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Welfare of equidae during transport

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Abstract

In the framework of its Farm to Fork Strategy, the Commission is undertaking a comprehensive evaluation of animal welfare legislation. This opinion deals with the protection of horses and donkeys during transport. While the opinion focuses primarily on road transport of horses, there are specific sections dealing with the transport of horses on roll-on–roll-off ferries, horses transported by air and the transport of donkeys. In addition, the opinion covers welfare concerns in relation to a specific scenario identified by the European Commission related to the transport of horses on long journeys to slaughterhouses. Current practices related to transport of horses during the different stages (preparation, loading and unloading, transit and the journey breaks) are described. Overall, 13 welfare consequences were identified as being highly relevant for the welfare of horses during transport based on severity, duration and frequency of occurrence: gastro-enteric disorders, handling stress, heat stress, injuries, isolation stress, motion stress, prolonged hunger, prolonged thirst, respiratory disorders, resting problems, restriction of movement, sensory overstimulation and separation stress. These welfare consequences and their animal-based measures are described. A variety of hazards were identified related to factors such as inexperienced/untrained handlers, lack of horse training, structural deficiencies of vehicles/facilities, poor driving skills/conditions, horse separation/regrouping, unfavourable microclimatic and environmental conditions and poor husbandry practices. The opinion contains general and specific conclusions in relation to the different stages of transport. Recommendations to prevent hazards and correct or mitigate welfare consequences have been developed. Recommendations were also developed to define quantitative thresholds for microclimatic conditions within the means of transport and for space allowance. The development of welfare consequences over time was assessed in relation to maximum journey time.

Keywords: Horse, transport, animal welfare, Farm to Fork Strategy, welfare consequences, animal-based measures, quantitative thresholds

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Summary

In the framework of its Farm to Fork Strategy, the Commission is undertaking a comprehensive evaluation of the animal welfare legislation, including Council Regulation (EC) No 1/2005[1]. The current EU legislation on the protection of animals during transport is based on a scientific opinion adopted in 2002. Against this background, the European Commission requested EFSA to give an independent view on the protection of animals during transport for different groups and categories of farmed animals. It also requested EFSA to propose detailed measures to prevent hazards and mitigate the welfare consequences for specific scenarios. This opinion deals with the protection of horses and donkeys during transport. While the opinion focuses primarily on road transport of horses, there are specific sections dealing with transport of horses on roll-on–roll-off ferries, transport of horses by air as well as transport of donkeys. In addition, the opinion covers welfare concerns in relation to a specific scenario identified by the European Commission related to transport of horses on long journeys to slaughterhouses.

The scientific assessment was carried out by breaking down the transport of horses into four distinct stages, namely preparation, loading/unloading, transit and journey breaks. For road transport, which is the most common transport practice, each stage was described in terms of current practice and assessed in terms of welfare consequences, animal-based measures (ABMs) and hazards leading to the welfare consequences. In addition, recommendations to prevent hazards and to correct or mitigate welfare consequences were developed. Recommendations on quantitative thresholds for microclimatic conditions within the means of transport and for space allowance were also developed. In addition, the development of welfare consequences over time was assessed in relation to maximum journey time.

According to TRACES, around 170,000 horses were transported between Member States per year in 2019–2021, across all means of transport. Road transport constituted around 85% of total horse transport in this period.

In total, 13 welfare consequences were selected as being highly relevant for the welfare of horses during transport based on literature and expert opinion considering severity, duration and frequency of occurrence. These were (i) gastro-enteric disorders, (ii) handling stress, (iii) heat stress, (iv) injuries, (v) isolation stress, (vi) motion stress, (vii) prolonged hunger, (viii) prolonged thirst, (ix) respiratory disorders, (x) resting problems, (xi) restriction of movement, (xii) sensory overstimulation and (xiii) separation stress. The occurrence of each type of welfare consequence varied depending on the stage and means of transport. Horses may experience one or more negative affective states associated with these welfare consequences, including fear, pain, discomfort, fatigue and distress. Specific ABMs were identified for each of the highly relevant welfare consequences, including behavioural, clinical and physiological ABMs. A definition and interpretation for each ABM is provided in the opinion. Some ABMs are relevant to more than one welfare consequence.

A wide variety of hazards were identified for the different welfare consequences and transport stages. These were related to factors such as inexperienced or untrained handlers, lack of training of the animals, horse temperament, horse breed, use of sedatives, structural deficiencies of vehicles and facilities, poor driving skills and conditions, separation from other horses, regrouping with unfamiliar horses, unfavourable microclimatic and environmental conditions and poor transport and husbandry practices.

Throughout the scientific literature, it is agreed that ensuring that animals are fit for transport before departure is of utmost importance. However, currently no agreed scientific definition of the concept of fitness for transport exists. In order to avoid doubt and misclassification of animals in relation to fitness for transport, the concept should be properly defined, owners and professional groups (including farmers, stockpersons, drivers, haulers, inspectors and veterinarians) should be well-educated and trained, and questions on responsibility between the groups should be clarified. Also, there are only few conditions leading animals to be unfit for transport, for which ABMs have been established and validated, including the establishment of thresholds. The main conditions rendering horses unfit for transport, and methods for assessing fitness for transport, are provided in the opinion. Guidelines based on ABMs for conditions leading to animals being unfit, including thresholds, should be established and validated.

[1] Regulation (EC) No 1/2005 of the European Council 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 1255/9.

The highly relevant welfare consequences during loading/unloading of horses are heat stress, handling stress, injuries, sensory overstimulation and restriction of movement. Across the highly relevant welfare consequences, the major hazards are inappropriate handling, unsuitable facilities and high effective temperatures. Delay in loading and/or unloading means prolonged exposure to the hazards. In case of lack of habituation to the relevant handling, most horses are expected to experience handling stress, which may be associated with fear and may lead to distress. The main preventive measures are training of horses, loading with an appropriate training method, establishment and maintenance of proper facilities and education and training of handlers.

During the transit stage, horses will be exposed to a number of hazards, either in isolation or in combination, leading to welfare consequences.

As regards microclimatic conditions during road transport of horses, in order to reduce the risk of welfare consequences due to exposure to high effective temperatures, the temperature inside vehicles transporting horses should not exceed the upper critical temperature estimated to be 25°C dry bulb temperature.

Horse welfare benefits from additional space with respect to the width as well as the length of a horse. Lateral space is necessary for spreading the legs to balance and adopt the excretory posture. The available evidence suggests that when transported individually, the width of the individual stall should be at least 40 cm more in total than the width of the widest point of the horse transported. Front/backspace is necessary for lowering the head for balancing, resting and clearing of airways, with additional space possibly required for the positioning of feeders and drinkers in vehicles. Extra space will also accommodate excretory postures. To accommodate this, a horse should have at least 40 cm of free space in addition to the body length of the horse (measured from the tail to the nose while the neck is parallel to the ground) plus 50 cm if feed in a hay net is provided in transit.

For unhandled horses, the limited available evidence suggests that a stocking density of no greater than 200 kg/m^2 leads to improved welfare as compared to higher stocking densities. The BUT test can be applied before loading animals to recognise whether a horse shows signs of being able to be tied or led by a halter without causing avoidable excitement, pain or suffering (called 'broken' in EC 1/2005[1]) or not. Horses showing signs of not being able to do so should be transported loose in a small group of familiar conspecifics.

The vertical space in a means of transport is important for horse welfare. Low vertical space can be associated with reduced ventilation, injuries to the head, lack of ability to move around and lack of space for natural movements, which should be prevented in order to avoid welfare consequences such as heat stress and restriction of movement. No studies have established a proper deck height for horses during transport, but it has been recommended that the minimum internal height of the compartment should be the height of the withers of the tallest animal in a compartment + 75 cm. Establishment of evidence-based thresholds constitutes a gap in knowledge.

The amount of time the animals are exposed to the hazards is dependent on the journey duration. The number and the severity of hazards that animals are exposed to during transport influence the resultant welfare consequences (continuous or semi-continuous, progressive and sporadic). On the basis of evidence on continuous welfare consequences involving stress and negative affective states, for the benefit of animal welfare, the journey duration and frequency, (and severity of the exposure to the hazards) should be kept to a minimum.

To limit the impact of transport on animal welfare, in an effort to reduce the exposure to hazards and related welfare consequences, it is recommended to consider that: animals experience motion stress and sensory overstimulation throughout the entirety of the journey potentially leading to fatigue, fear and distress; pain and/or discomfort from health conditions or injuries will worsen over time during transport and may lead to suffering; resting problems' severity is expected to increase with increasing duration and may lead to fatigue; clinical respiratory disorders can be present after journeys of 10–14 h; gastro-enteric disorders such as gastric ulceration can be seen after 12 h in unfed horses; physiological biomarkers indicative of prolonged hunger have been reported after 12 h of transport. Behavioural indicators of thirst have been reported after 3 h and physiological biomarkers of dehydration after as little as 1–3 h.

Per definition, breaks in journeys (either while vehicle is stationary or when animals are unloaded in e.g. control post) function to remove the animals from the hazards that they are exposed to during transit and to allow them to recover from the associated welfare consequences. The available evidence suggests that, in terms of the transport conditions currently practiced, the provision of water and/or feed on the vehicle while in motion can be ineffective, in that some animals do not drink, or consume feed. If they do, their consumption can be reduced compared with non-transported horses. There are also horses that are fully capable of eating during transport. However, for horses transported in single stalls, feeding and watering should be feasible while vehicles are stationary. During transport, horses should be provided with feed and water ad libitum or at least at regular intervals (of no more than 4 h) for a period of 30 min while the vehicle is stationary.

At control posts, along with the mitigation of welfare consequences, there is potential for exposure to hazards resulting in welfare consequences or interfering with the intended mitigation of other welfare consequences. In addition, control posts involve biosecurity risks as animals can be exposed to infectious diseases through direct or indirect contact with other animals and opportunistic pathogens. Across the categories of horses typically transported on journeys involving journey breaks, the scientific focus on control posts has been limited. This means that whether control posts in their current state fulfil their intended function is not known.

The specific scenario relevant to horses that EFSA was asked by the Commission to consider was the transport of horses on long journeys to slaughterhouses. Based on data recorded in the TRACES, in 2019, 2020 and 2021, 17,696, 17,329 and 10,647 horses, respectively, were recorded as being moved to slaughter in another MS. In essence, welfare concerns for this scenario are covered by the hazards and welfare consequences assessed during road transport of horses, but due to the duration of the journeys, the exposure time may be increased.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

In the framework of its Farm to Fork strategy, the Commission will start a comprehensive evaluation of the animal welfare legislation. This will include the following acts:

- Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes.
- Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens.
- Council Directive 2008/119/EC of 18 December 2008 laying down minimum standards for the protection of calves.
- Council Directive 2008/120/EC of 18 December 2008 laying down minimum standards for the protection of pigs.
- Council Directive 2007/43/EC of 28 June 2007 laying down minimum rules for the protection of chickens kept for meat production.
- 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 1255/976.
- Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing.

These acts are based on scientific opinions that are outdated. The current EU legislation on the protection of animals during transport is based on a scientific opinion adopted in 2002. Since then, the EFSA adopted opinions in 2004 (two opinions) and 2011.

In the context of possible drafting of legislative proposals, the Commission needs new opinions that reflect the most recent scientific knowledge.

