs)	Elevated body temperature Abnormal respiratory sounds Presence of pathogens
Pain & injury	Lameness, reluctance to move Abnormal body posture Skin lesions Swollen joints, feet	Inspection of affected areas (e.g. feet, suspected fractures) Hypersensitivity to touch
Fear	Vocalisation Escape behaviour Aggression (stamping, kicking) Urination and/or defaecation	Plasma corticosteroids Tonic immobility (poultry)

4. Impact on welfare due to transport associated disease transmission

Fitness for transport, animal welfare and infectious disease are intimately related. Animals that show clinical signs of infectious disease are self-evidently unfit to travel, both in the interests of their own welfare and that of the other animals on the vehicle. Stresses associated with handling and transport may cause latent infections with, for instance, *Salmonella* or *Pasteurella* sp. that proceed to clinical disease. Such animals are more likely to infect others during the journey or after arrival at their destination and in many cases (e.g. salmonellosis) this will also increase the risk to public health. This is the case for the whole panorama of the infectious animal diseases. The most important epizootic diseases are covered by globally accepted standards (OIE). However, in terms of animal welfare, the most important diseases are the endemic infections with the highest prevalence (e.g. enteric infections and the respiratory diseases associated with the "shipping fever" complex; IAASTD, 2009).

The risks for global spread of infectious diseases and the associated consequences for animal welfare by transport of animal remains, as well as infectious disease pandemics, vector invasion events and vector-borne pathogen importation have been identified as the major *consequences* (Tatem et al., 2006). The risks for spreading diseases also remain significant at the regional level, in particular, the trade of animals through markets (Robinson and Christley, 2007). This was found to be important in the spread of the foot-and-mouth disease (FMD) virus (e.g. during the 2001 outbreak in Great Britain; Mansley et al., 2003; Robinson and Christley, 2007). The chief veterinary officers (CVOs) of the EU Member States recently ranked movement of animals as the most important risk for spreading infectious diseases between farms (EC, 2009).

Prevention

The prevention of spreading infectious diseases by transport and thereby associated impaired animal welfare is well understood and current regulations at international and EU levels mitigate the risks involved, as reviewed by Fèvre et al. (2006). Typically, a total ban on the movement of animals is a standard procedure in emergency situations. However, such regulatory guided actions focus primarily on "listed diseases" and for the case of animal welfare it may be equally important to consider also the non-listed endemic diseases.

Several authors have proposed using social network analysis and network-based epidemiological models to analyse the animal transport data available in National Livestock Databases and assess risk and vulnerability of holdings in respect to trade patterns (for a review see: Martínez-López et al., 2009 and Dubé et al., 2009). The Joint Research Centre (JRC) developed an application using the National Animal Databases in order to analyse possible disease flows by transport from any given farm holding. This approach could identify the holdings most at risk during outbreaks and would allow increasingly selective biosecurity measures. The work also showed that selective movement restrictions for holdings most at risk would effectively reduce disease spread with consequent lower impact on trade (Natale et al., 2009).

Appropriate prevention involves different biosecurity measures. A crucial step is to prevent mixing of animals of uncontrolled origin and animals of a different health status. Priority should be given to direct transport without stop-overs that carry the risk for direct or indirect contact with animals from other holdings. An overall strategy is also, when possible, to avoid transport of live animals. Breeding animals may be replaced with the less risky use of semen or embryos and long distance transport of animals for finishing or slaughter may be replaced by the transport of carcasses and food products.

5. Control Posts

Major animal welfare risks when control posts are used relate to: (a) increased transmission of disease, especially if animals are unloaded, (b) poor welfare associated with unloading and loading, (c) fatigue and other poor welfare associated with journeys of longer duration than those allowed without use of a control post. The welfare aspects for each individual species and for disease transmission are described elsewhere in this report.

One possibility to deal with the negative aspects of the use of control posts is to introduce strategies that decrease the need for control posts. It could also be considered whether or not they are necessary at all.

If animals on vehicles are not unloaded at control posts, the welfare of the animals can be better because of reduced disease transmission and reduced loading and unloading stress. However, this would only be an advantage if good regulations concerning space allowance, resting time, watering facilities, and feeding facilities (see the relevant sections of this report) exist and these are complied with. If animals are to remain on vehicles at a control post, the space allowance on the vehicles would have to be increased in order that the needs of the animals for rest, food and water can be met (Broom, 2008). There may also be problems both on vehicles and after unloading at control posts because animals are unwilling to feed in unfamiliar circumstances (Hall et al., 1997, Parrott et al., 1998, Krawczel et al., 2008). However, leaving animals on board a stationary vehicle at control posts for extended periods could lead to poor welfare if insufficient care is taken of the animals due to a low level of compliance with EC Regulation 1/2005 (Marlin et al., 2011).

6. Description of methods for monitoring animal transport

The monitoring of animal friendly production – including live animal transport – is becoming increasingly recognised as an important attribute of food quality and quality assurance schemes (Blokhuis et al., 2008, Veissier et al., 2008). Besides the animal welfare aspect, there is an increasing demand for traceability along the food chain that is related to food safety (Ruiz-Garcia et al., 2010).

There are various indicators to draw upon for assessing the conditions under which animals are transported. Some of the indicators are, however, difficult to assess during on-the-spot checks or after transport has been completed if they are not systematically monitored and recorded along the journey. For better enforcement of the standards and increasing the traceability of long journey transport operations EC Regulation (EC) 1/2005 (EC, 2005) requires a monitoring document (journey log) for long journeys of domestic cattle, sheep, goats and pigs, as well as for domestic horses other than registered horses. In addition, road vehicles used for long journeys of these species have to be fitted with:

a) a temperature monitoring and recording system, as well as a warning system to alert the driver when temperatures in the animal compartments reach the maximum or minimum limit, and sensors must be located in parts of the lorry most likely to experience the worst climatic conditions;
b) a navigation system, providing a global, continuous, accurate and guaranteed timing and positioning service, allowing for recording and providing information equivalent to that in the journey log, Section 4, and information on opening/closing of the loading flap.

Although it is called a “navigation system” in the EC Regulation, from the requirements to record and provide information equivalent to Section 4 of the journey log and to the loading doors, its primary purpose is to act as a tracing system, monitoring whether the transport was executed to the stipulated requirements.

Despite the requirement that since 2007 new, and since 2009 all, long journey road vehicles should be equipped with “navigation and temperature monitoring systems”, there is widespread uncertainty regarding both the specifications and their implementation for official animal welfare controls (FVO 2009a, personal communication with transporters and Member State authorities). The competent authorities or designated bodies have to grant approval for the means of road transport used for long journeys when they comply with the legal requirements, including the requirements for a navigation and temperature monitoring system. However, competent authorities, as well as transport companies, frequently express that they are unable to verify if a system fulfils the requirements laid down.

Implementation of this regulation is compromised both by the lack of clarity in what is required and, subsequently, a lack of accepting effective monitoring systems. These points were emphasised repeatedly and with unanimity at the meeting with stakeholders (13/10/2010) representing a wide range of interests, including animal welfare charities, research scientists, veterinarians, companies involved in vehicle design and the international transport of animals. This section considers the design, validation and implementation of "autonomous" systems for monitoring and recording journeys, journey events and environmental conditions for the animals on the journey in the light of new evidence. The approach to monitoring the quality of journeys, described here, when taken together with Sections 2 and 3 of Annex I to Regulation (EC) 1/2005 which outlines procedures for monitoring animal welfare at the points of departure and destination, provide the scientific underpinning for the establishment of effective protocols for assessing compliance with the regulations contained within this Regulation, and any amendments that may arise from recommendations contained within this report.

6.1. Manual monitoring - Journey log

Detailed Community rules exist for the monitoring of long journeys. A number of actors are involved in the documentation, such as the organiser of the journey that completes the planning section, the animal keepers at the place of departure and destination, the transporter, the driver and the relevant competent authorities. After having completed the journey, a copy of the journey log should be returned to the competent authority at the place of departure and, on request, the corresponding sheet or printout of the tachograph should additionally be provided. The competent authorities at the destination can access some information concerning a planned journey through the integrated veterinary computer system TRACES.

For other journeys, Community legislation envisages that the organiser provides a person to be responsible for providing information on the planning, execution and completion of each journey to the competent authorities.

Reports from the Food and Veterinary Office (FVO 2008, 2009a, 2009b), from NGOs and from the Joint Research Centre on temperatures during transport (JRC, 2009b) indicate that manual monitoring and documentation in the journey logs are often incomplete and/or not returned to the competent authority of departure to allow for verifying compliance.

6.2. Tracing systems in animal transports

A wide range of devices and solutions for tracing and temperature monitoring systems can be found in commercial long journey animal road transport vehicles. Most systems derived from the tracing and tracking system services for the logistics industry consist of the following main elements in the transport vehicles.

- Receiver for a global navigation satellite system (GNSS) for exact timing and positioning;
- Sensors measuring certain parameters (e.g. temperatures, loading flap status);
- System control unit (on-board unit) with firmware and memory to monitor and record data provided by the peripheral devices;
- Output of the recorded information (e.g. module for regular data transmission to a remote receiver, a display and/or printer or port for electronic download).

Some systems also provide a cabin-user-interface whereby the driver can enter predefined information into the system, such as journey status events (start, break, and end), and receive information as to the status of the vehicle (e.g. mechanical ventilation or temperature warning). However, the system architecture, on-board architecture, functionalities and data availability vary considerably between different providers, which make it difficult to interpret the measured parameters and their relevance for animal welfare (JRC, 2009a).

JRC (2008) has outlined an approach for standardising the requirements of tracing systems in long journey transports. This would be based on automated monitoring and recording of journey durations, resting times and temperatures. The recordings would be accessible on the vehicle and also remotely.

Vehicle manufacturers and the majority of the national authorities favoured a tracing system that would permanently record (black box) and transmit data to a remote receiver in a decentralised architecture. With a view to data protection, according to the transporters and animal vehicle manufacturers, a decentralised architecture has clear advantages compared with a centralised system for the functioning and maintenance of on-board and remote equipment for around 6,000 long journey vehicles in circulation in the EU. This structure is already widely used in logistics. Under such architecture, private service providers would supply the transporters with the on-board devices, as well as receive and manage the data. The drivers would be able to assess the monitored transport conditions from the on-board system (display), the transporters could assess the transport conditions and status for their fleet at the remote receiver and the service providers would be able to transmit defined relevant animal welfare data to a dedicated service provider. Authorised competent authorities would have remote access to the information at this point.

Systems should automatically monitor and record the time, position, and temperatures in the compartment(s) at regular intervals (e.g. every 15 minutes), as well as the status changes of the loading door(s). In addition, a predefined set of entries from the driver could be handled, such as start, breaks, end of journey, species, category and number of animals loaded, and journey log/batch number(s). The recorded data would be stored on the systems memory and be made available to authorised users on the vehicle and remotely. A common communication standard (e.g. XML) and message structure from the intermediate service provider to a dedicated service provider would ensure a common data structure, irrespective of the origin of the vehicles and the tracing system.

In the transport industry, other requirements and systems apply or will apply (e.g. digital tachograph, monitoring of dangerous goods transport, e-call, and electronic road tolling). In 2009, the European Union commissioned a study regarding adoption of an open in-vehicle platform architecture for an intelligent transport system (ITS), which would include animal transport. According to the European Commission, ITS will address some of the pressing challenges that Europe faces in the transport sector (EC, 2009). Ljungberg et al. (2007) found that by route optimisation, the time and distance of transport could be reduced (e.g. for some routes, time savings of more than 20%).

6.3. Methods of automated monitoring and recording of legally required parameters

Exact time and position

Amongst the existing GNSS, the US global positioning system (GPS) is the most commonly used satellite navigation system worldwide that provides positioning and precise timing. However, a secure precise GPS positioning service is only available to military users, while the standard positioning service is free for civil and commercial users and frequently present in commercial animal transport vehicles.