Against this background, the Commission would like to request the EFSA to review the available scientific publications and possibly other sources to provide a sound scientific basis for future legislative proposals.

This request is about the protection of terrestrial animals during transport.

1.1.2. Terms of Reference

The Commission therefore considers opportune to request EFSA to give an independent view on the protection of animals during transport for the following groups and categories of farmed animals:

Free-moving animals (group 1):

1) Equids (horses, donkeys and their crossings),
2) Bovine animals (cattle and calves),
3) Small ruminants (sheep and goats),
4) Pigs,

Animals in containers (group 2):

5) Domestic birds (chickens for meat, laying hens, turkeys, ducks, geese, quails, etc.),
6) Rabbits.

The request refers to any journey, i.e., journeys of less than 8 h ("short journeys"), journeys of more than 8 h ("long journeys") and long journeys that need unloading and/or feeding ("very long journeys").

1.1.2.1. Assessment of common transport practices

For each category of animals (1–6), the EFSA will describe, based on existing literature and reports, the current practices regarding:

a) the preparation for transport (including catching and crating of poultry and rabbits), loading, unloading and handling of animals at all stages of the journey, including at destination,

b) the means of transport by road, roll-on–roll-off vessels, livestock vessels, the means of transport by rail and by air,
c) the conditions within the means of transport: space, microclimatic conditions, watering and feeding,
d) the journey duration and its circumstances as well as the resting of animals in the vehicle being stationary or being unloaded,
e) the conditions for areas where animals are unloaded and/or grouped as part of the journey (assembly centres, livestock markets, control posts, EU ports).

Additionally, for each of the above practices, the EFSA will:

- Describe the relevant welfare consequences for each category of animals during each step of the process. Relevance will not need to be based on a comprehensive risk assessment, but on EFSA's expert opinion regarding the severity, duration and occurrence of each welfare consequence,
- Define qualitative or quantitative measures to assess the welfare consequences during transport (animal-based measures),
- Identify the hazards leading to these welfare consequences,
- Provide recommendations to prevent, mitigate or correct the welfare consequences (resource and management-based measures).

1.1.2.2. Assessment of seven specific transport practices

For the following scenarios, the Commission has identified practical difficulties or insufficient information in ensuring the welfare of animals. At least for them, the EFSA is asked to propose detailed animal-based measures and preventive and corrective measures with, where possible, either qualitative (yes/no question) or quantitative (minimum/maximum) criteria (i.e. requirements to prevent and/or mitigate the welfare consequences):

1) 'Export by livestock vessels' – Transport of adult cattle, weaned calves and sheep over long journeys involving the combination road/livestock vessels;
2) 'Export by road' – Transport of adult cattle, weaned calves and sheep over long journeys by road involving the use of facilities where animals are unloaded and reloaded (control posts, livestock markets) or when animals are kept in stationary vehicles for hours (exit points) including in third countries;
3) 'Roll-on–roll off' – Transport of adult cattle, calves and sheep over long journeys involving the combination road/roll-on–roll-off vessels;
4) 'End-of-career animals' – Transport of end of career animals to slaughterhouses of dairy cows, breeding sows and laying hens;
5) 'Unweaned calves' – Transport of unweaned calves over long journeys; this scenario will particularly consider the risks regarding fitness for transport, watering, feeding and thermal comfort under Section c of the current practices associated with inappropriate drinkers and liquid feed for unweaned calves;
6) 'Horses' – Transport of horses on long journeys to slaughterhouses;
7) 'Special health status animals' – Transport of ruminants and pigs where unloading them before the final destination might jeopardise their health status.

For all scenarios, the EFSA will consider the risks regarding microclimatic conditions under Section c of the current practices associated with extremely high or low temperatures including the difficulty of measuring of temperature, humidity and gas concentration within animals' compartment.

1.2. Interpretation of the Terms of Reference

This scientific opinion concerns the protection of equids during transport. The fundamental premise of the work underlying this scientific opinion is that it is an accepted practice that humans breed animals for food, sports and leisure.

The scientific opinion focuses on horses, as they are transported in much higher numbers inside and outside EU than donkeys and crosses between donkeys and horses (mules and hinnies). The available information on donkey transport is summarised in a separate section focusing on selected welfare concerns for these animals. Scientific information on the transport of mules and hinnies are not available. For all animals included in the scientific opinion, the assessment does not go into details with the consequences of the housing system or production system, from which the animals to be transported are coming, even though it cannot be excluded that welfare consequences of transport to some extent differ depending on, e.g. the previous husbandry conditions.

The opinion will deal with the preparation, loading and unloading, transit and journey breaks. For the purpose of this opinion, the preparation phase involves all types of actions and animal management that take place during the interval from the decision to transport horses until the initiation of loading of the animals onto a vehicle or other means of transport. For horses, preparation including appropriate training for loading and transport correctly starts far before the decision to move the horse. In effect, in this opinion, the section on preparation of horses for transport describes also preparation practices, which commonly are conducted by horse industry members before moving their animals. Loading starts when the first animal is moved towards the ramp of the means of transport and ends when the last animal is loaded and up until the ramp is closing. Unloading starts when the ramp is open, and the first animal exits the means of transport, and ends when the last animal exits. Loading and unloading are dealt with together due to the similarities of the processes. The transit stage starts when the ramp has been closed and ends when the first animal unloads. Journey breaks conceptually apply to periods when the vehicle is stopped on the side of a road, or when animals are offloaded to other facilities for feeding, watering and resting, including control posts. Legislation regarding drivers of animal transport vehicles affect animal transport, especially on journeys with only one driver, as drivers must have rest breaks during which vehicles will be stationary (Table 1). As these breaks are not aimed to rest, feed and water the animals, for the purpose of the current assessment, they are not included in the 'journey break' stage.

Table 1: Overview of the EU legislation for the working time of truck drivers (Department for transport, UK 2014)

Drivers' hours rules Regulation (EC) 561/2006	Working time rules Directive 20002/15/EC
Driving • 9 h daily driving limit (can be increased to 10 h twice a week) • Max 56 h weekly driving limit • Max 90 h fortnight driving limit	**Working time (including driving)** • Working time must not exceed average of 48 h a week (no opt out)[1] • Max working time of 60 h in 1 week (provided average not exceeded) • Max working time of 10 h if night work performed.[2]
Breaks • 45 min break after 4.5 h driving • A break can be split into two periods, the first being at least 15 min and the second at least 30 min (which must be completed after 4.5 h driving)	**Breaks[3]** • A driver cannot work for more than 6 h without a break. A break should be at least 15 min long. • 30 min break if working between 6 and 9 h in total.[4] • 45 min break if working more than 9 h in total.
Rest • 11 h regular daily rest[5]; which can be reduced to 9 h no more than three times a week. • 45 h weekly rest, which can be reduced to 24 h, provided at least one full rest is taken in any fortnight. There should be no more than six consecutive 24-h periods between weekly rests.	**Rest** • Same rest requirements as EU' drivers.

(1): Normally calculated over a rolling 17-week period, but can be extended to 26 weeks under a collective or workforce agreement.
(2): Can be extended under a collective or workforce agreement.
(3): EC Regulation 561/2006 is directly effective and takes precedence over EC Directive 2002/15 – Article 2.4 Directive 2002/15. Therefore, EU drivers' hours break requirements take precedence when driving.
(4): After working for 6 h, a mobile worker must take a break of at least 15 min. However, if working more than 6 and up to 9 h in a shift, a mobile worker needs to take a break of in total at least 30 min – this could be two breaks of 15 min. Where a shift will contain more than 9 h of working time, a total of 45 min of break is needed.
(5): Alternatively, this regular daily rest period may be taken in two periods, the first of which must be an uninterrupted period of at least 3 h and the second an uninterrupted period of at least 9 h.

This opinion does not focus on the different types of premises defined in the transport Regulation (EC 1/2005)[1] (e.g. markets, auctions) but refers to them where appropriate. Destination is not covered, but only specified when important considerations are found.

Within equine species, different animal categories exist, such as foals and yearlings. This scientific assessment focuses on non-juvenile animals, but, when relevant and when information is available, specific categories are mentioned. When specific studies are referred to, the average body weight of the involved animals is mentioned (when available) as well as the animal category involved in the study. For most of the sections of the scientific opinion, conclusions and recommendations concerning non-juvenile horses of an unspecified weight or age are drawn.

The scientific assessment carried out by EFSA takes two forms. Firstly, for road transport practices, which is the most common practice, the transport stages are described and assessed in terms of welfare consequences (WCs), animal-based measures (ABMs) and hazards leading to the WCs. In addition, recommendations to prevent hazards and correct or mitigate WCs are provided. The preventive measures (PRE) relate to the hazards, and the corrective/mitigating measures refer to the WCs. Where possible, the assessment leads to the establishment of recommendations on quantitative thresholds for microclimatic conditions within means of transport (maximum temperature), and to spatial thresholds (minimum space allowance). In addition, the development of welfare consequences over time is assessed in relation to maximum journey time.

While the opinion mainly focuses on road transport, there are specific sections dealing with the following means of transport: Roll-on–roll-off ferries (RO-RO), air and rail.

Secondly, for the specific industry practices (specific scenario) listed in the mandate that relate to horses, EFSA examines selected welfare concerns (defined as an area or a topic to which special attention should be given in order to potentially avoid negative welfare consequences), and, where possible, suggests recommendations.

In Council Regulation (EC) No 1/2005[1], 'unbroken equidae' are defined as '*equidae that cannot be tied or led by a halter without causing avoidable excitement, pain or suffering*', and this category of horses must be transported in small groups ($n \leq 4$), and are currently not allowed to go for journeys exceeding 8 h. The term 'broken' is commonly used to indicate that a horse has been trained for riding or driving. Therefore, in this scientific opinion, 'equidae that cannot be tied or led by a halter without causing avoidable excitement, pain and suffering' are termed unhandled.

Currently, horses in the EU are classified as registered horses (with a studbook, according to Directive 90/426/EEC) and unregistered horses. Registered horses are exempted from the rules in the regulation regarding the watering, feeding interval, journey times and resting periods when transported for competition, races, cultural events or breeding, but this derogation does not apply when they are transported, directly or after transit through a market or marshalling centre, to a slaughterhouse for slaughter. However, as no scientific evidence can be found to document different effects of transport on the welfare of horses depending on their status as registered, in this scientific opinion, they are treated as one.

A list of WCs is selected among those reported in the guidance protocol published by EFSA (EFSA AHAW Panel, 2022a). These WCs can lead to negative affective states such as fear, pain and/or distress. For each transport stage, the highly relevant WCs are selected based on literature and expert opinion considering the severity, duration and frequency of occurrence of the WC. When possible, each WC is linked to one or more ABMs that are indicative of it.

During preparation for transport, animals might present health conditions (including WCs such as injuries or respiratory disorders) that may increase in severity during transport. Certain other physiological conditions, while not being a WC as such (e.g. pregnancy or certain age categories), are conditions that predispose the animal to experience WCs if transported. Rather than assessing all WCs that might occur at any given stage of transport due to animals being unfit for transport, a separate section in the scientific opinion, focusing on fitness for transport, is developed as part of the assessment of the preparation stage.

For the purpose of this scientific assessment, failure to implement or non-compliance with the current rules as specified in the transport regulation is not considered. This is outside the remit of EFSA as a risk assessor. During the work, the EFSA experts may include scientific information from practices currently prohibited in the EU.