The standard GPS positioning service is considered a useful tool for tracing, tracking and surveillance, although it has certain shortcomings. Some of the reported shortcomings would be of little importance in animal transport, such as the error of position, which is in the range of 15-20 metres, or the temporary absence of satellite signals (e.g. in urban areas with high buildings). However, other shortcomings may be of more relevance to animal transport, such as longer lasting disturbances of GPS signals by unintentional or deliberate jamming, when radio signals interfere with the satellite signals. The position and time can also be falsified by spoofing, which intercepts the calculation process for positioning and timing.

The European Geostationary Navigation Overlay Service (EGNOS) is intended to supplement GPS and other global satellite navigation systems (e.g. the existing GLONASS and the planned Galileo system) with improved reliability and accuracy (BNSC, 2009; Witte and Wilson, 2005). EGNOS and Galileo plan to offer a paid service with authentication of the position and time signals.

Temperatures

A large number of studies have involved temperature recording in vehicles during animal transports (e.g. Sällvik et al., 2005; Brüser-Pieper, 2006; Christensen et al., 2007; Knezacek et al., 2010, and other papers referred to in the species-based reviews in Section 2 of this document). However, differences in the type of vehicles and the number and location of the temperature sensors within a vehicle render comparisons difficult. A study recording temperatures in commercial transport vehicles on more than 900 animal journeys throughout Europe (JRC, 2009b) confirmed previous results that the temperatures within a transport vehicle may vary considerably both in time and space. In more than 7% of the records, differences between minimum and maximum temperature at a given time in the vehicles exceeded 10 ºC. The study proposed the number and position of temperature sensors to ensure a comparable representation of temperatures for different types of transport vehicles for horses, cattle, pigs, sheep and goats (mono-volume, multi-tier semi-trailers, truck and trailers). It was concluded that the preferable positions in semi-trailers would be one sensor in the front of the upper deck, one at the front on the lowest deck and one at the back on the upper deck, whereas in a truck and trailer configuration two sensors in the truck (one at the front on the lowest deck, one at the back on the upper deck) and three in the trailer (one at the front on the upper deck, one at the back of the upper deck and one at the back of the lowest deck).

The same study reported that out of all consecutive periods with temperatures above 30 ºC, at least for one sensor on the vehicles, 75% had durations of less than 2 hours and temperatures < 32 ºC. As regards the low temperature threshold, out of all consecutive periods with temperatures below 5 ºC, at least from one sensor in the vehicle, 75% had durations of less than 3.7 hours and temperatures > 2.6 ºC. The study concluded that temperature monitoring should not only consider the temperature thresholds but also the duration of out of range temperatures.

Allocating different acceptable temperature ranges for different species and categories of animals transported would require a temperature monitoring and warning system to monitor the temperatures against different thresholds. Some of the available tracing systems cater for different species/category settings.

As the temperatures recorded by sensors in the compartment(s) where the animals are loaded may not reflect the animals' temperature sufficiently, thermal imaging systems have been studied for recording the body temperature. Warriss et al. (2006) found good correlation in pigs between the mean ear temperature collected by thermal imaging in lairage and blood temperature at bleeding. Although thermal imaging was used in some transport studies, little experience is available as regards reliability and practicability of the devices when used in routine commercial animal transports. It may be more appropriate to use it as an assessment tool to monitor individual animals at unloading.

Electronic transponders (RFID devices) are described as another way of monitoring body temperature. There are transponders, injected or applied as a bolus, which besides the individual identification of an animal, such as that legally required in sheep and goats by Regulation (EC) 21/2004[8], are able to monitor its body temperature. Their use for continuous monitoring during commercial transports seems technically difficult though and recent studies concluded that improvements are needed regarding accuracy and practical application (Kort et al., 1997; Green et al., 2008; Ipema et al., 2008; Mash et al., 2008).

[8] Council Regulation (EC) No 21/2004 of 17 December 2003 establishing a system for the identification and registration of ovine and caprine animals and amending Regulation (EC) No 1782/2003 and Directives 92/102/EEC and 64/432/EEC. OJ L 5, 9.1.2004, p. 8–17.

Status of the loading flap

Many technical solutions are available for sensing the open and closed status of a loading flap. Contact sensors, which require a very close contact, in practice frequently show false 'flap open' status during driving due to twisting movements between the chassis and the flap. This is a particular problem for installations on older vehicles.

Journey events

Journey events during an animal journey can be recorded given that a journey is uniquely and unambiguously identified (e.g. by a journey number or a TRACES number). The start, rests and end of a journey can be replicated from the information recorded by the tracing system (time stopped at a given position with status of the loading flap) displayed on a geographical map and compared with the journey plan. With a cabin user interface, some tracing systems in animal transport also offer the possibility for the driver to enter such events into the system, as well as the species/category of animals loaded for setting the defined temperature thresholds, and information on the number of injured or dead animals. Such an interface can also display temperatures and other useful information, such as the status of the mechanical ventilation and an alert for the driver when a temperature limit is reached. The cabin user interface can also incorporate multiple other inputs, such as the number of animals loaded, identification of the batches loaded, information on the holdings or the individual animals (JRC, 2010), or inputs/information regarding fleet management.

6.4. Methods of monitoring other variables

A number of other variables could be considered for a transport monitoring system, such as relative humidity, airflow and ventilation, vehicle movement and vibration, total weight or animal behaviour. For some of these parameters, very little experience is available as regards reliability of the monitoring devices when used for routine commercial animal transports and what are the acceptable values.

Although output-based indicators, such as behavioural and physiological measurements may allow a more animal-based assessment of welfare (Matthews, 2008; Keeling, 2009), behavioural and physiological measurements during transport seem difficult to conduct (von Borell and Schaeffer, 2005) and analyse.

Humidity

Monitoring humidity in combination with temperature would allow a tracing system to automatically calculate the temperature-humidity index (THI). Monitoring humidity in commercial transport vehicles remains problematic due to, for example, jet spraying during vehicle washdown and disinfection that may harm the sensor's membrane, thus rendering the monitoring in the daily routine of commercial transports unreliable. Consequently there is a need to devise more robust sensors for temperature and relative humidity with more resistant membranes, such as a porous metallic membrane, for routine use in animal transports.

Airflow and ventilation

As temperature and humidity in the animal compartments are largely influenced by the airflow/ventilation, Christensen and Jonsson (2007) proposed that in pig transports at a temperature above 20 ºC inside the compartments, forced ventilation should automatically be switched on to improve the welfare of the animals, especially at loading and during stops. In a tracing system, the standardised temperature monitoring could be set to manage the mechanical ventilation system. Such tracing systems are used in vehicles for transport of perishable and refrigerated goods.

Vehicle movements, vibration

The effects of driver behaviour, driving style and road type on the transported animals have been described in different studies and there are indications that vibration may impair the welfare of livestock. With the low frequency vibration (0.01 to 0.2 Hz) measured in transport vehicles, pigs can exhibit signs of travel sickness, such as foaming, chomping, retching or vomiting. Vibration tests at different frequencies from 2 to 18 Hz provoked an increase of ATCH and plasma cortisol in piglets, as well as a reduced resting behaviour (Perremans et al., 2001). Vertical (up-down) vibration of 18 Hz seems to be less stressful than low frequency vibration (2-8Hz). During their tests, Christensen and Jonsson (2007) found frequencies within this range to a limited extent but with a tendency for low frequency vertical vibration to occur more often in the upper tier of the transport vehicle. When comparing normal, quiet and wild driving style for a small, towed, twin-axle farm trailer, Peeters et al. (2008) measured the lowest salivary cortisol increases for a wild driving style, whereas the quiet driving style resulted in a lower proportion of standing pigs and a consequent higher proportion of lying pigs. For the proportion of pigs standing and lying, a driving style-temperature interaction was described. The proportion of pigs standing decreased with increasing temperature and this decrease was smallest in a wild driving style.

Cockram et al. (2004) found that the driving style had a slight effect on the frequency of loss of balance in sheep but a more significant effect on resting behaviour during the journey.

Vibration and thermal stressor experiments in broiler chickens described aversion to vibration of 2 Hz and acceleration of 1.0 ms^{-2} (Abeyesinghe et al., 2001). These results supported previous work by Randall et al. (1997) which found root-mean-square weighted accelerations of 0.5-1.0 ms^{-2} as aversive and 2.0-4.0 ms^{-2} as extremely aversive to broiler chickens.

At least one commercially available tracing system for animal transports has an integrated tri-axial high precision accelerometer to routinely set the diesel consumption in relation to the driving behaviour (personal communication). The values monitored by the accelerometer could also feed in the assessment of animal welfare during commercial transports. However, as regards acceptable values of vibration at different locations of a commercial transport vehicle the knowledge is limited. More information would also be needed if and how low frequency vibrations could be minimised or avoided by constructive elements of the vehicles.

Total weight of load

Monitoring the weight in combination with the recording of species/category and number of animals loaded would allow the tracing system to provide an estimation of the loading density.

Several methods have been explored to automatically record the total weight of the animals when loaded (personal communication). So far, one method that senses the weight in the shock absorbers seems to be realistically applicable. However, the margin of error and fluctuation still lies at approximately ± 5% of the real weight (i.e. for a load of 20 tonnes ± 1 tonne).

Behaviour

While video surveillance of the animal behaviour during transport is used in many research projects, it is difficult to operate in routine commercial animal transports. When transporting adult cattle in 2 decks, or sheep and pigs in 3 or sometimes in 4 decks, the height of the deck and the reduced luminosity only gives very limited insight into a compartment. While values of measured parameters can be screened and analysed automatically, analysing images would require more sophisticated computer applications or an additional person to view the images.

6.5. Methods of visualisation and documentation of the recorded data

Printouts on the transport vehicle

According to system manufacturers and transporters, the veterinary services frequently request printouts of the temperature recordings of long journey transports. However, the layouts of the protocols vary widely and are sometimes difficult to analyse. While some printouts show an analysis if and when temperatures fall outside the legal thresholds, others list, in long paper rows, only the temperature records of each sensor.

Local data download and/or display

For locally analysing downloaded data from a tracing system a specific application is necessary. Without standardisation, each tracing system would require a different application for downloaded data. Depending on the architecture and memory of the on-board system of a tracing system, data of an on-going and of previous journeys could be displayed on the screen of the cabin user interface, comparing journey and temperature events with the legally accepted thresholds. In addition, some tracing systems available for animal transports allow an easy graphical comparison of the values.

Remote transmission and web access

Data transmitted to a private service provider would allow almost real time corrective actions by the transporter, if necessary, as well as straightforward documentation and provision of information for customers and suppliers along the livestock supply chain. Remote data management would also allow for further processing of the data (e.g. against thresholds, with automatic calculation of the THI) if deemed necessary.

In addition, with web access, certain data could become available remotely in almost real time to the competent authorities in order to verify legal conformance of ongoing or completed transports.

A decentralised system with private service providers could reduce the administrative and organisational burden but they would require certification and auditing to ensure data integrity for official purposes.

CONCLUSIONS AND RECOMMENDATIONS

1. Annex I of EC Regulation 1/2005

In order to assess the risks for the welfare of transported animals according to the provisions of the present EC Regulation 1/2005, new scientific evidence and data have been arranged following the structure of Annex I of the Regulation. Conclusions and Recommendations have been ordered following the same structure.

In order to allow the reader to immediately link the conclusions and consequent recommendations with the provisions of Annex I of EC Regulation 1/2005, the piece of legislative text corresponding to Annex I has been placed (grey box) before the conclusions and recommendations corresponding to each Annex I chapter or section.

Chapter I
FITNESS FOR TRANSPORT

1. No animal shall be transported unless it is fit for the intended journey, and all animals shall be transported in conditions guaranteed not to cause them injury or unnecessary suffering.