The assessment is not split according to the thresholds of the current legislation, e.g. specifying that 8 h as journey duration is the threshold between short and long journeys (each with specific legislative requirements). Alternatively, the assessment is performed based on a journey carried out in the EU of an unspecified length and duration.

2. Data and methodologies

2.1. Data

2.1.1. Data from literature

The information contained in the scientific papers and reports identified as relevant during the literature search was used as a basis for the text of this scientific opinion. Additional sources were added by the EFSA experts when dealing with specific sections.

2.1.2. Data from Public Consultation

To consult interested parties and gain feedback on EFSAs interpretation of the transport mandate, a public consultation was launched in the period from 15 April to 10 June 2021. In particular, EFSA called for interested parties to:

- identify current transport practices of particular concern not already identified by EFSA in the interpretation of mandate;
- describe the practical difficulties or insufficient information in ensuring the welfare of animals, for the specific transport practices listed in the request from the European Commission and for any other additional practices of concern that might be identified;
- provide any available recorded data from road or sea transport, e.g. from a data logger, related to the microclimatic environment (temperature, humidity and ammonia levels). The data should demonstrate a link between the microclimatic conditions and any adverse welfare consequences that are experienced by the animals during transport.

The information received in the public consultation was considered by the EFSA experts as part of its work on this opinion (See Annex A: Report of the Public Consultation on the Protection of Animals during Transport, published under 'Supporting Information' in the Opinion on transport of small ruminants).

2.2. Methodologies

This scientific opinion follows the guidance protocol that was developed by the AHAW Panel to deal with all the mandates in the context of the Farm to Fork Strategy revision (EFSA AHAW Panel, 2022a).

To address the terms of reference of the mandate, the AHAW panel translated the assessment questions into more specific subquestions. These are interrelated, meaning that the outcome of each subquestion is necessary to proceed to the next subquestion. The approach to develop the subquestions was based on evidence from the scientific literature and expert opinion. The translation of the assessment questions into subquestions is mapped in Table 2.

Table 2: Specific assessment questions and subquestions of the mandate

Assessment Questions		Subquestions	
i.	**Describe the current transport practices**	***1.*** *Identify and select relevant transport scenarios (common animal transport practices per species and animal category)*	***2.*** *Describe the transport practices*
		Aim: Animal transport practices to be considered in the assessment are identified and selected to be common (representative of the current practice) in the EU. Approach: Expert opinion via group discussion. Relationship with assessment question: This subquestion is necessary for the overall assessment question requiring the description of the practices.	Aim: All the animal transport practices per animal category identified and selected from subquestion 1 are described narratively. Approach: Literature review. Relationship with assessment question: This corresponds to the assessment question and is necessary for the next assessment question.
ii.	**Describe the relevant welfare consequences that may occur due to the practices**	***3.*** *Identify the welfare consequences common for all mandates and provide their definitions*	***4.*** *Select the highly relevant welfare consequences for the selected animal transport practices*
		Aim: To identify the welfare consequences and provide a definition for them. EFSA generates a list of welfare consequences common for all mandates. Approach: Expert opinion via group discussion (see focus and full resulting list in Section 3.2). Relationship with assessment question: The list of all possible welfare consequences is necessary for the next assessment question asking to identify the most relevant ones per each system.	Aim: To select the most relevant welfare consequences for each of the previously defined animal transport scenarios per species or animal category. Approach: Expert opinion via Expert Knowledge Elicitation (EKE) (see Section 2.2.1). Relationship with assessment question: This corresponds to the assessment question, is related to subquestion 1 in which relevant welfare consequences are identified only for current transport scenarios.
iii.	**Define qualitative or quantitative animal-based measures (ABMs) to assess these welfare consequences**	***5.*** *Identify the feasible ABMs for the assessment of the most relevant welfare consequences*	***6.*** *Describe the feasible ABMs for the assessment of the most relevant welfare consequences*
		Aim: The ABMs for the assessment of the welfare consequences previously identified as relevant are selected (only for feasible ABMs). Approach: Expert opinion via group discussion. Relationship with assessment question: This corresponds to the assessment question and is related to subquestion 4 in which ABMs are identified only for the most relevant welfare consequences.	Aim: The ABMs for the assessment of the welfare consequences previously identified as the most relevant are described. Approach: Literature review. Relationship with assessment question: related to subquestion 5.

Assessment Questions		Subquestions	
iv.	**Identify the hazards leading to these welfare consequences**	***7.*** *Identify the hazards leading to the most relevant welfare consequences*	***8.*** *Describe the hazards leading to the most relevant welfare consequences*
		Aim: The hazards leading to the most relevant welfare consequences are identified. Approach: Expert opinion via group discussion. Relationship with assessment question: This corresponds to the assessment question and is related to subquestion 4 in which hazards are identified only for the most relevant welfare consequences.	Aim: The hazards are described. Approach: Literature review. Relationship with assessment question: related to subquestion 6.
v.	**Provide recommendations to prevent, mitigate or correct the hazards**	***9.*** *Identify the preventive and corrective measures for the most relevant welfare consequences*	***10.*** *Describe the preventive and corrective measures for the most relevant welfare consequences*
		Aim: Preventive and corrective measures for the most relevant welfare consequences for the previously defined transport scenarios per animal category are identified. Approach: Expert opinion via group discussion. Relationship with assessment question: This corresponds to the assessment question and is related to subquestion 4 in which preventive and corrective measures are identified only for the most relevant welfare consequences.	Aim: Preventive and corrective measures are described. Approach: Literature review. Relationship with assessment question: related to subquestion 8.

2.2.1. Experts' opinion

The data obtained from the literature and public consultation were complemented by the opinions of the EFSA experts. As described in Table 2, expert opinion was mainly used for the subquestions requiring the identification of transport practices, WCs, ABMs, hazards, preventive and corrective or mitigative measures. Expert opinion was mainly elicited via discussion among EFSA experts. However, for the identification of the highly relevant WCs, an informal, structured Expert Knowledge Elicitation (EKE) was carried out.

As explained above (subquestion 4), the mandate requested the identification of the highly relevant welfare consequences for each of the defined animal transport practices.

The starting point was the list of 33 specific WCs identified under subquestion 3 (for details, see Section 3.1.1.3 of the protocol, EFSA AHAW Panel, 2022a). The exercise was carried out separately for each of the animal transport stages per species or animal category resulting from subquestion 1.

The exercise consisted of selecting the highly relevant WCs out of these 33 per each of these combinations (species/animal category × transport stage).

For each combination, EFSA experts classified, based on an estimate of their magnitude, the 33 WCs into four categories of relevance: (i) non-applicable, (ii) slightly relevant, (iii) moderately relevant and (iv) highly relevant. Appendix B contains an example of this process. The magnitude of a WC was defined as the product of three parameters: severity, duration and frequency of occurrence (EFSA AHAW Panel, 2012). Owing to the lack of published data on these three parameters, the experts expressed their qualitative expert opinion on the magnitude of WCs.

Expert opinion is elicited in three phases:

1) First phase: The experts go individually through the list of welfare consequences and identify those that would fall in the 'non-applicable' or 'slightly relevant' categories. Their individual judgements are then be collated, and those WCs unanimously identified as belonging to these two categories are removed and not considered for further assessment. Those WCs for which there is no consensus whether they are considered 'non-applicable' or 'slightly relevant' remain for further assessment and require an open group discussion to find a consensus.
2) Second phase: the experts individually went through the list of remaining WCs and identified those that fell in the category of 'highly relevant'. These were kept for further assessment. Similarly, as during the first phase in case discrepant opinions emerged, consensus was sought through group discussion.
3) Third phase: The experts are asked to rank individually all the remaining WCs in the list that are not already identified as highly relevant (and thus kept) or non-applicable or slightly relevant (and thus removed) from the highest to the least relevant. Their individual rankings are then discussed again in an open group discussion with the aim to assign the remaining WCs into the category 'highly relevant' or in the category 'moderately relevant'.

The scientific opinion only report, for each of the defined animal transport stages, those WCs that were selected to be highly relevant from this exercise (since the mandate asks for the 'most relevant' WCs in each identified transport practice).

Expert opinion was also part of the syntheses involved in the development of the quantitative recommendations for specific conditions within the means of transport (i.e. space allowance, microclimatic conditions and journey duration) relevant to the assessment.

2.2.2. Literature searches

As described in Table 2, literature searches were carried out for the subquestions requiring the description of transport stages, WCs, ABMs, hazards, preventive and corrective or mitigative measures.

First, broad literature searches were carried to provide information on current practices on transport of the animal categories and species included in the 'free-moving' mandate. Restrictions were applied in relation to the date of publication, considering only those records published after a previous EFSA scientific opinion on the topic (EFSA AHAW Panel, 2011).

Following the broad searches, more specific searches were carried out focusing on WCs, ABMs, hazards, preventive and corrective or mitigative measures.

For horses, the search results (general + specific) yielded a total of 590 (103 + 487) records that were exported to an EndNote library together with the relevant metadata (e.g. title, authors, abstract).

Titles and abstracts were screened to remove irrelevant publications (e.g. related to species, processes and research purposes that were out of scope of this opinion) and duplicates, and successively to identify their relevance to the topic. The screening led to 191 relevant records for the search concerning publication dates from 2011 to 2021. Experts screened these papers and selected 86 for further assessment. Full texts were retrieved and made available to the experts.

The search terms were saved in Web of Science and rerun with any results (records) subsequent to 2021 screened and added to the pool of papers available to the experts. In addition, the experts selected relevant references starting from scientific papers, including review papers, book chapters, non-peer-reviewed papers known by the experts themselves or retrieved through non-systematic searches, until the information of the subject was considered sufficient to undertake the assessment by the EFSA experts. If needed, relevant publications before 2011 were considered.

3. Assessment

3.1. The transport of horses in the EU

Transport of animals between MS and exports from the EU is recorded in the TRAde Control and Expert System (TRACES), which is the European Commission's multilingual online platform for sanitary and phytosanitary certification required for intra-EU trade and importation of animals, semen, embryos, food, feed and plants (https://ec.europa.eu/food/animals/live_animals_en). However, movements within an MS (i.e. to slaughterhouses or between farms) are not recorded in this database (Rojek, 2021).

According to TRACES, around 170,000 horses were transported between MS per year in 2019–2021, across all means of transport. Road transport constituted around 85%. Horses are the most frequently transported species in the EU in proportion to their population (EP report, 2016). The frequency, distance and means of transport to which the horse is subjected are largely dependent on the use to which a horse is put. Horses are transported for many different reasons: competition, breeding, leisure activities, sale or slaughter are common uses for transported horses.

3.2. Welfare consequences associated with transport of horses

During the last decades, several scientific reviews (e.g. Nielsen et al., 2011; Padalino, 2015), textbooks and book chapters (e.g. Driessen et al., 2022; Padalino and Riley, 2022a) and international organisations (e.g. WOAH, 2011) have described and discussed the consequences of animal transport in terms of animal welfare. In general, it is agreed that animal transport can lead to severe negative animal welfare consequences. Transport of animals is known as a complex stressor involving many aspects (related to the condition of the animals, their general biological characteristics, as well as the conditions under which the transport takes place including journey duration), the majority of which to some extent may influence animal welfare. Thus, when analysed in detail, a highly complex picture emerges and transport must be considered as a multifactorial stressor.

Across the different stages of the transport of horses, the following WCs were selected as highly relevant: gastro-enteric disorders, handling stress, heat stress, injuries, isolation stress, motion stress, prolonged hunger, prolonged thirst, respiratory disorders, resting problems, restriction of movement, sensory overstimulation and separation stress (Table 3) It is clear from this table that all transport stages involve several WCs.

For the purpose of this scientific opinion, the WC injuries, was created as a combination of the WCs 'Soft tissue lesions and integument damage' and 'bone lesions' (see Section 3.1.1.3. of the protocol, EFSA AHAW Panel, 2022a).

Table 3: WCs selected as 'highly relevant' per each of the transport stages involved in this opinion

WCs and definitions		Transport stages			
		Preparation	Loading/ unloading	Transit	Journey break
Gastro-enteric disorders	The animal experiences negative affective states such as discomfort, pain and/or distress due to impaired function or lesion of the gastro-intestinal tract resulting from, e.g. nutritional deficiency, infectious, parasitic or toxigenic agents.			X	X
Handling stress	The animal experiences stress and/or negative affective states such as pain and/or fear resulting from human or mechanical handling (e.g. sorting and vaccination of newly hatched chicks, loading/unloading, catching and crating of animals to be transported, inversion).	X	X		X
Heat stress	The animal experiences stress and/or negative affective states such as discomfort and/or distress when exposed to high effective temperature.		X	X	
Injuries	The animal experiences negative affective states such as pain, discomfort or distress due to physical damage to somatic tissue types (bones, joints, skin, muscles). This can be due to injuries or pathological changes.	X	X	X	X
Isolation stress	The animal experiences stress and/or negative affective states such as frustration and/or fear resulting from the absence of or from limited social contact with conspecifics.	X			
Motion stress	The animal(s) experience motion sickness, stress and/or fatigue due to the forces exerted as a result of acceleration, braking, stopping, cornering, gear changing, vibrations and uneven road surfaces during transport.			X	
Prolonged hunger	The animal experiences craving or urgent need for food or a specific nutrient, accompanied by a negative affective state and eventually leading to a weakened condition as metabolic requirements are not met.			X	
Prolonged thirst	The animal experiences craving or urgent need for water, accompanied by an uneasy sensation (a negative affective state) and eventually leading to dehydration as metabolic requirements are not met.			X	
Respiratory disorders	The animal experiences negative affective states such as discomfort, pain, air hunger and/or distress due to impaired function or lesion of the lungs or airways.			X	X

WCs and definitions		Transport stages			
		Preparation	Loading/ unloading	Transit	Journey break
Resting problems	The animal experiences stress and/or negative affective states such as discomfort, and/or frustration due to the inability to lie/rest comfortably or sleep. (e.g. due to hard flooring, inability to perch or vibration during transport). This may eventually lead to fatigue.			X	X
Restriction of movement	The animal experiences stress and/or negative affective states such as pain, fear, discomfort and/or frustration due to the fact that it is unable to move freely, or is unable to walk comfortably (e.g. due to overcrowding, unsuitable floors, gates, barriers).		X	X	
Sensory overstimulation	The animal experiences stress and/or negative affective states such as fear, discomfort due to visual, auditory or olfactory overstimulation by the physical environment.	X	X	X	X
Separation stress	The animal experiences stress and/or negative affective states such as fear and/or frustration resulting from separation from conspecifics.	X			

3.2.1. Negative affective states

The description of each WC reported in Table 4 refers to one or more negative states involving affective components (e.g. pain, fear, fatigue). The states listed in Table 5 are the major negative affective states that derive from the occurrence of the WCs, and that may potentially lead to animal suffering. A list and description of the negative affective states as derived from literature, and also described in the guidance protocol document (EFSA AHAW Panel, 2022a) is reported in Table 4.

Table 4: List and description of the negative states that animals may experience, when exposed to at least one of the WCs listed above

Negative affective state	Description
Boredom	Boredom is an unpleasant emotion including suboptimal arousal levels and a thwarted motivation to experience almost anything different or more arousing than the behaviours and sensations currently possible (adapted from Mason and Burn, 2011).
Discomfort	Discomfort can be physical or psychological and is characterised by an unpleasant feeling resulting in a natural response of avoidance or reduction of the source of the discomfort. Pain is one of the causes for discomfort, but not every discomfort can be attributed to pain. Discomfort in non-communicative patients is assessed and measured via behavioural expression, also used to describe pain and agitation, leading to discomfort being interpreted as pain in some conditions (Ashkenazy and DeKeyser Ganz, 2019).
Stress[1] & Distress	STRESS[1]: Stressors are events, internal or external to the body involving real or potential threats to the maintenance of homoeostasis. When stressors are present, the body will show stress responses (biological defence to re-establish homoeostasis – e.g. behavioural, physiological, immunological, cognitive and emotional). Stress is a state of the body when stress responses are present (Sapolsky, 2002). DISTRESS: Distress is a conscious, negatively valenced, intensified affective motivational state that occurs in response to a perception that current coping mechanisms (involving physiological stress responses) are at risk of failing to alleviate the aversiveness of the current situation in a sufficient and timely manner (McMillan, 2020).

Negative affective state	Description
Fatigue	Physiological state representing extreme tiredness and exhaustion of an animal (EFSA AHAW Panel, 2020).
Fear	The animal experiences an unpleasant emotional affective state induced by the perception of a danger or a potential danger that threatens the integrity of the animal (Boissy, 1995).
Frustration	Negatively valenced emotional state consecutive to the impossibility to obtain what is expected or needed. Frustration is very often triggered by restriction of natural behaviours thus resulting in thwarted motivation to perform these behaviours.
Pain	An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage (Raja et al. 2020).

(1): The term stress does not describe a negative affective state in itself, but it is mentioned and defined in the table as it is a prerequisite of distress.

3.2.2. Definition and interpretation of ABMs for highly relevant welfare consequences during animal transport

Only a few studies have evaluated systems for assessment of welfare during transport for livestock species. Messori et al. (2016) proposed a tool to assess welfare of horses at unloading, while Padalino et al. (2018a) proposed an ethogram to quantify transport stress of horses during journeys and some ABMs were proposed for specific WCs (Dai et al., 2020a,b). However, no tool for assessment of welfare before, during and after transport has been validated. Consequently, no benchmark values documenting optimal animal welfare status during or after transport exist to inform this scientific opinion.

The feasibility of an ABM may be defined as the practicality to carry out an assessment of the ABM under field conditions. Feasibility does not relate to the sensitivity, specificity or repeatability of recording of an ABM. A feasible ABM for use during transport should be able to be recorded quickly, not using any specialised equipment or laboratory test, at a low cost, be minimally invasive for the animal, and with no (or only minimal) interference with normal operation procedures (Llonch et al., 2015; Messori et al., 2015). Llonch et al. (2015) divided feasibility into three categories: *high* (easy and quick recording without any special needs/tools), *medium* (extra time and/or space needed for recording) and *low* (not able to record under 'field conditions').

Some ABMs may have acceptable feasibility when it comes to recordings as part of research projects, but not when it comes to recordings during routine transport, especially in the transit stage. No studies have evaluated whether an ABM is of low, medium or high feasibility during horse transport. Feasibility is therefore not further addressed in this scientific opinion. A similar lack of knowledge exists with reference to sensitivity and specificity of ABMs in the context of animal transport and so these characteristics will not be dealt with in this scientific opinion.

One of the main feasibility challenges in relation to ABMs during animal transport is the access to, and visibility of, the animals during particular stages, especially the transit stage. It can be difficult to see and access any single horse when they are in a vehicle or other means of transport. This problem can be partially overcome by the use of cameras and/or other types of sensors. However, the mere presence of cameras or sensors does not overcome all these challenges, as data generated by such sensors need to be analysed in some way leading to an interpretation of the practice in question. Currently, technological tools within this area are in development (e.g. AlZubi et al., 2016). For example, recent studies have evaluated the use of facial expression to assess transport stress in horses (Lundblad et al., 2021) and the possibility of automated interpretation of this (Andersen et al., 2021). Another aspect of transport stress that may be mitigated in the future by use of sensor technology is motion stress caused by movements of the vehicle during the transit stage (as described by Morris et al. (2021) in pigs). However, there have been almost no studies that address the efficacy and feasibility of these new technologies for use in horse transport, and further research is required to develop practical sensors and associated interpretative or alarm systems.

During preparation, loading, unloading and journey breaks on or off the vehicle, the animals can be properly inspected and ABMs utilised. Among these could be visually recognised indicators, but potentially also auditory indicators, or physiological biomarkers that can be obtained from e.g. saliva, or even behavioural tests of e.g. latency to eat/drink from a bucket. At present, however, these potential tools need further development and validation for use in animal transport. There is also a subset of ABMs that are not feasible during transport even when the animals can be inspected (Llonch et al., 2015). Examples of these are physiological indicators requiring invasive procedures. Tables 5–16 below contain information on the definition and interpretation of ABMs, including ABMs considered potential candidates for future inspection, as well as ABMs that so far have only been used in scientific studies underlying the conclusions of the scientific opinion.

i) ABMs for the assessment of the welfare consequence gastro-enteric disorders

Table 5: ABMs for assessment of gastro-enteric disorders in horses in transit and journey breaks

ABM	Definition and interpretation of the ABM
Diarrhoea	**Definition:** Faeces are discharged from the bowels frequently and in an abnormal form (e.g. liquid form). Sometimes signs of diarrhoea are visible on the wall of the boxes or horses' legs and tails are dirty. **Interpretation:** Gastro-enteric disorders may alter the gastrointestinal absorption process and gastrointestinal motility leading to an increased volume and frequency of defecation often not shaped properly.
Colic clinical signs	**Definition:** Clinical signs of colic include changes in behaviour or activity that indicate abdominal pain. Horses may paw, roll, look at the belly, stop eating and drinking, may urinate and defecate less, assume abnormal posture during urination and defecation. Heart and respiratory rate and sweat score increase. **Interpretation:** A pain composite scale, including a score for the behavioural and physiological parameters listed above, and a scale for facial assessment of pain have been validated for colic (van Loon and Van Dierendonck, 2015).