2. Animals that are injured or that present physiological weaknesses or pathological processes shall not be considered fit for transport and in particular if:

- (a) they are unable to move independently without pain or to walk unassisted;
- (b) they present a severe open wound, or prolapse;
- (c) they are pregnant females for whom 90 % or more of the expected gestation period has already passed, or females who have given birth in the previous week;
- (d) they are new-born mammals in which the navel has not completely healed;
- (e) they are pigs of less than three weeks, lambs of less than one week and calves of less than ten days of age, unless they are transported less than 100 km;
- (f) they are dogs and cats of less than eight weeks of age, unless they are accompanied by their mother;
- (g) they are cervine animals in velvet.

3. However, sick or injured animals may be considered fit for transport if they are:

- (a) slightly injured or ill and transport would not cause additional suffering; in cases of doubt, veterinary advice shall be sought;
- (b) transported for the purposes of Council Directive 86/609/EEC (1) if the illness or injury is part of a research programme;
- (c) transported under veterinary supervision for or following veterinary treatment or diagnosis. However, such transport shall be permitted only where no unnecessary suffering or ill treatment is caused to the animals concerned;
- (d) animals that have been submitted to veterinary procedures in relation to farming practices such as dehorning or castration, provided that wounds have completely healed.

4. When animals fall ill or are injured during transport, they shall be separated from the others and receive first-aid treatment as soon as possible. They shall be given appropriate veterinary treatment and if necessary undergo emergency slaughter or killing in a way which does not cause them any unnecessary suffering.

5. Sedatives shall not be used on animals to be transported unless strictly necessary to ensure the welfare of the animals and shall only be used under veterinary supervision.

6. Lactating females of bovine, ovine and caprine species not accompanied by their offspring shall be milked at intervals of not more than 12 hours.

7. Requirements of paragraphs 2(c) and 2(d) do not apply for registered Equidae if the purpose of the journeys is to improve the health and welfare conditions of birth, or for newly born foals with their registered mares, provided that in both cases the animals are permanently accompanied by an attendant, dedicated to them during the journey.

In horses, rabbits, pigs, sheep and goats there is no new evidence to support any conclusions and recommendations linked with the provisions of Annex I of EC Regulation 1/2005.

Cattle:

Conclusion

- Repeated humane handling of cattle during rearing, and in particular immediately prior to transport, can minimise aversive reactions at the time of transport.

Recommendation

- There should be repeated humane handling of cattle during rearing and immediately prior to transport, in order to minimise aversive reactions during transport.

Poultry:

Conclusions

- The type and age of birds determine the potential for reduced welfare in transport. Due consideration must be paid to the potential for the presence of metabolic disease and injuries in both broilers and laying hens, the effects of which may be exacerbated by hostile transportation conditions and poor handling;
- Under current commercial conditions, birds with both “old” injuries, catching-induced injuries and pre-existing pathologies may be loaded and transported.

Recommendation

- There should be careful inspection of both broiler chickens and laying hens prior to transport to ensure that they are fit for transport. Inspection responsibilities and procedures should be specified.

Recommendation for further research

- Pre-transport inspection/assessment procedures for end of lay hens and broiler chickens.

Chapter II
MEANS OF TRANSPORT

1. Provisions for all means of transport

1.1. Means of transport, containers and their fittings shall be designed, constructed, maintained and operated so as to:

a) avoid injury and suffering and to ensure the safety of the animals;
b) protect the animals from inclement weather, extreme temperatures and adverse changes in climatic conditions;
c) be cleaned and disinfected;
d) prevent the animals escaping or falling out and be able to withstand the stresses of movements;
e) ensure that air quality and quantity appropriate to the species transported can be maintained;
f) provide access to the animals to allow them to be inspected and cared for;
g) present a flooring surface that is anti-slip;
h) present a flooring surface that minimises the leakage of urine or faeces;
i) provide a means of lighting sufficient for inspection and care of the animals during transport.

1.2. Sufficient space shall be provided inside the animals' compartment and at each of its levels to ensure that there is adequate ventilation above the animals when they are in a naturally standing position, without on any account hindering their natural movement.

1.3. For wild animals and for species other than domestic Equidae or domestic animals of bovine, ovine, caprine and porcine species where appropriate, the following documents shall accompany the animals:

a) a notice indicating that the animals are wild, timid or dangerous;
b) written instructions about feeding, watering and any special care required.

1.4. Partitions shall be strong enough to withstand the weight of animals. Fittings shall be designed for quick and easy operation.

1.5. Piglets of less than 10 kgs, lambs of less than 20 kgs, calves of less than six months and foals of less than four months of age shall be provided with appropriate bedding material or equivalent material which guarantees their comfort appropriate to the species, the number of animals being transported, the journey time, and the weather. This material has to ensure adequate absorption of urine and faeces.

1.6. Without prejudice to Community or national rules on crew and passenger safety, where transport on a vessel, an aircraft or a rail wagon is to last more than three hours, a means of killing suitable for the species shall be available to the attendant or a person on board who has the necessary skill to perform this task humanely and efficiently.

In pigs, sheep, goats and cattle there is no new evidence to support any conclusions and recommendations linked with the provisions of Annex I of EC Regulation 1/2005.

In the case of rabbits, new scientific evidence confirms the previous conclusions of EFSA (2004a) on the size and height of the crates, type of floor, mixing unfamiliar animals, thermal stress and lack of ventilation.

Horses:
Conclusions

- Equidae differ markedly from other commonly transported farmed species, such as sheep and cattle, in terms of inter-animal behaviour and, in particular, the levels of aggression during transport;
- Equid animals find it relatively difficult to maintain their posture during sudden vehicle movements because of their high centre of gravity. They are at relatively high risk of injury during all journeys and exhaustion after long journeys;
- The only exceptions to individual stalls or pens are for mares travelling with their foals and groups of semi-feral, unbroken ponies already accustomed to living as a social group. The current maximum group size of 4 allowed for this type of pony under EU legislation appears to be the optimum;
- Equidae can have problems with thermoregulation, especially at high temperature. They rely on sweating to thermoregulate at high temperatures and this can be compromised when ventilation within a load is restricted.

Recommendations

- Since there is a high potential level of aggression between animals, equid animals transported for commercial purposes should always be transported in individual stalls or pens (except for mares travelling with their foals), whether by road, rail, air or sea, for all journeys;
- Since there are high levels of injury associated with contact with lorry structures, as well as the vulnerability of equid animals to thermal stress, the partitions used between stalls should protect and isolate (physically and socially) each animal but should not impair ventilation within a load.

Recommendation for further research

- Work is required to define acceptable partition design for equid animals, especially to avoid overheating, and this needs to be determined in conjunction with acceptable stall/pen materials, dimensions and orientation to also avoid physical damage.

Pigs:

Conclusions

- New research confirmed that pigs show maladaptation to stressful situations due to a relatively small heart size in relation to body mass;
- At low environmental temperatures pigs remain close together (huddle) during air transport, even though they have plenty of space;
- Increased drinking post-transport and evidence of dehydratation indicate that water intake of pigs while vehicles are in motion is low, despite the fact that water is provided in the vehicle;
- There is a lack of scientific information about the handling of pigs during transport by air, such as fatigue, heat and cold stress, and fear in darkness.

Recommendation

- Pigs should be fasted before transport. Water should always be available at the farm, assembly point and lairage. During long transports (over 8 h) water should be provided at rest stops. It is unnecessary to provide water continuously while the vehicle is in motion.

Recommendation for research

- Research related to fatigue, heat and cold stress and fear during darkness in aircraft and during their stay in the animal control post is needed.

Sheep:

Conclusion

- Driving events, such as acceleration, braking, stopping, cornering, gear changes and uneven road surfaces can have a major negative influence on welfare by affecting the risk of injury and by disturbing the ability of the animals to rest and ruminate during the journey. These events can be monitored using suitable accelerometers installed within the vehicle.

Recommendations

- During transport, driving events such as acceleration, braking, stopping, cornering, gear changes and uneven road surfaces should be avoided;
- On long journeys, driving quality should be monitored and recorded using accelerometers installed in the vehicles.

Poultry:

Conclusions

- Genetic selection of broiler chickens has resulted in possible predisposition to metabolic diseases and pathologies that may render these birds more susceptible to some of the stresses imposed by handling, catching and transportation. These conditions include skeletal injuries and muscle pathologies that may be exacerbated by 'transport stress';
- Thermal conditions in transport pose the biggest threat to bird welfare. The severity of these stresses increases with journey length;

- Heat stress in broiler chickens may result from elevated external temperatures, high heat and moisture production of the very large numbers of birds carried on commercial vehicles;
- Passive ventilation systems on many commercial poultry vehicles cannot achieve optimal temperature conditions while vehicles are both stationary and in motion. Problems of heat stress can be reduced by reducing stocking density but cannot be eliminated in the absence of mechanical ventilation systems;
- Cold stress may occur in end of lay hens, in broilers that are wet in cold conditions and in birds exposed to excess air movement.

Recommendations

- Specific thermal limits should be defined for broilers, point of lay hens and end of lay hens;
- The upper temperature limit in a transport container for broilers should be 24-25 ºC, assuming a relative humidity of 70% or higher. The lower temperature limit for broilers in containers should be 5 ºC;
- Localised high air velocities should be avoided on passively ventilated vehicles by close attention to curtain construction and air inlet control;
- Measures should be taken to prevent wetting of broilers and laying hens prior to or during transport;
- For journeys of 4 hours or over, poultry vehicles should be equipped with mechanical ventilation systems with the capacity to regulate both air temperature and humidity within prescribed limits.

Recommendation for further research

- Specific thermal limits for point of lay hens and end of lay hens.

2. Additional provisions for transport by road or rail

2.1 Vehicles in which animals are transported shall be clearly and visibly marked indicating the presence of live animals, except when the animals are transported in containers marked in accordance with paragraph 5.1.

2.2 Road vehicles shall carry suitable equipment for loading and unloading.

2.3 When assembling trains and during all other movement of rail wagons every precaution shall be taken to avoid jolting of a rail wagon containing animals.

There is no new evidence to support any conclusions and recommendations linked with the provisions of Annex I of EC Regulation 1/2005.

3. Additional provisions for transport on roll-on-roll-off vessels

3.1 Before loading onto a vessel the master shall verify that when vehicles are loaded:
(a) on enclosed decks, the vessel is equipped with an appropriate forced ventilation system and it is fitted with an alarm system and an adequate secondary source of power in case of failure;
(b) on open decks, adequate protection from sea water is provided.

3.2 Road vehicles and rail wagons shall be equipped with a sufficient number of adequately designed, positioned and maintained securing points enabling them to be securely fastened to the vessel. Road vehicles and rail wagons shall be secured to the vessel before the start of the sea journey to prevent them being displaced by the motion of the vessel.

Horses:

Conclusion

- It has been demonstrated that equid animals find transport over rough terrain more physically demanding than other species. Transport by sea in rough conditions will also be physically demanding for equid animals and cannot be considered as a resting period.

Recommendation

- For animal welfare reasons, the time spent on a lorry loaded onto a vessel should not be considered as a resting period but as journey time.

Cattle:

Conclusion

- Heat stress is a major cause of poor welfare and increased mortality of cattle transported by sea during hot, humid conditions. The provision of electrolytes can reduce adverse effects.

Recommendations

- Ventilation systems in vessels used to transport cattle by sea should have the capacity to prevent excessive heat load. In some circumstances mechanical ventilation is essential;
- Electrolyte solutions should be made available to cattle on long sea journeys when there is a risk of heat stress.