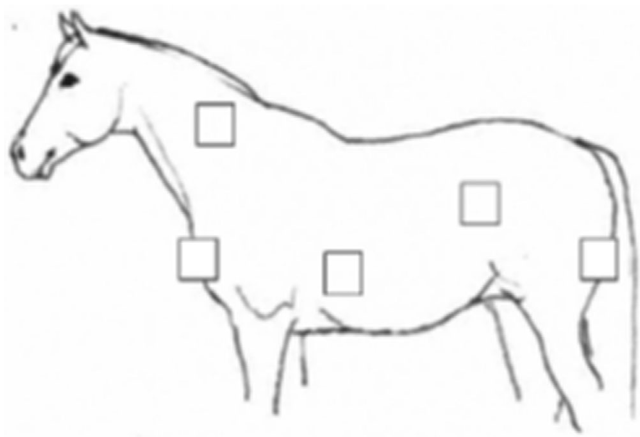
ii) ABMs for the assessment of the welfare consequence handling stress

Table 6: ABMs for assessment of handling stress in horses during preparation, loading/unloading and the journey break stages

ABM	Definition and interpretation of the ABM
Aggressive behaviour towards handler (rearing, kicking, biting)	**Definition:** Aggression is defined as threats or harmful actions directed towards another individual/animal and can include threat displays, rearing, kicking, pushing and biting. In animals, aggressive behaviours are a means of communication, and often horses use high pitch vocalisation and tail wishing to alert before showing aggressive behaviour. **Interpretation:** Aggressive behaviour is part of the flight or fight response when the horse is handled improperly and tries to escape from the handler using aggressive behaviour.
Avoidance behaviour	**Definition:** Refusal to move forward and/or moving away from a source of the aversive situation (e.g. too much pressure applied by the handler, fear of the ramp). **Interpretation:** During loading/unloading, horses may be scared of the ramp, or unable to respond to the pressures given by the handler. They try to avoid handler pressure or threats perceived from the vehicle.
Respiratory rate (RR)	**Definition:** Frequency of breathing, usually measured as the number of breaths per minute (bpm) (Mills et al., 2010). Normal range is considered between 8 and 12 bpm (Reed et al., 2017). **Interpretation:** Handling stress leads to the release of catecholamines, which will lead to an increased RR. Respiratory rate is correlated well with the level of handling and the handling stress experienced by unhandled horses in comparison with handled horses (Menchetti et al., 2021; Padalino et al., 2019). However, horses may show increased RR due to other reasons for activation of the sympathetic system (e.g. exercise, heat stress).

iii) ABMs for the assessment of the welfare consequence heat stress

Table 7: ABMs for assessment of heat stress in horses during loading/unloading and in transit

ABM	Definition and interpretation of the ABM
Sweating (sweat score)	**Definition**: Evaporation of sweat is one of the mechanisms that horses use to lose heat and keep their core internal temperature. Sweating is a way of thermoregulation. Evaporation is the primary process with the body tissues conducting heat to the skin, via the bloodstream and skin capillaries, transferring it to the external environment (Marlin, 2008). The presence of sweat can be scored by the Sweat score (from 0 to 5), as per methods described by Holcomb et al. (2013), adding the presence of sweat at five specific body regions of the horse; the neck, chest, girth, flank and hindquarters (as below Figure 1).  **Figure 1:** Area where horses must be assessed to calculate the sweat score (from 0 to 5) proposed by Holcomb et al. (2013) **Interpretation:** When the effective temperature increases above the comfort zone (defined in Section 3.5.3.1), the horse will start to sweat. Further increases in the effective temperature will see increased rates of sweating. Sweating can also be due to other factors such as exercise, fear or distress.
Rectal temperature (RT)	**Definition:** The core temperature of the horse is estimated from the measurement of rectal temperature, as these two are correlated. The normal RT range in horses is 37.2–38.2°C with an average of 37.6°C for stallions and 37.8°C for mares (Reece et al., 2015). **Interpretation**: RT tends to increase when the animal loses the ability to thermoregulate. Rectal temperature can increase also due to other factors such as exercise or disease (e.g. respiratory disorders (Yousuke and Masaaki, 2019)).
Heart rate (HR)	**Definition:** Number of heart beats per unit of time, usually per minute (Mills et al., 2010). The normal HR range for horses is 30–36 bpm. **Interpretation:** Heat stress leads to an increased HR in an attempt to preserve homoeothermy; however, the specific effect of heat on HR is likely variable depending on exposure length. Horses experiencing heat stress show an increased heart rate (Padalino et al. 2019). However, horses may show increased heart rate due to other reasons for activation of the sympathetic system (e.g. exercise, fear, anxiety).
Respiratory rate (RR)	**Definition:** Frequency of breathing, usually measured as the number of breaths per minute (bpm) (Mills et al., 2010). Normal range is considered between 8 and 12 bpm (Reed et al., 2017). **Interpretation:** Heat stress leads to an increased RR in an attempt to increase respiratory loses; however, the specific effect of heat on RR is likely variable depending on exposure length (Sapkota et al., 2016). Horses experiencing heat stress show an increased respiratory rate (Padalino et al. 2019). However, horses may show increased RR due to other reasons for activation of the sympathetic system (e.g. exercise, fear, anxiety) and respiratory disorders (e.g. transport pneumonia, EHV) (Ainsworth and Hackett, 2004).

iv) ABMs for the assessment of the welfare consequence injuries

Table 8: ABMs for assessment of injuries in horses during preparation, loading/unloading, transit and journey break stages

ABM	Definition and interpretation of the ABM
Superficial skin lesions	**Definition:** Superficial tissue damage such as bruises, and scratches particularly on the tail, head, back and legs (EFSA AHAW Panel, 2012; Mansmann and Woodie, 1995). **Interpretation:** Skin lesions are often caused by behavioural problems during loading and unloading (refusal to load, kicking, rearing, etc.). They may also result from the actions of handlers or another horse.
Wounds and sores	**Definition:** Deep damage to the skin, muscle or bone tissue. **Interpretation:** Horses get injured mainly by hitting against part of the vehicle, falling down the ramp, tripping or sliding on the ramp or being kicked or bitten by another horse.
Post-mortem carcass lesions	**Definition:** Skin damage on carcases is assessed subjectively along the dressing line by using different photographic scales. The assessment can be conducted as a whole, or separately in different parts of the carcass using visual inspection or thermography (Roy and Cockram 2015, Roy et al., 2015a; Roy et al., 2019). Bruises are not visible on a live horse, but are visible on a carcass. **Interpretation:** Post-mortem carcass lesions are considered as evidence of poor handling and inadequate facilities during all stages of the journey, but may also have happened prior to travel.

v) ABMs for the assessment of the welfare consequence motion stress

Table 9: ABMs for assessment of motion stress in horses in transit

ABM	Definition and interpretation of the ABM
Transport-related problem behaviours (TRPBs)	**Definition:** Any transport-related behaviour that impedes welfare or safety of the horse or handler during the transportation process (Dai et al., 2021). Reported TRPBs are vocalisation, head tossing, pawing, scrambling, head-turning, kicking out at the vehicle, biting and kicking directed at travelling companions and reduced feeding/drinking. **Interpretation**: During travel, TRPBs are generally exhibited during the first hour, due to the need of the horse to adapt to the vehicle and the motion (York et al., 2017; Dai et al., 2021).
Balance-related behaviours	**Definition:** Sum of the behavioural events related to balance: forward, lateral and backward movements, leaning on stall rails, loss of balance/dashing on the partitions (Padalino et al., 2018b). **Interpretation**: When horses travel in better conditions, with more space allowance and backward orientation, the frequency of balance-related behaviours decreases (Padalino and Raidal, 2020).

vi) ABMs for the assessment of the welfare consequence prolonged hunger

Table 10: ABMs for assessment of prolonged hunger in horses in transit

ABM	Definition and interpretation of the ABM
Licking/chewing	**Definition:** Opening of mouth with extension and retraction of tongue, lip-smacking without tongue extension, lateral jaw movements involving the partial opening of the lips (McGreevy, 2012). **Interpretation**: Horses fasted in transit have shown a higher frequency of licking and chewing (Padalino et al., 2018a,b,c,d). However, this is considered a stress-related behaviour and may be caused also by other stressors.

ABM	Definition and interpretation of the ABM
Stomach ulcer	**Definition:** Inflammation and disruption of the gastric mucosa, usually found in the non-glandular squamous mucosa of the stomach (Andrews et al., 2005) **Interpretation:** Stomach ulcers were seen after 12 h of travelling without food, but not when hay was available. Ulcers are caused not only by prolonged hunger but also by other stressors.
Latency to feed after unloading	**Definition:** The time passed from unloading the horses until they start eating. **Interpretation:** When food is offered in transit, the duration of the feeding behavioural patterns changes (Padalino and Riley, 2022a). Latency time for feeding after unloading is very short if the horses were not fed in transit.
Body weight	**Definition:** Difference in body weight between departure and arrival. **Interpretation:** Body weight loss is positively related to the hours of fasting and prolonged hunger during transport. It is mainly due to the loss of water and gastro-internal content and it can be easily measured using specific weight tape (Padalino et al., 2017b).

vii) ABMs for the assessment of the welfare consequence prolonged thirst

Table 11: ABMs for assessment of prolonged thirst in horses in transit

ABM	Definition and interpretation of the ABM
Capillary refill time	**Definition:** This test indicates how quickly the capillaries refill at the surface of the horse's body, evaluated by gently pressing on the gum with a fingertip and timing how long it takes the blanched area to become pink again; normally < 2 s (Dalla Costa et al., 2014). **Interpretation**: A prolonged capillary refill time suggests poor blood pressure and circulatory function. This can be caused by dehydration but also other illnesses.
Abnormal drinking behaviour/ Bucket test	**Definition:** Particular signs that horses are strongly motivated to drink include drinking for extended periods of time, gulping water, taking unusually long draughts or drinking until the trough is dry (Anon, 2014). A bucket test has been validated for horses (AWIN, 2015a). **Interpretation:** The latency time for drinking and the quantity of water drunk during the bucket test is used to assess thirst in the welfare assessment of horse on farm (AWIN, 2015a).

viii) ABMs for the assessment of the welfare consequence respiratory disorders

Table 12: ABMs for assessment of respiratory disorders in horses in transit and journey breaks

ABM	Definition and interpretation of the ABM
Rectal temperature	**Definition:** The core temperature of the horse is estimated from the measurement of rectal temperature, as these two are correlated. The normal RT range in horses is 37.2°C–38.2°C with an average of 37.6°C for stallions and 37.8°C for mares (Reece et al., 2015). **Interpretation:** Horses experiencing respiratory problems may show an increased rectal temperature after 20 h of travelling (Maeda and Oikawa, 2019). However, the rectal temperature may increase also due to other transport-related diseases (Padalino et al., 2015).
Nasal discharge	**Definition:** The horse presents with a discharge (serous, mucus, purulent) from its nostrils **Interpretation:** Due to the development of respiratory disorders, horses may develop a nasal discharge. The turbidity of the nasal discharge and the tracheal mucus is related to the number of bacteria in their lungs. However, some horses may develop pneumonia without showing it (Raidal, 1995).
Abnormal respiration	**Definition:** Dyspnoea (laboured breathing), tachypnoea (rapid breathing) and hyperpnoea (increased ventilation). **Interpretation:** A horse with a respiratory disorder may present with laboured and deeper breathing at a faster rate than normal.

ABM	Definition and interpretation of the ABM
Cough	**Definition:** Coughing is a vigorous inspiratory contraction, followed by a rapid exhalation, with the genesis of a sound vibration (AWIN, 2015a). **Interpretation:** A horse with a respiratory disorder may cough, and different coughing sounds may be related to the involvement of different parts of the respiratory system. Horse with transport pneumonia may not cough.

ix) ABMs for the assessment of the WC resting problems

Table 13: ABMs for assessment of resting problems in horses in transit and the journey breaks

ABM	Definition and interpretation of the ABM
Resting behaviour	**Definition:** Time that the horses rest in tripodal standing posture, lateral or sternocostal decubitus. Horses show those behaviour only if they are quiet and they feel safe and comfortable (Padalino, 2022). **Interpretation:** Despite the motivation, the horse cannot rest due to the prolonged need to stand, need to rebalance constantly, fear of falling or due to insufficient space allowance.
Blood muscle enzyme (Creatine kinase (CK), Aspartate Aminotransferase (AST), lactate)	**Definition:** CK, AST and lactate levels measured in blood (serum or plasma), which can increase after muscle problems and journeys due to excessive muscle work (Padalino et al., 2018a). **Interpretation:** Fatigue/exhaustion occurs when horses have to stay standing over a long period and have to rebalance constantly to adapt to the movement of the vehicle. Consequently, measuring CK, AST and lactate can indirectly determine whether the horses experience resting problems during travelling.

x) ABMs for the assessment of the WC restriction of movement

Table 14: ABMs for assessment of restriction of movement in horses during loading/unloading and in transit

ABM	Definition and interpretation of the ABM
Unable to walk comfortably/freely	**Definition:** Number of un-natural steps due to the fact that the horse's movement is restricted. **Interpretation**: Movement may be impeded by insufficient space or inappropriate ramp design (too steep, unstable, etc.) during loading and unloading and/or inappropriate handling and/or by equipment (e.g. bandages, rugs, short lead-rope).
Unnatural posture	**Definition:** Time spent by the horse assuming unnatural standing positions or standing continuously on four legs instead of assuming tripodal position (See Section 3.5.3.2). **Interpretation:** Insufficient space (length, width or height) causes the horse to stand uncomfortably and have difficulties balancing.
Superficial skin lesions	**Definition**: Superficial tissue damage such as bruises, scratches and wounds (EFSA AHAW Panel, 2012; Mansmann and Woodie, 1995). **Interpretation:** Skin lesions can be caused by hitting the walls or by another part of the vehicle (i.e. partitions). Often skin lesions are under the halter or on the tail, due to lack of space or too short rope, with impaired balance behaviour with the head (Padalino, 2022).

xi) ABMs for the assessment of the WC sensory overstimulation

Table 15: ABMs for assessment of sensory overstimulation in horses during preparation, loading/unloading, transit and journey breaks

ABM	Definition and interpretation of the ABM
Alert behaviour	**Definition**: In the alert pose, horses mostly freeze, have their ears towards the stimulus and eyes wide open. This can be accompanied with a snorting noise from their nostrils meaning they are insecure or frightened. When they get agitated/scared, they start to breathe fast and when more extreme start to sweat. **Interpretation**: Every time horses might experience novel objects, sound, smell or lighting may show alert behaviour, and experience excitement and anxiety.
Avoidance behaviour	**Definition:** Refusal to move forward and/or moving away or moving away from a source of the aversive situation (e.g. aversive smell, like adrenaline (Siniscalchi et al., 2015)). **Interpretation:** During transport, horses might experience novel obstacles, reflections or sound, which leads to avoidance reactions such as running away, rearing, shaking or trembling and wall climbing.
Sweating (Sweat score)	**Definition:** Horses start sweating under the activation of the sympathetic system (i.e. any stressful and fearful situation). The presence of sweat can be scored Sweat score (from 0 to 5), as per methods described by Holcomb et al. (2013), with the presence of sweat at five specific body regions of the horse; the neck, chest, girth, flank and hindquarters (See Figure 1 in Table 8). **Interpretation:** Horses may sweat in response to sensory overstimulation.
Heart Rate (HR)	**Definition:** Number of heart beats per unit of time, usually per minute (Mills et al., 2010). The normal HR range for horses is 30–36 bpm. **Interpretation:** Sensory overstimulation leads to an increased heart rate due to the release of catecholamines. However, horses may show increased HR due to other reasons (e.g. exercise, heat stress).

xii) ABMs for the assessment of separation/isolation stress

Table 16: ABMs for assessment of the WC separation stress and isolation stress in horses during preparation

ABM	Definition and interpretation of the ABM
Loud vocalisations	**Definition:** Horses have a large range of auditory signals. Neighs and whinnies are loud, prolonged call, typically of 1–3 s, beginning high pitched and ending lower pitched. The whinny is associated with alert behaviour (McGreevy, 2012). **Interpretation:** When a horse is isolated, agitated or is separated from his familiar groups he tends to emit neighs and whinnies.
Heart rate (HR)	**Definition:** Number of heart beats per unit of time, usually per minute (Mills et al., 2010). The normal HR range for horses is 30–36 bpm. **Interpretation:** Horses may show increased heart rate due to many reasons for activation of the sympathetic system (e.g. exercise, fear, anxiety).
Heart rate variability (HRV)	**Definition:** HRV is a non-invasive method of assessing the variance in time between heart beats. Knowledge about HRV gives information on the influence from the sympathetic and parasympathetic parts of the autonomic nerve system (Von Borell et al., 2007; Shaffer and Ginsberg, 2017). **Interpretation:** HRV variables change in response to stress induced by various stressors, and across the different indices of HRV, a decrease has been associated with exposure to stress (Ohmura et al., 2006).

3.3. Preparation of horses for transport

Careful preparation of horses for transport can substantially improve the welfare impact of transport. However, across horse categories as well as journey types and journey durations, the preparation of horses for transport may differ substantially. For the purpose of this scientific opinion, the preparation phase involves all types of actions and animal management that take place during the interval from the decision to transport horses until the initiation of loading of the animals onto a vehicle or other means of transport. Assessment of fitness for transport is included in the preparation stage.

Since horses may be transported many times during their life (Padalino, 2015), the preparation for transport should start soon after foaling with appropriate handling and training for loading and transport (Houpt, 1982). However, although breeding/competing horses are often transported, they may also develop TRPBs (York et al., 2017). Through associative learning, horses recognise features of the preloading routine (e.g. fitting of protective equipment and the presence of a transport vehicle) and associate them with past transport experiences (Weeks et al., 2012). Horses which have experienced problematic transport, such as falls or whipping, tend to exhibit increased TRPBs during preloading (Leadon et al., 2008). Preloading TRPBs include signs of anxiety such as vocalisation, pawing, heightened locomotion and trembling (Waran et al., 2007a,b). Unsurprisingly, as preloading, handling can be the moment when horses start to interact with people, the relationship between the horse and handler contributes to the risk of the horse displaying a TRPB (Padalino et al., 2017a). To prevent preloading TRPBs, it is important to apply an effective and proper handling routine underpinned by knowledge that recognises and mitigates stress. Even well-experienced horses may experience transport stress and their stress responses are strongly related to the transport conditions (Padalino, 2015).

3.3.1. Training for loading and transport

From an animal welfare perspective, all horses should be trained for loading and transport (York et al., 2017). However, when equine industry members were surveyed in different countries, around 50–60% of respondents reported to train horses for loading and transport (Padalino et al., 2016a (based on a survey among 797 respondents in Australia) and Padalino et al. (2017a) based on a survey among 1,000 respondents in New Zealand); and Dai et al. (2021) based on a survey among 201 respondents in Italy.

Different types of training programmes, such as habituation, can be used to train horses for loading and transport (as reviewed by York et al., 2017). This view is shared by Yngvesson et al. (2016), whose work with habituating Icelandic horses before transport showed that the time taken to load decreased significantly with the number of times attempted, when this type of training was performed.

3.3.2. Current practices

Horses are kept in a variety of husbandry systems from the extensive (an unfenced pasture (Insausti et al., 2021)) to intensive (kept indoors, in a single loose box stall or in groups in pens (Mills and Clarke, 2007)). The means of gathering for transport and the type of premises will depend on the type of horse and the type of journey being prepared for. In general, preshipment pens are used to assist in sorting, weighing and loading horses, particularly for horses transported to slaughter. Sport horses transported for competitions, breeding or other purposes, are often subjected to specific transport-related practices (see Section 1.2) and are then moved from their stall to the loading ramp.

The transport of horses from one farm to another may involve the rounding up of the horses to holding pens in a yard or shed where they will await loading. This might also involve short journeys by road in a trailer. If horses are being transported from one EU MS to another for slaughter, they are likely to be brought by truck, or jeep and trailer, to an assembly centre from where they will be officially consigned and transported in an authorised truck (see systematic review by Fletcher et al., 2022). If the horses are to be exported from the EU, they will likely have to spend time in quarantine premises in order to meet the requirements of the relevant health certificate. These horses all have to be transported by road to the quarantine premises before the quarantine process can begin. Moving horses between farms may involve them passing through a market. Such sales involve horses being transported to a market and then from the market to another farm in a different vehicle and sometimes with different horses. Horses transiting through live markets are at particular risk of poor welfare due to improper handling (and the associated slips, falls), hurried loading and, less commonly, isolation (Marlin et al., 2011). They are also at increased risk of being infected with pathogens. However, markets are not specifically dealt with in this scientific opinion.

Horses being transported by air have several transport stages, initially by vehicle, then onto the airplane and finally by vehicle again. Air transport is covered in detail in Section 3.9.

Horses are also transported by roll-on/roll-off (RO-RO) ferries. This will include first a road journey, followed by a journey via RO-RO ferry, followed by another road journey. This will be specifically covered in detail in Section 3.8.

For matters related to logistics, paperwork and planning, such as, e.g. route planning, readers are recommended to check recommendations from the EU Transport Guides (Consortium of the Animal Transport Guides Project (2017a).

In a questionnaire conducted in Australia, it was asked what practices were conducted before the journey of sport horses to more than 700 equine industry members who had transported horses in the previous 2 years (Padalino et al., 2016a). The answers are shown in Figure 2.

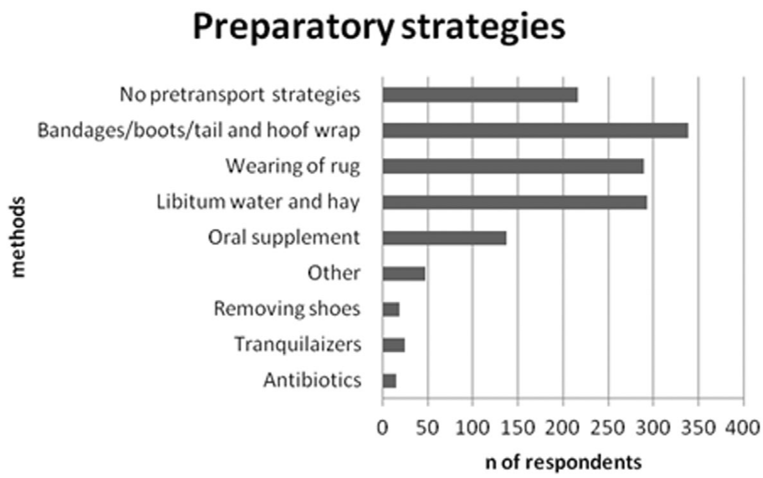


Figure 2: Preparatory actions reported in a questionnaire on horse transport-related issues and practices conducted in Australia (Padalino et al., 2016a)

Even if those preparatory practices are quite common in the equine industry, they may not always be appropriate, such as, e.g. the administration of antimicrobials and sedation (Padalino et al., 2016a). There are several supplements that have been suggested to help in reducing anxiety and stress related to transport, but the findings are conflicting. The use of prejourney immunostimulants seemed to be beneficial in reducing shipping fever (i.e. transport pneumonia mainly caused by *Pastorella* spp., *Staphylococcus* spp., *Strepococcus* spp., which can be fatal) after transport (Nestved, 1996, Akai et al., 2008, Endo et al., 2017). Rugs should not be worn since they may impair thermoregulation in transit (See Section 3.5.2) and wearable equipment (head-bumpers, tail guards, bandages, leg wraps or boots) can move and get misplaced, and also contribute to a risk of overheating (Padalino et al., 2016a).