> **4 Additional provisions for transport by air**
>
> 4.1 Animals shall be transported in containers, pens or stalls appropriate for the species, which comply with International Air Transport Association (IATA) live animals Regulations, in its version referred to in Annex VI.
>
> 4.2 Animals shall be transported only in conditions where air quality, temperature and pressure can be maintained

There is no new evidence to support any conclusions and recommendations linked with the provisions of Annex I of Regulation 1/2005.

> **5 Additional provisions for transport in containers**
>
> 5.1 Containers in which animals are transported shall be clearly and visibly marked, indicating the presence of live animals and with a sign indicating the top of the container.
>
> 5.2 During transport and handling, containers shall always be kept upright and severe jolts or shaking shall be minimised.
> Containers shall be secured so as to prevent displacement due to the movement of the means of transport.
>
> 5.3 Containers of more that 50 kg shall be equipped with a sufficient number of adequately designed, positioned and maintained securing points enabling them to be securely fastened to the means of transport where they are to be loaded. Containers shall be secured to the means of transport before the start of the journey to prevent displacement due to the motion of the means of transport.

Rabbits:

Conclusion

- There is insufficient information on temperature regulation in rabbits during transport in containers.

Recommendation for further research

- Further information is needed concerning adequate crate design for thermal regulation and if there is the need to use specific containers for rabbits.

Chapter III
TRANSPORT PRACTICES

1. Loading, unloading and handling

1.1 Due regard shall be paid to the need of certain categories of animals, such as wild animals, to become acclimatised to the mode of transport prior to the proposed journey.

1.2. Where loading or unloading operations last for more than four hours, except for poultry:

(a) appropriate facilities shall be available in order to keep, feed and water the animals outside the means of transport without being tied;

(b) operations shall be supervised by an authorised veterinarian and particular precautions shall be taken to ensure that the welfare of the animals is properly maintained during these operations.

Facilities and procedures

1.3. Facilities for loading and unloading, including the flooring, shall be designed, constructed, maintained and operated so as to:

(a) prevent injury and suffering and minimise excitement and distress during animal movements as well as to ensure the safety of the animals. In particular, surfaces shall not be slippery and lateral protections shall be provided so as to prevent animals from escaping;

(b) be cleaned and disinfected.

1.4. (a) Ramps shall not be steeper than an angle of 20 degrees, that is 36,4 % to the horizontal for pigs, calves and horses and an angle of 26 degrees 34 minutes, that is 50 % to the horizontal for sheep and cattle other than calves. Where the slope is steeper than 10 degrees, that is 17,6 % to the horizontal, ramps shall be fitted with a system, such as provided by foot battens, which ensure that the animals climb or go down without risks or difficulties;

(b) lifting platforms and upper floors shall have safety barriers so as to prevent animals falling or escaping during loading and unloading operations.

1.5. Goods which are being transported in the same means of transport as animals shall be positioned so that they do not cause injury, suffering or distress to the animals.

1.6. Appropriate lighting shall be provided during loading and unloading.

1.7. When containers loaded with animals are placed one on top of the other on the means of transport, the necessary precautions shall be taken:

(a) to avoid, or in the case of poultry, rabbits and fur animals, to limit urine and faeces falling on the animals placed underneath;

(b) to ensure stability of the containers;

(c) to ensure that ventilation is not impeded.

Handling

1.8. It shall be prohibited to:

(a) strike or kick the animals;

(b) apply pressure to any particularly sensitive part of the body in such a way as to cause them unnecessary pain or suffering;

(c) suspend the animals themselves by mechanical means;

(d) lift or drag the animals by head, ears, horns, legs, tail or fleece, or handle them in such a way as to cause them unnecessary pain or suffering;

(e) use prods or other implements with pointed ends;

(f) knowingly obstruct any animal which is being driven or led through any part where animals are handled.

1.9. The use of instruments which administer electric shocks shall be avoided as far as possible. In any case, these instruments shall only be used for adult bovine animals and adult pigs which refuse to move and only when they have room ahead of them in which to move. The shocks shall last no longer than one second, be adequately spaced and shall only be applied to the muscles of the hindquarters. Shocks shall not be used repeatedly if the animal fails to respond.

1.10. Markets or assembly centres shall provide equipment for tethering animals when necessary. Animals not used to being tied shall remain untied. Animals shall have access to water.

1.11. Animals shall not be tied by the horns, the antlers, the nose rings nor by legs tied together. Calves shall not be muzzled. Domestic Equidae older than eight months shall wear halters during transport except for unbroken horses.
When animals need to be tied, the ropes, tethers or other means used shall be:
(a) strong enough not to break during normal transport conditions;
(b) such as to allow the animals, if necessary, to lie down and to eat and drink;
(c) designed in such a way as to eliminate any danger of strangulation or injury, and so as to allow animals to be quickly released.

Separation
1.12. Animals shall be handled and transported separately in the following cases:
(a) animals of different species;
(b) animals of significantly different sizes or ages;
(c) adult breeding boars or stallions;
(d) sexually mature males from females;
(e) animals with horns from animals without horns;
(f) animals hostile to each other;
(g) tied animals from untied animals.
1.13. Points (a), (b), (c) and (e) of paragraph 1.12. shall not apply where the animals have been raised in compatible groups, are accustomed to each other, where separation will cause distress or where females are accompanied by dependent young.

Pigs:
Conclusions
- Loading pigs onto a truck in groups of no more than 5 or 6 pigs reduces the heart rates and takes the same amount of time as when larger group sizes are loaded;
- Both aggressive handling during loading and driving pigs long distances, adversely affect rectal temperature and blood-acid balance;
- Transport of sows and/or entire boars together causes aggression and increases the risk of injury. 'Birth to slaughter' systems, where litters of pigs are kept together from birth to slaughter, including transport and pre-slaughter lairage, minimises skin damage (fighting).

Recommendations
- Groups of animals should be kept stable and limited to 6 pigs during loading;
- Sows and boars should be handled separately and transported in separate compartments. 'Birth to slaughter' systems, where litters of pigs are kept together from birth to slaughter, including transport and pre-slaughter lairage, are recommended.

Recommendation for research
- Effects of ventilation in relation to the level of stress of the pigs are lacking. Research with effects of mechanical and air conditioning or other cooling systems on cooling should be performed.

Goats:
Conclusions
- Mixing goats unknown to one another will disturb the social hierarchy and lead to an increase of agonistic behaviour;
- Transport of individuals in isolation from their group members is very stressful for goats.

Recommendations
- Groups of goats should be kept stable, repeated regrouping should be avoided, and the introduction of new individuals should be monitored closely, especially in the first 24 h after regrouping;

- Horned and hornless goats should be kept separate unless they have previously been reared together;
- Positive daily contact between humans and goats should be encouraged;
- Goats should not normally be isolated. If animals have to be isolated for management purposes, they should be provided with olfactory, vocal, and visual contact with their group members.

Cattle:

Conclusion

- Partition of vehicles for cattle reduces the risk of injury, allows faster loading and unloading and allows animals to settle better during transport and thereafter at lairage.

Recommendation

- Cattle should be transported in vehicles fitted with partitions so that the animals can be transported, loaded and unloaded in small groups.

Rabbits:

Conclusion

- Physiological stress responses in rabbits are more influenced by transport and handling, than by the different loading methods or crate position in the truck.

Recommendations

- Proper handling is crucial for rabbit welfare during loading and unloading. Provision and regulation of proper training is required;
- Rabbits should be put into or taken out of crates only if the person handling can see into the crates.

Recommendation for further research

- Effects of early handling experience on fear and flight responses of rabbits during loading for transport need further investigation.

> **2. During transport**
> 2.1. Space allowances shall at least comply with the figures laid down, in respect of the animals and the means of transport referred to, in Chapter VII.
> 2.2. Domestic Equidae except mares travelling with their foals shall be transported in individual stalls when the vehicle is loaded onto a Roll-on-Roll-off vessel. Derogation to this provision may be granted under national rules provided that they are notified by the Member States to the Standing Committee on the food Chain and Animal Health.
> 2.3. Equidae shall not be transported in multi-deck vehicles except if animals are loaded on the lowest deck with no animals on higher deck. The minimum internal height of compartment shall be at least 75 cm higher than the height of the withers of the highest animal.
> 2.4. Unbroken Equidae shall not be transported in groups of more than four individuals.
> 2.5. Paragraphs 1.10. to 1.13. shall apply mutatis mutandis to the means of transport.
> 2.6. Sufficient ventilation shall be provided to ensure that the needs of the animals are fully met taking into account in particular the number and type of the animals to be transported and the expected weather conditions during the journey. Containers shall be stored in a way which does not impede their ventilation.
> 2.7. During transport, animals shall be offered water, feed and the opportunity to rest as appropriate to their species and age, at suitable intervals and in particular as referred to in Chapter V. If not otherwise specified, Mammals and Birds shall be fed at least every 24 hours and watered at least every 12 hours. The water and feed shall be of good quality and presented to the animals in a way which minimises contamination. Due regard shall be paid to the need of animals to become accustomed to the mode of feeding and watering.

Goats:

Conclusion

- Exposure of goats to heat stress may cause longer term disruption of their homeostatic control mechanisms.

<u>Rabbits</u>:

Conclusion

- Thermal stress can be detrimental for the welfare of rabbits. Effects of heat stress are exacerbated in transport conditions, such as long journeys and location of the crates on the bottom and middle floor position on the truck.

Recommendation

- Adequate ventilation during transport has to be ensured to maintain the inside crate temperature within a range of 5-20 ºC.

Recommendation for further research

- Further research is needed to better define the allowed minimum space allowance and thermal range during transport of rabbits.

<u>Poultry</u>:

Conclusions

- Journey times and space allowances for newly hatched chicks are adequate;
- Newly hatched chicks are particularly vulnerable to cold stress in transport. Heat stress will deplete water and energy reserves more rapidly. Both these stresses pose an immediate risk to welfare and a long term risk to productivity;
- Optimum vehicle temperatures for newly hatched chicks are currently proposed to be 24-25 ºC with limits of 22-28 ºC and container temperatures of 30-31 ºC;
- Adequate ventilation of chick vehicles and transport containers is vital to maintain welfare in transport.

Recommendation

- Temperature limits for newly hatched chicks during transportation should be introduced.

Recommendations for further research

- There is a need for research to define ventilation regimes and loading strategies to ensure optimal air movement throughout vehicles for transportation of newly hatched chicks;
- Effect of space allowance, container design and phenotype on the welfare of newly hatched chicks during transport;
- The utilisation of yolk sac substrates and reserves in newly hatched chicks from modern commercial broiler lines should be examined and the effects of transportation on this process should be characterised in order to underpin recommendations for any changes in maximum journey times for this category of birds.

Chapter IV
ADDITIONAL PROVISIONS FOR LIVESTOCK VESSELS OR VESSELS TRANSPORTING SEA CONTAINERS

There is no new evidence to support any conclusions and recommendations linked with the provisions of Annex I of EC Regulation 1/2005.

Chapter V
WATERING AND FEEDING INTERVAL, JOURNEY TIMES AND RESTING PERIODS

1. **Domestic Equidae, domestic animals of bovine, ovine, caprine and porcine species**

1.1. The requirements laid down in this Section apply to the movement of domestic Equidae, except registered Equidae, domestic animals of bovine, ovine, caprine and porcine species, except in the case of air transport.
1.2. Journey times for animals belonging to the species referred to in point 1.1. shall not exceed eight hours.
1.3. The maximum journey time in point 1.2. may be extended if the additional requirements of Chapter VI are met.
1.4. The watering and feeding intervals, journey times and rest periods when using road vehicles which meet the requirements in point 1.3. are defined as follows:

(a) Unweaned calves, lambs, kids and foals which are still on a milk diet and unweaned piglets must, after nine hours of travel, be given a rest period of at least one hour sufficient in particular for them to be given liquid and if necessary fed. After this rest period, they may be transported for a further nine hours;
(b) Pigs may be transported for a maximum period of 24 hours. During the journey, they must have continuous access to water;
(c) Domestic Equidae may be transported for a maximum period of 24 hours. During the journey they must be given liquid and if necessary fed every eight hours;
(d) All other animals of the species referred to in point 1.1. must, after 14 hours of travel, be given a rest period of at least one hour sufficient for them in particular to be given liquid and if necessary fed. After this rest period, they may be transported for a further 14 hours.