3.3.3. Highly relevant welfare consequences

The highly relevant WCs selected during the preparation of horses for transport are handling stress, injuries, isolation and separation stress, sensory overstimulation. The selected ABMs that can indicate the WCs are shown in Section 3.2.2. Below, hazards (in bold), preventive (PRE), corrective and mitigating measures are described.

i) Handling stress

Proper handling before transport is important to minimise horse stress responses and to prevent preloading TRPBs. The main hazards leading to handling stress during the preparation phase are:

Inexperienced, untrained handlers or handlers using coercive methods/tools: Inexperienced, untrained handlers or handlers using coercive methods/tools have been identified as a risk factor for TBRPs and subsequent injuries in horses and handlers (York et al., 2017). The use of such tools has been identified as a risk factor for injuries and TRPBs during preloading (Padalino et al., 2017a).

— PRE: To prevent this hazard, suitable and educated staff should be selected to manage the process. This includes them paying attention to detail in the planning process and, when handling horses, doing it properly using proper handling based on animal learning theory

(Baragli et al., 2015). The response of animals to handling and transport also depends on their sensory capabilities, the visual field and flight zone. Identifying and managing stressful situations by responding to key behaviours indicative of discomfort, such as vocalisation, attempts to escape, kicking or struggling, is essential (Siniscalchi et al., 2014,b). The ability to recognise behavioural indicators of distress in horses reduces the risk of injury to the horse and handler and can have a significant effect on equine welfare (Padalino et al., 2018a).

Poor handling facilities: Poorly designed or poorly maintained facilities can be a stressor for handlers and animals. In these conditions, handlers are less efficient, tire more quickly and may experience occupational accidents, as well as become rougher in their handling of the animals.

- PRE: To prevent this hazard, handling facilities should be fit for purpose.

Time pressure: Rushed handling, not allowing horses enough time for adjustment/exploration increase the risk of fearful and agitated horses, and thus increase the risk of slipping, falling and skin lesions.

- PRE: Fast movements and time pressure should be minimised when working with horses (McGreevy et al., 2014). Horses should be given enough time to explore during movement.

Corrective/mitigating measure for handling stress

Corrective measures include the removal of the specific person employing inappropriate handling or provision of assistance to him/her on the spot. There are several supplements (e.g. tryptophan, alpha-casozepine) that have been suggested to reduce anxiety and preloading stress, but the findings are conflicting (Burk and Williams, 2008; Ijichi et al., 2019). Horses showing TRPBs should not be loaded and should undergo a retraining programme (York et al., 2017).

ii) Injuries

Many of the same factors that cause handling stress will also increase the risk of injury.
Inexperienced, untrained handlers: See Section 3.3.3i [handling stress].
Lack of prior training: see Section 3.3.1. Lack of appropriate training of the horse for loading and transport has been identified as a risk factor for preloading injuries (Padalino et al., 2017a).

- PRE: To prevent this hazard, horses should be trained in advance of loading and transport using effective and proper handling based on animal learning theory (Baragli et al., 2015). Through associative learning, horses recognise features of the preloading routine (e.g. fitting of protective equipment and the presence of a transport vehicle) and associate them with past transport experiences (Weeks et al., 2012). Horses who have experienced problematic transport, such as falls, tend to exhibit increased TRPBs during preloading (Leadon et al., 2008). Preloading TRPBs include signs of anxiety such as vocalisation, pawing, heightened locomotion and shaking (Waran, 2007a; Padalino, 2015).

Poor handling facilities: See Section 3.3.3i [handling stress].

Corrective/mitigating measure for injuries

Corrective measures include removal of the horse from the transport process, appropriate treatment and veterinary care.

iii) Isolation and separation stress

Important stressors during preloading include leaving a familiar environment and conspecifics and being isolated or sometimes grouped or regrouped with unfamiliar horses, depending on the type of journey planned and the prejourney housing.
Separation from other horses: Unhandled youngsters display anxiety when not transported in a group, and for this reason, their individual transport is not recommended (Knowles et al., 2010), as they are not used to being isolated, handled or restrained.

- PRE: To prevent this hazard, horses should be kept in their social groups during preparation for transport whenever possible (Broom, 2008).

Being isolated: Horses are social animals and when isolated, if not previously habituated to this situation, may be very agitated and anxious (McGreevy, 2012).

— PRE: To prevent this hazard, whenever possible, horses should be kept in their social groups (Broom, 2008) or properly trained to be isolated and kept individually for a long period.

(Re)grouping with unfamiliar horses: Grouping or regrouping horses before loading is a common practice to form groups based on their future destination, for logistic reasons. (Re)grouping can be done just before loading or a few days before at the stable or assembly centre. However, mixing of groups disturbs the social cohesion between the horses in the original groups (Van Dierendonck and Spruijt, 2012) and may result in fighting (i.e. biting and kicking) to establish a social hierarchy, potentially leading to bruises (Houpt and Wickens, 2014), especially in groups transported loose. Furthermore, (re)grouping can interfere with the loading of the horses because stressed animals often are more difficult to handle.

— PRE: To prevent this hazard, horses should be kept in their social groups during preparation for transport whenever possible (Broom, 2008).

Corrective/mitigating measure for isolation or separation stress

Horses are social animals and tend to bond to particular conspecifics, sometimes named 'buddies' (McGreevy, 2012). If a horse is showing signs of isolation stress, it should be brought back into the familiar group, or a 'buddy' should be brought along.

iv) Restriction of movement

Important stressors during preloading include physical restraint which is often part of the handling procedure. Age, sex and physiological condition influence the behaviour of horses during preloading, as will their prior experience. Foals and yearlings are usually not trained extensively and can be more difficult and riskier for themselves and for the handlers (risk of injuries) than older animals. Although stallions are generally assumed to be more difficult to handle than geldings, this difference may also be age dependent.

Haltering and tethering animals: Horses that have not been handled before, should not be lead and tethered.

— PRE: Horses that are tethered should be able to lower their head below wither height. Unhandled horses should be kept free to move and without wearing halters and any other type of restrictions (rope, collar, hobbles (devices to prevent or limit the movement of the horse by tethering one or more legs)). When kept loose, horses should have at least the space to turn around.

Corrective/mitigating measure for restriction of movement

Tethering should be stopped and horses should go back to their pens/stalls.

v) Sensory overstimulation

The transport situation as a whole involves many stimuli, which can be unfamiliar to the horse, such as novel environments, noises and odours.

Noises: Horses can be startled by auditory inputs that are inaudible to people. During handling, noises arise from sources such as human voices, whips, animal vocalisations (e.g. barking dogs), noisy machinery, alarm bells/klaxon and compressed air brakes. Horses may react adversely during all transport stages before the surrounding humans perceive the source of the disturbance.

— PRE: Shouting or any disturbing auditory input from equipment or other animals (i.e. dogs barking) should be avoided during all transport stages.

Visual stimuli (i.e. handler position, moving objects): 'Horses have wide-angle vision and they can see nearly 360° around themselves. A flight zone or safety zone is the space around an animal within which the animal feels safe. If the animal turns away from an approaching handler, it means that the handler has entered the flight zone and the animal is trying to re-establish a comfortable distance. The size of the flight zone depends on the tameness of the animal: when a horse becomes more fearful, its flight zone will increase. The centre of the flight zone circle ("point of balance") is usually at the animal's shoulder. All species of livestock will move forward if the handler stands behind the point of balance. They will back up if the handler stands in front of the point of balance. An approximation of the flight zone can be made by approaching the animal and noting at what distance the animal moves away. Animals have a widening blind spot located right behind them and a narrowing one in front (Figure 3). If a handler positions himself in the blind spot, animals can get nervous as they cannot see what is happening' by Consortium of the Animal Transport Guides Project (2017a).

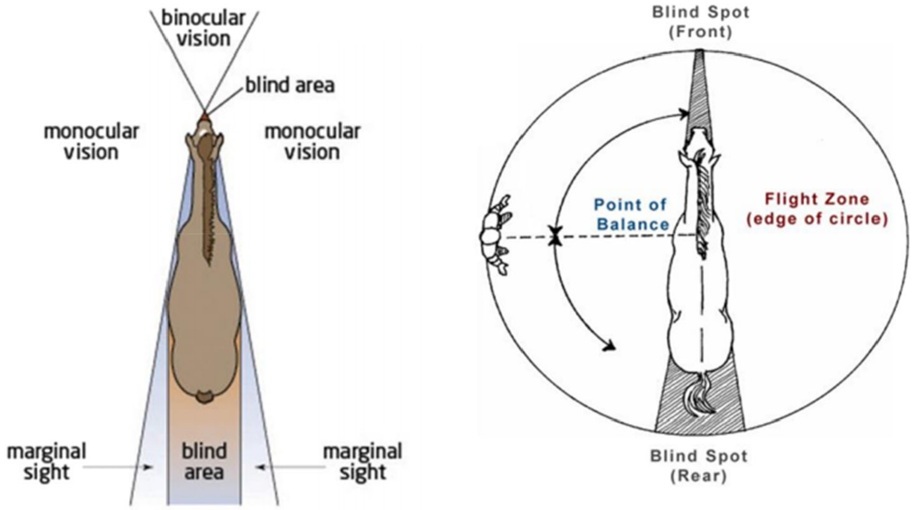


Figure 3: Horse vision and flight zone (Consortium of the Animal Transport Guides Project (2017a))

Horses are very sensitive to movements around them. They can instantly change their focus from near to far objects. Therefore, horses cock their head in different ways to detect how far objects are. This also explains why most horses are flightier on windy days; things that are normally stationary are now moving and likely perceived as potential threats. Excess of movement around them can lead to sensory overstimulation.

— PRE: Excessive visual stimuli should be avoided in the handling areas. Handlers should be appropriately trained to know flight zones and how to approach horses. When horses are moved from a darker to a lighter area, they should be given time to accommodate their vision, and light contrasts and shadows should be avoided or limited as much as possible. Lighting of the inner part of the animal compartment in the vehicle should be provided.

Unfamiliar olfactory stimuli: Horses have a well-developed sense of smell. The vomeronasal organ is receptive to non-volatile and poorly volatile molecules. Familiar input can calm a horse, unfamiliar or aversive smells, e.g. smell of a predator, can lead to a flight response. Horses may show vigilance behaviour when exposed to an unknown odour e.g. a disinfectant. Exposure to a combination of sensory stressors causes significantly increased heart rate responses and a tendency to a longer latency to a calm situation (Christensen and Rundgren, 2008).

— PRE: The presence of unfamiliar olfactory stimuli should be avoided in handling areas.

Unfamiliar sensory experiences: Horses encounter many environmental stimuli for the first time during the transport process, starting in the preparation phase but continuing in the subsequent phases. Habituation to these during the preparation phase can reduce the fearfulness of subsequent experiences.

— PRE: Habituation training can help in reducing this WC (see Section 3.3.1).

Corrective/mitigating measure for sensory overstimulation

If a horse is showing signs of sensory overstimulation, the area should be checked to see if any sensory stressors are present, such as frightening auditory stimuli, unfamiliar/aversive odours or visual disturbances, and these should be removed.

3.3.4. Fitness for transport

3.3.4.1. Introduction

Throughout the scientific literature, it is agreed that – in terms of animal welfare – making sure that animals are fit for transport before departure is of utmost importance (Grandin, 2001;

Cockram, 2019). However, currently no agreed scientific definition of the concept of fitness for transport exists (as discussed by Herskin et al. (2021)).