1.5. After the journey time laid down, animals must be unloaded, fed and watered and be rested for at least 24 hours.
1.6. Animals must not be transported by train if the maximum journey time exceeds that laid down in point 1.2. However, the journey times laid down in point 1.4. shall apply where the conditions laid down in points 1.3. and 1.4, except for rest periods, are met.
1.7. (a) Animals must not be transported by sea if the maximum journey time exceeds that laid down in point 1.2, unless the conditions laid down in points 1.3. and 1.4, apart from journey times and rest periods, are met.
(b) In the case of transport by sea on a regular and direct link between two geographical points of the Community by means of vehicles loaded on to vessels without unloading of the animals, the latter must be rested for 12 hours after unloading at the port of destination or in its immediate vicinity unless the journey time at sea is such that the voyage can be included in the general scheme of points 1.2. to 1.4.
1.8. In the interests of the animals, the journey times in points 1.3, 1.4. and 1.7(b) may be extended by two hours, taking account in particular of proximity to the place of destination.
1.9. Without prejudice to the provisions of points 1.3. to 1.8, Member States are authorised to provide for a maximum non-extendible journey time of eight hours for the transport of animals destined for slaughter, where the transport is carried out exclusively from a place of departure to a place of destination both situated on their territory.

Horses:
Conclusions

- Findings indicate that an hour is an adequate time for a water stop to allow most of the horses to drink at least once. However, there was a trend for horses even with proper access to water during transport to lose weight, indicating that they do not consume an adequate amount of water and therefore are likely to become severely dehydrated after journeys lasting 18-20 hours;
- The effects of transport on horses are profoundly influenced by their previous experience of transport and their state of health before transport. Recent surveys have shown that horses destined for slaughter are often in a poor state of health before transport and also show a relatively high prevalence of injuries;

- There is evidence of increased pyrexia in horses transported for 10 hours and immunosuppression in horses transported for 12 hours. There was a large increase in clinical signs of acute respiratory disease in horses transported for slaughter after road journeys in excess of 12 hours.

Recommendations

- When untrained horses of uncertain health status are transported for slaughter, the journey time should not normally exceed 12 hours;
- At least one hour should be allowed during the watering stop to allow animals to drink and, if necessary, feed;
- During the whole of any rest period all horses should have continual access to an unrestricted supply of clean drinking water. All horses should have continual access to an unrestricted supply of clean drinking water for a period of one hour before transport and for one hour immediately following transport.

Recommendations for further research

- The definition of unweaned animals in the literature is inconsistent. A suggestion for a more consistent definition is, "An unweaned animal is any neonate that in the absence of its dam requires supplemental bottle feeding to survive". However, this may need further consideration;
- Given the conclusion for adult equid animals with regard to maximum journey time, research is required to investigate a suitable maximum journey time for unweaned animals, most importantly, for those travelling without the dam.

Pigs:

Conclusions

- Eight hours of rest allows pigs to regain some weight due to rehydration;
- Although length of transport is usually considered a major risk factor for the welfare of pigs, other hazards attributable to vehicle design (e.g. drinkers, suspension), driving style, stocking density and inadequate ventilation may cause greater stresses whatever the length of the journey;
- Short and long transports may have the same effect on the stress level and physical fatigue in weaned piglets;
- Weaners may show different stress responses to transport from those seen in older pigs;
- In relation to journey duration, mortality increased when the pigs had not been fasted before the journey, but that duration had little effect when the pigs had been fasted, even for journeys lasting 24 hours.

Recommendation

- For journeys exceeding 24 hours, feed should be available every 24 hours at staging points followed by 6 hours rest.

Recommendations for research

- The 'optimal' journey time for weaners should be further investigated;
- To determine optimal watering intervals, especially for weaner pigs;
- To avoid spilling of water the drinking behaviour and new systems need to be examined;
- More research is necessary to examine the conditions during transport of weaners.

Sheep:

Conclusions

- Healthy adult sheep, transported under good conditions can tolerate transport durations and associated feed and water withdrawal periods of up to 48 h, without undue compromise to

their welfare. However, exposure to heat stress increases water loss principally through thermal panting and this increases the risk of significant dehydration;
- Sheep may not drink water from unfamiliar sources in novel environments. However, provision of a 24 h rest stop is sufficient to ensure adequate drinking and rest;
- Sheep that experience dehydration during a journey may be less able to respond effectively to other environmental challenges such as an increase in environmental temperature during a journey;
- Off-trailer rest stops with feed and water during long distance transport at high ambient temperatures eliminated signs of food deprivation and dehydration but did not alleviate transport stress and evidence of immunosuppression. Off-loading can increase the stress associated with handling, loading, unloading, and possibly social changes, after exposing the animals to another novel environment.

Cattle:

Conclusions

- For cattle, journeys of 12 to 24 hours will lead to fatigue and the physiological changes reported may take 24 hours or longer to return to normal levels, depending on the category of animal and feeding regime during the recovery period;
- After transport of 29 hours heifers show significant signs of fatigue;
- Bulls kept in lairage for 24 h after transport of 25-29 h show significant signs of muscular fatigue.

Recommendations

- During journeys of 8 to 29 hours, cattle should be offered water during rest periods. This is especially important in hot conditions;
- Adult cattle should not be transported on a journey of longer than 29 hours, even when ventilation is good and space allowance adequate. After this time there should be a 24 hour recovery period with access to appropriate food and water.

Recommendations for further research

- Information on the impact of journey time and thermal environment in the vehicle on the welfare of calves;
- Strategies for provision of rest, feed and water on the vehicles;
- The length of resting periods in cattle transport should be reviewed.

2. Other species

2.1. For poultry, domestic birds and domestic rabbits, suitable food and water shall be available in adequate quantities, save in the case of a journey lasting less than:
(a) 12 hours disregarding loading and unloading time; or
(b) 24 hours for chicks of all species, provided that it is completed within 72 hours after hatching.
2.2. Dogs and cats being transported shall be fed at intervals of not more than 24 hours and given water at intervals of not more than eight hours. There shall be clear written instructions about feeding and watering.
2.3. Other species other than those referred to in point 2.1. or 2.2. shall be transported in accordance with the written instructions about feeding and watering and taking into account any special care required.

Rabbits:

Conclusions

- Rabbits exposed to both fasting and transport lost more live weight compared with those that merely fasted;
- When applying the current definition of a journey there is usually a delay between arrival at the slaughterhouse and removal from the container;
- For animals transported in containers, such as rabbits, provision of water and feed as stated in EC Regulation 1/2005 is not possible either during the journey, or during the resting periods and lairage;

- Time spent in the containers represents a severe stress to rabbit welfare, whether on a truck or in lairage. Length of time from loading to slaughter, including time in lairage, constitutes the main hazard measured in terms of animal welfare and mortality, as well as carcass yield and quality.

Recommendations

- Time spent inside the containers during lairage should not be considered as a resting period but as journey time. In the case of rabbits transported in containers and so unloaded and kept on arrival for lairage, journey time should be defined as commencing when the first animal is loaded into a container and as ending when the last animal is unloaded from a container;
- In order to reduce stress and mortality, journeys should not exceed 7 hours.

Poultry:

Conclusion

- Temperature multiplied by journey duration is an important determinant for deaths in transport resulting from thermal stress. Thus, journeys of over 4 hours for broiler chickens and end of lay hens constitute a greater risk to welfare from thermal stress (heat or cold) than shorter journeys, particularly in more severe weather conditions.

Recommendations

- Journey times should be minimal and before a journey is undertaken the weather conditions should be taken into account;
- For journeys longer than 4 h for broilers and end of lay hens, vehicles should be equipped with mechanical ventilation with the capacity to maintain satisfactory thermal environments. The thermal environment within the animal accommodation should be monitored and recorded. An alarm system should be installed to notify the driver in the event of conditions predisposing to heat or cold stress. Journey time should include loading and unloading, and standing periods.

Chapter VI
ADDITIONAL PROVISIONS FOR LONG JOURNEYS OF DOMESTIC EQUIDAE AND DOMESTIC ANIMALS OF BOVINE, OVINE, CAPRINE AND PORCINE SPECIES

There is no new evidence to support any conclusions and recommendations linked with the provisions of Annex I of EC Regulation 1/2005.

Chapter VII
SPACE ALLOWANCES

Space allowances for animals shall comply at least with the following figures:

A. Domestic Equidae

Transport by rail

Adult horses	1.75 m^2 (0.7 × 2.5 m) (*)
Young horses (6 - 24 months) (for journeys of up to 48 hours)	1.2 m^2 (0.6 × 2 m)
Young horses (6 - 24 months) (for journeys over 48 hours)	2.4 m^2 (1.2 × 2 m)
Ponies (under 144 cm)	1 m^2 (0.6 × 1.8 m)
Foals (0 - 6 months)	1.4 m^2 (1 × 1.4 m)

(*) The standard useable width of wagons is 2 to 2 m.

Note: During long journeys, foals and young horses must be able to lie down

These figures may vary by a maximum of 10 % for adult horses and ponies and by a maximum of 20% for young horses and foals, depending not only on the horses' weight and size but also on their physical condition, the meteorological conditions and the likely journey time.

Transport by road

Adult horses	1.75 m^2 (0.7 × 2.5 m)
Young horses (6 - 24 months) (for journeys of up to 48 hours)	1.2 m^2 (0.6 × 2 m)
Young horses (6 - 24 months) (for journeys over 48 hours)	2.4 m^2 (1.2 × 2 m)
Ponies (under 144 cm)	1 m^2 (0.6 × 1.8 m)
Foals (0 - 6 months)	1.4 m^2 (1 × 1.4 m)

Note: During long journeys, foals and young horses must be able to lie down

These figures may vary by a maximum of 10% for adult horses and ponies and by a maximum of 20% for young horses and foals, depending not only on the horses' weight and size but also on their physical condition, the meteorological conditions and the likely journey time.

Transport by air

Loading density of horses in relation to surface area

0 - 100 kg	0.42 m^2
100 - 200 kg	0.66 m^2
200 - 300 kg	0.87 m^2
300 - 400 kg	1.04 m^2
400 - 500 kg	1.19 m^2
500 - 600 kg	1.34 m^2
600 - 700 kg	1.51 m^2
700 - 800 kg	1.73 m^2

Transport by sea

Live weight in kg	m^2/animal
200 - 300	0.90 - 1.175
300 - 400	1.175 - 1.45
400 - 500	1.45 - 1.725
500 - 600	1.725 - 2
600 - 700	2 - 2.25

Horses:

Conclusion

- Horse welfare during transport is poorly associated with stocking density when it is defined in terms of m^2/animal. There is a much stronger relationship with stocking density when it is defined in terms of m^2/kg. This is particularly important when animals are transported in groups of heterogenous weight or are of heterogenous body condition.

Recommendation

- Space allowances should be given in terms of kg/m^2 instead of m^2/animal where animals are likely to differ significantly in weight or body condition.

B. Bovine animals

Transport by rail

Category	Approximate weight (in kg)	Area in m^2/animal
Small calves	50	0.30 to 0.40
Medium sized calves	110	0.40 to 0.70
Heavy calves	200	0.70 to 0.95
Medium sized cattle	325	0.95 to 1.30
Heavy cattle	550	1.30 to 1.60
Very heavy cattle	> 700	> 1.60

These figures may vary, depending not only on the animals' weight and size but also on their physical condition, the meteorological conditions and the likely journey time.

Transport by road

Category	Approximate weight (in kg)	Area in m^2/animal
Small calves	50	0.30 to 0.40
Medium sized calves	110	0.40 to 0.70
Heavy calves	200	0.70 to 0.95
Medium sized cattle	325	0.95 to 1.30
Heavy cattle	550	1.30 to 1.60
Very heavy cattle	> 700	> 1.60

These figures may vary, depending not only on the animals' weight and size but also on their physical condition, the meteorological conditions and the likely journey time.

Transport by air

Category	Approximate weight (in kg)	Area in m^2/animal
Calves	50	0.23
	70	0.28
Cattle	300	0.84
	500	1.27

Transport by sea

Live weight in kg	m^2/animal
200 - 300	0.81 - 1.0575
300 - 400	1.0575 - 1.305
400 - 500	1.305 - 1.5525
500 - 600	1.5525 - 1.8
600 - 700	1.8 - 2.025

Pregnant animals must be allowed 10 % more space.

Cattle:

Conclusions

- Space allowances for cattle calculated according to the allometric equation A = 0.021W0.67m^2 are satisfactory for journeys no longer than 12 hours. Cattle are given sufficient space to allow them to lie down without risk, or fear of injury when space allowances are calculated according to the equation A = 0.027W0.67 m^2. Cattle with horns require 7% more space than their polled or dehorned counterparts. Cattle offered feed and drink on a vehicle, as well as space to rest, require a space allowance calculated according to the equation $A=0.0315W^{2/3}m^2$;
- Bruising injuries are significantly increased when the ceiling of the compartment is less than 20 cm above the withers height.

Recommendations

- Cattle should be provided with sufficient space to stand without contact with their neighbours and to lie down if the journey is more than 12 hours. The equations that allow for this are A = 0.021W0.67 m^2 for journeys of up to 12 hours and A = 0.027W0.67 m^2 for journeys of over 12 hours. For cattle with horns, the space allowance should be 7% higher. If cattle are to be offered feed and drink on a vehicle as well as space to rest, space allowances should be calculated according to the equation $A=0.0315W^{2/3}m^2$;
- Ceiling height should be at least 20 cm above the withers height of the tallest animal.

C. Sheep

Transport by rail

Category	Weight in kg	Area in m^2/animal
Shorn sheep	<55	0.20 to 0.30
	>55	>0.30
Unshorn sheep	<55	0.30 to 0.40
	>55	>0.40
Heavily pregnant ewes	<55	0.40 to 0.50
	>55	> 0.50

The surface area indicated above may vary depending on the breed, the size, the physical condition and the length of fleece of the animals, as well as on the meteorological conditions and the journey time.

Transport by road

Category	Weight in kg	Area in m^2/animal
Shorn sheep and lambs of 26 kg and over	<55	0.20 to 0.30
	>55	>0.30
Unshorn sheep	<55	0.30 to 0.40
	>55	>0.40
Heavily pregnant ewes	<55	0.40 to 0.50
	>55	> 0.50

The surface area indicated above may vary depending on the breed, the size, the physical condition and the length of fleece of the animals, as well as on the meteorological conditions and the journey time. As an indication: for small lambs, an area of under 0.2 m^2 per animal may be provided.

Transport by air

Loading density for sheep in relation to surface area

Average weight (in kg)	Surface area per sheep (in m^2)
25	0.2
50	0.3
75	0.4

Transport by sea

Live weight in kg	m^2/animal
20 - 30	0.24 - 0.265
30 - 40	0.265 - 0.290
40 - 50	0.290 - 0.315
50 - 60	0.315 - 0.34
60 - 70	0.34 - 0.39

Sheep:

Conclusions

- The minimum space allowances given in EC Regulation 1/2005 do not allow sheep to adopt their preferred spacing strategy and this leads to greater loss of balance, slips and falls;
- Allometric equations of the form $A = kW^{2/3}$, where A is the area in m^2, k is a constant and W is the liveweight in kg, can be used to estimate the space an animal occupies as a consequence of its mass. The value of the constant k will be determined by the spacing strategy of the sheep, whether they want to lie in transport, and whether all animals need to lie at the same time.

Recommendation

- Space allowances for sheep should be based on allometric equations relating size to body weight. For journeys of up to 6 h, the recommended empirical coefficient (and space allowances) are: (i) shorn ewes, k = 0.026 (0.44 m^2 for 67 kg), (ii) fleeced ewes and lambs, k = 0.033 (0.56 m^2 for 65 kg, 0.4 m^2 for 40.5 kg), and (iii) shorn lambs, k = 0.029 (0.3 m2 for 32.5 kg).

D. Pigs

Transport by rail and by road

All pigs must at least be able to lie down and stand up in their natural position.

In order to comply with these minimum requirements, the loading density for pigs of around 100 kg should not exceed 235 kg/m^2.

The breed, size and physical condition of the pigs may mean that the minimum required surface area given above has to be increased; a maximum increase of 20% may also be required depending on the meteorological conditions and the journey time.

Transport by air

The loading density should be relatively high to preclude injury on takeoff or landing or in the event of turbulence, although all animals must still be able to lie down. The climate, total journey time and hour of arrival should be taken into account in deciding on the loading density.

Average weight (in kg)	Surface area per pig
15 kg	0.13 m^2
25 kg	0.15 m^2
50 kg	0.35 m^2
100 kg	0.51 m^2

Transport by sea

Live weight in kg	m^2/animal
10 or less	0.20
20	0.28
45	0.37
70	0.60
100	0.85
140	0.95
180	1.10
270	1.50

Pigs:

Conclusions

- Mechanical ventilation is suitable for cooling pigs in vehicles in most circumstances provided that it has adequate capacity. In conditions when it is inadequate to prevent heat stress, additional measures, such as sprinklers or air-conditioning, can be used to cool pigs;
- Floor space per pig on the trailer and transport conditions can affect pig welfare and transport losses;

- Different space allowances are suggested for different pig groups. In general, these may be derived from the allometric equation $A=kW^{2/3}$.

Recommendations for research

- Studies of the space allowances required for good welfare of piglets, feeder pigs, sows and boars in order to validate allometric equations for different vehicles and thermal conditions;
- Fan-assisted ventilation should have adequate capacity to ensure thermal comfort. The capacity of fan assisted ventilation should be determined under different thermal conditions.

E. Poultry

Densities applicable to the transport of poultry in containers

Minimum floor areas shall be provided as follows:

Category	Area in cm^2
Day-old chicks	21 - 25 per chick
Poultry other than day-old chicks: weight in kg	Area in cm^2 per kg
< 1.6	180 - 200
1.6 to < 3	160
3 to < 5	115
> 5	105

These figures may vary depending not only on the weight and size of the birds but also on their physical condition, the meteorological conditions and the likely journey time.

Poultry:

Conclusion

- Current recommendations for stocking densities for transport of poultry are adequate, however, these recommended stocking densities can predispose to heat stress in warm or hot weather and on long journeys.

Recommendation

- Limits for stocking densities of broilers in transport containers should be related to thermal conditions. Numbers should be limited in conditions when external temperatures exceed the proposed acceptable range (e.g. > 22 ºC) and on long journeys.

2. Impact on welfare due to transport associated disease transmission

Conclusions

- As transport of animals is considered a major risk for the spread of infectious animal diseases in the EU, efforts are continually required to prevent such spread and also as a means to prevent associated poor animal welfare;
- Although the current regulatory framework on infectious animal diseases mainly focuses on the control of "listed diseases", it is equally important to consider prevention of the non-listed endemic diseases as a means to prevent poor animal welfare caused by transport-associated disease outbreaks.

Recommendations

- Measures should be taken to reduce the transport-associated outbreaks of non-listed (by the OIE) endemically occurring infectious diseases;
- Priority should be given to direct transport, without stop-overs that carry the risk for direct or indirect contact with animals from other holdings;
- In order to reduce the risk of transport-associated disease outbreaks, strategies should be developed to reduce the volume of transport (e.g. replacing the transport of breeding animals by using semen or embryos), and long distance transport of animals for finishing or slaughter (e.g. by the transport of carcasses and food products) or reducing journey time (e.g. by slaughtering animals as close as possible to the site of production).

Recommendation for further research

- Further investigation in practical applications and tools for the analysis of movement and on-farm mortality data from the national animal databases to allow preventive measures and timely reactions in the case of a disease outbreak in order to reduce disease propagation with minimal restrictions to trade.

3. Control Posts

Conclusions

- In addition to the risk of increased disease transmission at control posts there is an increased risk of poor welfare associated with unloading and loading, fatigue and other poor welfare associated with journeys of longer duration than those allowed without use of a control post;
- If animals on vehicles are not unloaded at control posts, the welfare of the animals can be better than if they are unloaded because of reduced disease transmission and reduced loading and unloading stress;
- If animals are to remain on vehicles at a control post, the space allowance on the vehicles would have to be increased in order that the needs of the animals for rest, food and water can be met.

Recommendations

- Measures should be taken to reduce the need for using control posts;
- There should be measures to avoid disease transmission at control posts through the implementation of strict biosecurity measures, in particular for those animals that are unloaded.

4. Methods for monitoring animal transport

Conclusions

- From the experience available, the manual monitoring and documentation of long animal journeys does not sufficiently achieve its intended goals to allow better verification of journey compliance and improve the enforcement of welfare requirements;
- Tracing systems which monitor and record time, position and parameters such as air temperature can improve the routine assessment of welfare during transport and reduce the administrative burden related to the journey log. The driver (on-the-spot) and the transporter (remotely) can assess possible risks in real time, react to correct an adverse situation and document conformance with regulations. Giving competent authorities access to relevant data could improve welfare in transport by allowing a wider and more harmonised verification of compliance and enforcement across the EU;
- Tracing systems for long animal journeys are not yet sufficiently used for a better traceability of transport operations and for the enforcement of welfare requirements, although a number of suitable systems are commercially available. The use of such systems is hindered by uncertainties as to what they should and could achieve, and differences regarding availability of the monitored data;
- Besides the legally required parameters, monitoring of parameters such as relative humidity, vibration and total loaded weight could provide additional information for assessing welfare during transport. However, much of the equipment (e.g. that for measuring relative humidity) is still not sufficiently robust or accurate enough for routine application in commercial transports;
- The automatic control of mechanical ventilation and misting devices by the monitored temperature of a tracing system is technically feasible and new evidence suggests that it would be beneficial in pig transports;
- A more comprehensive tracing system could address animal welfare, animal health and logistic aspects, ensuring full traceability of animal transport.

Recommendations

- For clarity of its intended use, the navigation systems referred to in Regulation (EC) 1/2005 should be called ‘tracing systems in long animal journeys’, and incorporate a temperature monitoring and warning system;
- Common minimum standards should be set for tracing systems on long journeys. These should set out the entities to be monitored (journey or batches), the system architecture to be followed (i.e. recording and transmission to remote receivers), and on-board features (e.g. number/position of temperature sensors in the animal compartments, minimum recording intervals, memory, display or printout) to allow a more harmonised assessment of the monitored parameters;
- Tracing systems for commercial long journey transports of domestic cattle, pigs, sheep, goats and horses, other than registered horses, should at least monitor and record the exact time and position when the first animal of the first batch is loaded, the time the last animal of the last batch is unloaded, start and end of rests, temperatures in the animal compartments and status of the loading doors.

Recommendations for future research

Further studies are suggested for improving the welfare assessment by a tracing system:

- Acceptable temperature corridors or variations during transport in relation to the temperature at origin (on loading);
- Relevance of out of range temperature over time (duration of non-compliant temperatures) during animal transports;
- Humidity sensors which are sufficiently robust for commercial animal transports to allow reliable humidity monitoring;
- Acceptable THI (temperature-humidity index) values over time (values and duration) for different species of livestock;
- Design and location of accelerators for monitoring vibration in commercial animal vehicles: design of vehicles to minimise low frequency vibration;
- Evaluation of weight monitoring sensors in commercial animal transporters to record loading density and weight loss during transport;
- Evaluation of methods to integrate digital tachograph and recording of electronic identification of loaded animals with animal transport tracing systems;
- Development and evaluation of transport tracing systems for vehicles used for poultry and rabbit transports;
- Development and evaluation of simple tracing systems for vehicles used to transport livestock on short journeys (usually less than 8 hours).

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APPENDICES

A. IDENTIFICATION OF ANIMAL BASED INDICATORS OF WELFARE DURING TRANSPORT FOR THEIR POSSIBLE USE AS AN ALTERNATIVE TO THE PRESENT LEGISLATIVE REQUIREMENTS (EC REGULATION 1/2005)

Table A1. Observational and clinical indicators of welfare adverse effects related to transport – Equidae
(For full details of behavioural indicators associated with pain and distress in Equidae see Ashley et al., 2005).

Adverse effect	Observational indicators	Clinical indicators
Heat stress	Panting and/or sweating, extreme thirst (high drive to drink), collapse, high body condition score, (see also exhaustion)	Elevated body temperature Elevated haematocrit
Exhaustion	Inability/reluctance to rise, lack of reaction to an external stimulus, chin resting, depressed or exhausted demeanour	Blood enzymes, CPK, postmortem muscle colour and pH
Dehydration	Extreme thirst (high drive to drink), skin 'tenting', wrinkled skin, congested mucous membranes	Elevated haematocrit, Abnormal blood pH, pCO2, urine specific gravity/colour
Fear	Vocalisation, escape behaviour, turning away, appearance of eye, head lowering (without air inhalation or expulsion), ears flat, dilated nostrils, wide eyed, urination and/or defecation, sniffing ground with audible sniffing/blowing, stamping hooves, head tossing	Corticosteroids in plasma or saliva elevated, heart rate elevated by more than 20-30% without exercise
Disease	Nasal discharge, cough, diarrhoea, abdominal discomfort, posture. lameness	Elevated body temperature Presence of pathogens
Isolation distress	Isolated, pawing at ground, head turning, vocalisation, ears laid back, wide eyed	Elevated haematocrit Elevated plasma/salivary cortisol
Poor ventilation	Unusual sweat patterns, collapse, nasal discharge	Elevated body temperature Presence of pathogens
Injury	Presence of aggressive conspecifics, bite marks, bleeding, laceration, lameness, friction injuries, grazing	Elevated plasma/salivary cortisol CPK, bruising/injury evident on carcass, postmortem
Pain	<u>Posture</u> Head bent down/ears low, rolling, abdominal muscles tight, spinal column bent low, arts widened, fixed stare, dilated nostrils, clenched jaw <u>Behaviour</u> Aggressive behaviour, escape behaviour, restlessness, agitation, anxiety, rolling, flank watching with colicky pain, limb guarding,	Elevated plasma/salivary cortisol CPK Heart rate elevated by more than 20% in the absence of exercise

	anorexia, quidding, food pocketing (dental pain) Vocalisations Blow/neighing, groaning Movements Reluctance to move/weight shifting, Limp/'walking on eggshells', Ataxia/inactivity, rigidity, kicking at abdomen, 'pointing', hanging and rotating limbs, head shaking Others Reacts to the touch of the painful area, self-injury, dilated pupils	

Table A2. Observational and clinical indicators of welfare adverse effects related to transport – Rabbits (For a detailed description of the main behavioural indicators of pain in rabbits see Leach, 2006; Leach et al., 2009)

Adverse effect	Observational indicators	Clinical indicators
Heat stress	Laboured breathing/panting, flaring of the nostrils, extreme thirst (high drive to drink), posture (rabbit stretched out), moisture around face and/or drooling, mucous membranes engorged, bloody discharge from mouth/nose, enlarged blood vessels in ears and mouth turning blue, collapse /stroke (see also exhaustion), mortality	Elevated body temperature Elevated haematocrit Plasma osmolarity Heart rate
Cold stress	Shivering	Low body temperature, CPK, LDH
Exhaustion	Inability/reluctance to move, lack of reactions	Blood enzymes/CPK Postmortem muscle colour and pH
Dehydration	Extreme thirst (high drive to drink), "skin-pinch" test, skin 'tenting' test*, dark urine	Elevated haematocrit, Abnormal blood pH, pCO2, urine specific gravity/colour
Fear	Vocalisation (loud teeth grinding noises, piercing screams), flight behaviour/freezing, urination and/or defecation	Plasma corticosteroids Heart rate
Disease	Eye and nasal discharge, dyspnoea, respiratory sounds, diarrhoea, etc.), abnormal posture and mobility, (see also exhaustion)	Elevated body temperature Presence of pathogens
Mixing distress	Aggressive vocalisations (grunts, growls, loud teeth grinding bruxism), stamping of the hind feet, freezing/fighting	Elevated haematocrit Elevated plasma/salivary cortisol
Poor ventilation	Collapse, nasal discharge	Elevated body temperature Presence of pathogens
Injury	Mixing with unfamiliar conspecifics, bite marks, wounds, scratches, abrasions, back injuries – unable to move, mortality rate/abnormal mobility	Elevated plasma corticosteroids Bruising/injury evident on carcass Broken bones
Pain	Main behaviours potential indicators of pain varies with site (Leach, 2006; Leach et al., 2009). Twitch, flinch, wince, stagger, press, arched back, quivering, writhing, fall, eyes closed or slit, elongated stance, bruxism	Elevated plasma corticosteroids CPK, heart rate

*This test indicates approximately the severity of dehydration. A fold of skin on the dorsum of the neck is lifted and released. Ordinarily, the skin should return to its position within 1-2 seconds; however, if a rabbit is dehydrated, the return to normal position will take longer (Suckow and Douglas, 1997).

Table A3. Description of the main behaviours - potential indicators of pain – Rabbits (after Leach, 2006; Leach et al., 2009).

Behaviour	**Description**
Twitch	Rapid movement of fur on back
Flinch	Body jerks upwards for no apparent reason
Wince	Rapid movement of the back in a rocking motion accompanied by eye closing and swallowing action
Stagger	Partial loss of balance
Press	Abdomen pushed towards floor, usually before walking
Arching	Full arching of the back upwards
Quivering	Slow rhythmic side-to-side movement
Writhe	Contraction of the oblique flank muscles
Fall	Complete loss of balance when moving
Eyes closed/Eyes slit	Eyes tightly closed or semi-closed
Elongated stance	Standing stance in which the forward part of the body is placed as far from the hind limbs as possible so the body is stretched out horizontally
Bruxism	Teeth grinding

Table A4. Observational and clinical indicators of welfare adverse effects related to transport – Sheep and goats

Adverse effect	Clinical / observational indicators
Hunger	- Weight loss (in long journeys)
Dehydration	- Skin-pinch test - Extreme thirst
Lack of comfort around resting	- Tired animals - Lack of space to lie down all at the same time - Lying down behaviour - Lack of space above the top of the head - Dead animals by asphyxia
Heat stress	- Panting - Respiration rate (low heat stress: 40–60 breaths/min, medium heat stress: 60–80 breaths/min, high heat stress: 80–200 breaths/min, severe heat stress: over 200 breaths/min (Silanikove, 2000) - Drooling - Position of animals (isolated individuals or group clumped or dispersed) - Increased body temperature - Mortality - Extreme thirst, high drive to drink
Cold stress	- Shivering - Stand in physical contact with one another - Reduced body temperature
Exhaustion	- General lethargy - Apathy - Lack of reaction - Inability/reluctance to rise
Disease	- Incoordination - Posture - Limping - Teeth gnashing/grinding/bruxism - Incapacity to walk - Head tilt gait - Eye and nasal discharge - Difficult to breath - Respiratory sounds - Coughing - Lethargy - Apathy - Faecal characteristics
Injury	- Visible signs of injury (bite marks, wounds, bruises, scratches, abrasions)
Pain	- Evidence of pain on palpation of “injured” area - Increased heart rate - Bruxism - Panting

Locomotion problems	- Slipping and falling events - Stiffness in gait - Shorter strides - Slower walking speed - Lameness
Fear	- Increase in heart rate - Increase in respiration rate - Head alert - Stand still - Escape behaviour - Turning away - Freezing behaviour - Reluctance to move - Moving backwards - Urination and defecation - Jump events (goats) - Alarm vocalisation (goats) - Kicking (goats) - Bruxism
Isolation distress	- Isolated - Vocalisation - Bruxism

Table A5. Observational and clinical indicators of welfare adverse effects related to transport – Pigs

Adverse effect	**Observational indicators**	**Clinical indicators**
Heat stress	Open-mouth breathing Panting + extra sounds Skin decolouration (red/blue or rash) Lying apart	Respiration rate (raised > 20%) Heart rate (raised > 20%) Rectal temperature raised by 2/3 degrees
Cold stress	Skin decolouration (red/blue or rash) Trembling, shivering Lying close together (huddling) Hair position	Respiration rate Heart rate Rectal temperature
Exhaustion	Inability/reluctance to stand up (downer pigs) Lack of reaction, apathy Balking	Dark, firm dry carcass Blood pH O2 CO2 Glucose Lactate Leukocytes
Dehydration	Loss of skin tension Capillary refill time in mucous membranes	Hb Ht Urine pH Urine SG Osmolarity
Fear	Balking, screaming Defecation Hiding, avoidance	Cortisol
Disease	Vigilance Nasal discharge Abdominal respiration Muscle tremor Position of the head Red mucous membranes Condition Cachexia Injuries, sores Unusual locomotion, swollen joints, lameness Creeping nose/eyes/vulva, high body temperature	All above
Mixing distress	Fight and flight behaviour Bite marks Vocalisation	
Poor ventilation	Open-mouth breathing Panting Skin decolouration (see above)	
Injury	Aggression Bite marks, skin lesions Vocalisation Locomotion abnormalities, lameness	

Pain	Muscle tremor Vocalisation Balking	
Motion sickness	Vomiting	

Table A6. Observational and clinical indicators of welfare – Poultry

It is important to recognise that the "classes" of poultry routinely transported for commercial purposes will differ widely in their susceptibility to the various stresses potentially imposed in transport. The consequences of the hazards to which the birds are subjected will depend upon the age and type of bird concerned. Thus, whilst the hazards may be generally applicable to all categories, the clinical and scientific indicators may vary between the different classes and the frequency with which they may be observed will also depend upon the type and age of the birds in question. Different indicators are thus required for day old chicks, broiler chickens and turkeys (transported to slaughter) and laying hens (pullets transported to farms) and spent laying hens to slaughter. Specific examples of the important differences are given in the additional Table A6b.

Table A6a. Observational and clinical indicators of welfare adverse effects related to transport – Poultry

Adverse effect	Observational indicators	Clinical indicators
Heat stress	Panting (exaggerated chest and abdominal movements) High respiration rates High saliva production/drooling Gaping Gular flutter Excited behaviour in container, scrambling (may cause "back scratching") Prostration Glazed expression Dull eyes Bloody discharge from beak Excessive defecation (moist/wet) Collapse Moribund animal/increased in transport mortality	Elevated body temperature or hyperthermia Increased plasma osmolarity Decreased blood pCO_2 Increased blood pH Elevated haematocrit Elevated comb/wattle, skin temperatures approaching normal core temperature Elevated plasma activities of CPK, LDH, AST, (meat quality changes e.g. pH, PSE and ecchymoses and petechial haemorrhages) Blood APP changes H/L ratio increased Plasma corticosterone increased
Cold stress	Shivering Huddling Pteroerection (feather erection) or fluffing Lethargy Pale comb/wattle skin (bluish) Skin discolouration Prostration Dull eyes Collapse	Reduced body temperature or hypothermia Low skin temperature (unfeathered) approaching air temperature Elevated plasma activities of CPK, LDH, AST (meat quality changes/pH) Plasma corticosterone increased
Exhaustion (often with hyperthermia)	Inability/reluctance to rise Lack of reaction Prostration Apathy	Elevated plasma activities of CPK, LDH, AST Postmortem muscle colour and pH
Dehydration (often with hyperthermia)	Lethargy Collapse/prostration Pinched facial appearance Skin tension changes	Elevated haematocrit Increased plasma osmolarity Abnormal blood pH, pCO_2

Fear	Vocalisation Freezing Tonic immobility Escape behaviour Eye fixed and pupil dilated Defecation (often wet) Trembling or shivering	Elevated heart rate Risk of arrhythmias Corticosterone increased in plasma H/L ratio increased
Disease	Lethargy Prostration (lateral lying) Respiratory sounds Discharge from beak or nasal passages or vent Discharge from eyes Diarrhoea Posture Lameness Blood in faeces Blood in auditory meatus or saliva/beak Red mucous membranes	Elevated body temperature Changes in differential haematology profiles (H/L ratio, monocytes, basophils) Blood APP changes
Injury	Lameness (Immobility and "awkward posture") Fractures to wings, legs, keel bone Dislocation to hip and wing Skin haemorrhages, cuts and scratches Vent damage Sores Swollen joints and/or feet	Elevated plasma corticosterone and POMC/APP Bruising/injury/fractures evident on carcass, postmortem
Pain (often little reaction in broilers to quite severe and painful injuries)	<u>Posture</u> Prostration or "Sitting very still" Wing or leg extended and in abnormal position relative to body <u>Behaviour</u> Little activity/ataxia Dilated pupils Panting Trembling <u>Vocalisations</u> Low volume distress calls <u>Movements</u> Reluctant to move Limping or lameness	Elevated heart rate, elevated plasma enzymes depending on extent, location and age of injury/pathology

Table A6b. Observational indicators subdivided by poultry type and age.

Adverse effect	**Type and age of poultry**	**Observational indicators**
Heat stress	Most likely in broilers at high stocking densities in transport containers / crates in summer conditions May occur in day old chicks in poorly ventilated "boxes" in transport or when "stacked".	Distress calls in chicks Panting (exaggerated chest and abdominal movements) High respiration rates High saliva production/drooling Gaping Gular flutter Excited behaviour in container, scrambling (may cause "back scratching) Prostration Glazed expression Dull eyes Bloody discharge from beak Excessive defecation (moist/wet) Collapse Moribund animal/increased in transport mortality
Cold stress	Most likely in: (a) Day old chicks (b) Spent layers in poor feather condition	Distress calls in chicks Shivering Huddling Pteroerection (feather erection) or fluffing Lethargy Pale comb/wattle skin (bluish) Prostration Dull eyes Collapse
Exhaustion (often with hyperthermia)	Most likely in :- (a) Day old chicks (b) Spent layers (long journeys and food deprivation)	Inability/reluctance to rise Lack of reaction Prostration Apathy
Dehydration (often with hyperthermia)	May occur in broilers, turkeys, layers and chicks when hyperthermia is a risk and journey times are extended	Lethargy Collapse/prostration Pinched facial appearance
Fear	Will be most apparent in day old chicks Tonic immobility may be rare in commercial transport situations	Vocalisation Freezing Tonic immobility Escape behaviour Eye fixed and pupil dilated Defecation (often wet)

Disease	May occur in all classes of poultry High likelihood of cardiovascular pathology in broilers (e.g. heart failure, ascites syndrome and "sudden death syndrome"), possibly skeletal and muscle pathology Risk of necrotic enteritis in broiler birds. Metabolic disease likely in spent layers	Lethargy Prostration (lateral lying) Respiratory sounds Discharge from beak or nasal passages or vent Posture Lameness Blood in faeces Blood in auditory meatus or saliva/beak
Injury	Poultry at risk of skeletal diseases and damage thus: (a) Broilers subject to fractures and dislocations during catching and handling (b) Spent laying hens have a very high risk of skeletal pathology, bone weakness, old and new fractures and dislocations, pecking damage and catching and handling induced injuries	Lameness (immobility and "awkward posture") Fractures to wings, legs, keel bone Dislocation to hip and wing Skin haemorrhages, cuts and scratches Vent damage
Pain (often little reaction in broilers to quite severe and painful injuries)	Despite a high incidence of apparently serious or severe injuries and pathologies associated with catching, and handling of broilers and turkeys and laying hens (often associated with pre-existing pathology) these birds do no readily exhibit obvious signs of pain and suffering and thus special attention should be paid to all other indicators.	Posture Prostration or "Sitting very still" Wing or leg extended and in abnormal position relative to body Behaviour Little activity/ataxia Dilated pupils Panting Vocalisations Low volume distress calls Movements Reluctant to move Limping or lameness

B. List of hazards based on expert opinion with highest impact on the welfare of animals during transport

For each of the farm animal species, experts from the working group were asked to consider a wide range of hazards and their impact on the risk of poor welfare during transport. The hazards were categorised in order of impact on animal welfare and those most highly ranked are listed by species below.

Horses

- Poor inspection of equid animals prior to transport resulting in the transport of animals diseased, injured and otherwise unfit to travel;
- Lack of appropriate individual penning resulting in aggression between conspecifics, injury and exhaustion;
- Lack of appropriate penning resulting in reduced ventilation leading to heat stress, exhaustion and disease;
- Lack of appropriate penning leading to inability to balance or maintain posture resulting in injury, exhaustion and disease;
- Lack of appropriate penning causing direct physical injury and exhaustion;
- Poor watering provision at all stages in the transport process resulting in dehydration, heat stress and exhaustion;
- Journey length and exacerbation of journey length by poor driving and/or road/sea conditions resulting in disease, injury and exhaustion.

Pigs

- Inadequate natural and mechanical ventilation when the vehicle is stationary resulting in heat stress when ambient temperature is high. The problem is exacerbated when stocking density is at the maximum prescribed limit;
- Inadequate planning of the journey leading to prolonged journey times causing exhaustion;
- Prolonged restriction of water before transport, resulting in dehydration and heat stress when slaughter pigs are transported at high ambient temperatures or cold stress in weaner pigs transported at low ambient temperatures;
- Prolonged fasting before transport, resulting in exhaustion and cold stress.

Sheep

- Inadequate floor space resulting in exhaustion and injury, and increasing risk of heat stress when sheep are transported at high ambient temperatures;
- Inadequate ventilation, resulting in heat stress when sheep are transported at high ambient temperatures, especially when the vehicle is stationary;
- Slippery floors combined with erratic driving resulting in fear, exhaustion and injuries.

Goats

- Mixing of unfamiliar animals resulting in fear and injury.

Cattle

- Inadequate inspection prior to transport; failure to detect injury and latent disease;
- Inadequate vehicle design; especially inadequate ventilation, slippery floors, lack of partitions, resulting in heat stress, injuries and exhaustion;
- Inadequate loading facilities (driveways, ramps and gates) resulting in fear and injury;
- Inadequate planning and execution of long journeys, resulting in heat stress, dehydration and exhaustion;
- Erratic driving, causing exhaustion and injuries.

Rabbits

- Inadequate container design and lack of ventilation resulting in death, bruising, heat stress;

- Poor inspection prior to transport resulting in the transport of rabbits diseased, injured and otherwise unfit to travel and predisposition to spread of infection;
- Poor loading, unloading and handling, in addition to improper facilities, untrained handlers and mixing unfamiliar animals resulting in injury, bruising, pain and death;
- Inadequate space allowance, worsened by mixing unfamiliar animals, adverse terrain and environmental extremes resulting in hyperthermia, exhaustion and disease spread;
- Lack of feeding and watering provision at all stages of the transport process, including lairage, resulting in dehydration, heat stress and exhaustion;
- Journey length and exacerbation of journey length by poor driving and length of the loading, unloading and lairage time resulting in heat stress and exhaustion;
- All of these hazards may cause death, severe stress and disease and thus constitute major welfare concerns.

Poultry

Broiler chickens

- The presence of existing injuries and disease exacerbated by poor catching and handling;
- Exposure to heat or cold stress in transport containers due to poorly controlled passive ventilation regimes (low or excessive local air flows);
- The risk of cold stress will be increased if birds are wetted prior to or during transport.

End of lay hens

- Presence of existing injuries and disease, particularly skeletal injuries exacerbated by poor depopulation and handling;
- Exposure to heat or cold stress in transport containers due to poorly controlled passive ventilation regimes (low or excessive local air flows);
- The risk of cold stress will be increased if birds are wetted prior to or during transport or when stocking densities are low and hens poorly feathered.

Newly hatched chicks

- Exposure to heat or cold stress in transport containers due to poorly controlled ventilation regimes of ‘chick boxes’ (low or excessive local air flows);
- The risk of cold stress will be increased if chick space allowance is high or bird numbers small;
- Hypothermia is the major risk to this category of birds.

GLOSSARY AND ABBREVIATIONS

Glossary

Container
Any crate, box, receptacle or other rigid structure used for the transport of animals which is not a means of transport.

Detusking
Removal of part or all of the tusks of a boar to prevent injury to other pigs and people.

Equidae
Domestic horses, mules and asses.

Journey
The entire transport operation from the place of departure to the place of destination, including any unloading, accommodation and loading occurring at intermediate points in the journey.

Livestock vessel
Means a vessel which is used or intended to be used for the carriage of domestic Equidae or domestic animals of bovine, ovine, caprine or porcine species, other than a roll-on roll-off vessel, and other than a vessel carrying animals in moveable containers.

Long journey
Journey that exceeds 8 hours, starting from when the first animal of the consignment is moved.

Means of transport
Road or rail vehicles, vessels and aircraft used for the transport of animals.

Navigation systems
Satellite-based infrastructures providing global, continuous, accurate and guaranteed timing and positioning services or any technology providing services deemed equivalent for the purpose of this Regulation.

Place of departure
Means the place at which the animal is first loaded on to a means of transport provided that it had been accommodated there for at least 48 hours prior to the time of departure.

Place of destination
Place at which an animal is unloaded from a means of transport and (i) accommodated for at least 48 hours prior to the time of departure; or (ii) slaughtered.

Rest stop
A pause in the journey for the driver or the animals.

Roll-on roll-off vessel
Sea-going vessel with facilities to enable road or rail vehicles to roll-on and roll-off the vessel.

Transport
Movement of animals effected by one or more means of transport and the related operations, including loading, unloading, transfer and rest, until the unloading of the animals at the place of destination is completed.

Transporter
Any natural or legal person transporting animals on his own account, or for the account of a third party.

Tusk
An elongated pointed tooth, usually one of a pair, extending outside of the mouth in certain animals such as the walrus, elephant, or wild boar.

Unbroken Equidae
Equidae that cannot be tied or led by a halter without causing avoidable excitement, pain or suffering.

Vehicle
Means of transport fitted with wheels which is propelled or towed.

Abbreviations

ACTH	Adrenocorticotrophic hormone
CK	Creatine kinase
CPK	Creatine phosphokinase
DFD	Dark, firm and dry (meat)
DHEA	Dehydroepiandrosterone
DOA	Dead on arrivals
ECG	Electrocardiogram
EFSA	European Food Safety Authority
EMG	Electromyographic
HCT	Haematocrit
HGB	Haemoglobin
HR	Heart rate
HRV	Heart rate variability
LDH	Lactate dehydrogenase
MFRS	Multi-floor cage rolling stand
NEFA	Non-esterified fatty acids
PSE	Pale, soft and exudative (meat)
RBC	Red blood cells (also referred to as erythrocytes)
RO-RO	Roll-on roll-off
SCAHAW	Scientific Committee on Animal Health and Animal Welfare
THI	Temperature-humidity index
WHC	Water holding capacity