Ensuring that horses are fit before transport is highly important for the welfare of the horses during the loading, transit, unloading and post journey stages. In addition, a physically fit horse is more capable of handling stress than an unfit horse (Vermeulen, 2019). Transport associated exacerbation of infectious diseases (either through contact with unfamiliar pathogens and/or immunological impairment due to transport-associated stressors) is common in horses (Dalin et al., 1993; Stull and Rodiek, 2002).

3.3.4.2. Conditions rendering horses unfit for transport

Across animal species one concern for animal fitness for transport is pregnancy (Velarde et al., 2021). The concern for animal welfare in relation to transport of pregnant females is twofold and includes the pregnant female as well as the fetus/newborn:

Concerns for the welfare of the pregnant female relates to:

i) The stress and WCs associated with the different transport stages when carrying a fetus;
ii) The risk of going into labour or giving birth during transport; and
iii) The risk of abortion and health consequences thereof,

Concerns for the welfare of the fetus/newborn relates to:

i) Prenatal stress associated with having been transported if the pregnant female is not slaughtered prior to giving birth;
ii) The risk of being born during transport.

Across livestock species, the biology of the species in question is likely to influence the fitness for transport of pregnant females. The biology of horses, giving birth to one precocial foal means that the investment – in terms of energy resources and weight – of pregnant mares differ from species, such as rabbits, where the young are born in an altricial state (as reviewed by Nowak et al. (2000)).

A mare is pregnant for ~ 11 months (Peugnet et al., 2016), and pregnancy does not in itself make a mare unfit for transport. In industry guidelines, it may be recommended not to move mares in the early period of pregnancy (first month), but Baucus et al. (1990b) did not detect signs of early embryonic death in pregnant mares transported for 10 or 12 h, even if a significant increase of cortisol was detected.

In a very small study, involving only four control animals, Nagel et al. (2020) found that transport (of 3 h) increased the risk of premature onset of parturition in late-pregnant mares. The experiment was started when mares were well prepared but not yet ready for foaling (last month of pregnancy). Relative time of foaling was calculated as time from precolostrum pH decrease to 6.5 to onset of parturition. Transport not only elicited increased cortisol concentration but also significantly advanced the time of foaling (42 ± 9 h in transported and 116 ± 28 h in control mares. From Table 18, it is clear that guidelines, including the existing Council Regulation (EC) No 1/2005[1], specify that pregnant mares should not be transported beyond 90% of pregnancy. The results from Nagel et al. (2020) support this threshold. However, as illustrated from Figure 4, the development of the fetal growth curve of foals, and thus, the weight carried by the pregnant mare, is increasing during approximately the last half of pregnancy.

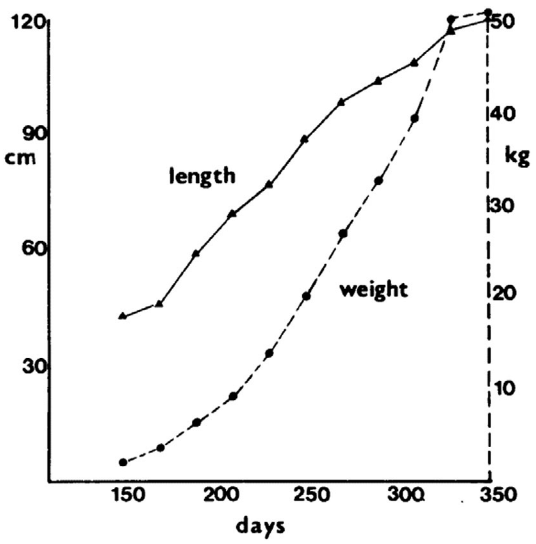


Figure 4: Mean fetal growth curve in thoroughbred from day 150 of gestation to term. (Source: Platt, 1978)

Fetuses may be exposed to prenatal stress, that has the potential to affect them later in life (Braastad, 1998), and across the studied animal species, gestational periods especially sensitive to prenatal stress have been identified. The available literature on prenatal stress in horses is limited (e.g. Peugnet et al., 2016) but, as in other mammalian species, prenatal stress can have implications for offspring welfare and performance.

EFSA AHAW Panel (2017) concluded that livestock fetuses in the last third of gestation have the anatomical and neurophysiological structures required to experience negative affect. However, regarding welfare of fetuses during transport, EFSA AHAW Panel (2017) concluded, with 66–99% likelihood, that the neurophysiological situation of the livestock fetuses throughout pregnancy (e.g. inhibitory and excitatory systems) does not allow for perception of pain or another negative affect as long as the fetus is *in utero*. If this is correct, it means that welfare concerns for the fetus during transport are most likely of minor relevance. However, if this is incorrect, the fetuses might experience negative affective states while in utero. If this lesser possibility should not be precluded, pregnant females should not be transported within the last trimester.

3.3.4.3. Assessment of fitness for transport in horses

Research in Australia (Padalino et al., 2016a) showed that professionals (i.e. people who gain an economic profit from horse related activities) assess the health and fitness of the horses more frequently prior to and following transport than amateurs (e.g. people who keep horses for hobby). Professionals also more often consulted veterinarians when in doubt of whether a horse was fit for transport or not. The same study showed that visual examination was the most common method (90%) for assessing fitness, followed by monitoring of feeding and drinking behaviour pre- (58%) and post-loading (74%). Rectal temperature, body weight and heart rate were determined by only 30%, 20% and 15% of respondents (respectively) both pre- and post-loading, with higher frequencies for professionals and veterinarians than amateurs. The same research showed that body condition score (not as such an ABM for any of the highly relevant WC listed in Section 3.2.2, but potentially useful in the assessment of fitness for transport), lameness and rectal temperature were rarely considered. Research showed that an elevated risk of transport-related muscular problems was observed when pretransport health checks were lacking (Padalino et al., 2016b). Similar results have been found in New Zealand, United Kingdom and Italy (Padalino et al., 2018b, Riley et al., 2022, Hall et al., 2020, Dai et al., 2021).

Conditions affecting fitness for transport are listed in several regulations (Government of Canada, 2022; Council Regulation (EC) No 1/2005[1], US Transport Quality Assurance (2021), Transport within New Zealand 2016), and there are several guidance documents and decision trees available to assist in the assessment of the fitness of horses (AAWSG (Australian Animal Welfare Standards and Guidelines), 2012, Equine Canada, (2018), WOAH, 2021).

Table 17 provides an extensive list of examples of conditions that would make a horse unfit for transport. However, the full list has not been scientifically validated. In addition, such a list of conditions is not the complete answer to issues related to fitness. There are difficulties in readily identifying the conditions and in making a judgment on whether the severity of each condition is sufficient to make a horse unfit in relation to the intended journey.

Table 17: List of conditions that can make a horse unfit for transport

General condition	Specific condition		References
Sickness/illness	Not specified further		WOAH, ECA, UST
	Pathological processes		CR2005
	Laboured breathing (rapid, shallow)		ECA
	Generalised nervous system disorder		ECA
	Shock or dying		ECA
	Fever		ECA
Pathophysiological state	Weakness		ECA, CR2005, WOAH, UST
	Emaciation/ Severely emaciated		AAWSG, ECA
	Fatigue/exhaustion		ECA, WOAH, UST
	Visible dehydration		AAWSG, ECA
	Distress		AAWSG, TNZ
	Body condition would result in poor welfare because of the expected climatic conditions		WOAH
Injury	Not specified further		AAWSG, UST, CR2005, WOAH
	Open or infected wound		CR2005, ECA
	Disabled		WOAH
	Recent major surgery		ECA
	Severe head injury		ECA
	Profuse bleeding		ECA
	Penis injuries		ECA
Prolapse	No further specified		CR2005
	Uterine, vaginal or rectal prolapse		ECA
In pain	Cannot be moved/transported without causing additional suffering		AAWSG, WOAH
	Present signs of unreasonable pain (e.g. Unresponsive to surroundings or repeatedly looking at abdomen, rolling or kicking at abdomen)		TNZ
	Fracture		ECA
	Until 3 weeks after a painful procedure (e.g. castration)		TNZ
Lameness	Unable to bear weight on each leg		AAWSG, ECA, WOAH, TNZ, UST
	Unable to move independently without pain or to walk unassisted		CR2005, AAWSG, UST
Non-ambulatory			ECA, CR2005, WOAH
Eye lesion	Blind in both eyes		WOAH, AAWSG, UST, ECA
Reproductive state	**Pregnancy**	Final 10% of gestation period (corresponding to 33 days)	WOAH, CR2005, TNZ
		Heavily gestating, likely to give birth (i.e. Wax-like beads or milk drops present, or relaxation hindquarters and tail muscles)	UST, ECA, TNZ
		Within 2 weeks of giving birth	AAWSG
	Recent given birth without a foal	Given birth within the previous 48 h	ECA, WOAH
		Given birth within previous week	AAWSG, CR2005

General condition	Specific condition	References
Newborn	Unhealed navel (e.g. red and swollen, moist or with fluid)	CR2005, WOAH
	Less than 6 months	UST
	Less than 4 months	TNZ

Wording used in source material has where appropriate been modified for consistency and clarity

- AAWSG: Australian Animal Welfare Standards and Guidelines (2012).
- ECA: Equine Canada (2013).
- CR2005: European Council (2005).
- TNZ: Transport within New Zealand (2016).
- UST: US transport Quality assurance.
- WOAH: World Organisation for Animal Health (2011).

In Europe, Practical Guidelines to Assess Fitness for Transport of Equidae were developed by a group of stakeholders (World Horse Welfare et al., 2015) to identify the conditions that should be considered to render horses unfit for transport. A summary of those detailed guidelines is presented in Table 18.

Table 18: Adapted table from Fitness Guidelines (World Horse Welfare et al., 2015) on conditions that render horses unfit for transport or require further investigation

CONDITIONS	ABMs to declare horses UNFIT FOR TRANSPORT	FURTHER ASSESSMENT REQUIRED
Difficulty standing, moving, maintaining balance or lameness, deformity	Stumbling, staggering or falling. Animal unable to: Stand or remain standing; maintain balance; move without difficulty; bear any weight on one of its legs. Severe lameness. Reluctance to stand or move.	Mild lameness. Weight shifting. Abnormal posture.
Wounds	Exposed body cavity, muscle, deeper tissue or bone. Large reopened wound (including surgical wounds). Infected open wound. Difficulty moving. Pain	Multiple wounds. A wound that may reopen. Transport likely to aggravate the wound.
Bleeding	Profuse and/or continuous bleeding. Blood squirting out under pressure.	Nose bleed or other bleed that has stopped
Prolapse	Red or pink mass protruding from the vulva or anus. Bleeding from vulva or anus.	
Late pregnancy or recently foaled	Mares beyond 90% (300 days) of gestation period, or foaled in the previous week. Wax-like beads or droplets of milk on tips of teats.	Enlarged abdomen. Full or enlarged udder.
New-born foal	Navel not healed: moist; fluid dripping from end; redness and swelling.	
Pain	Repeated rolling; kicking or looking at abdomen. Unresponsive to surroundings. Inability to stand or difficulty standing. Horse grimace scale = 2	Restlessness. Weight shifting. Profuse sweating. Facial tension. Horse grimace scale = 2
Dehydration	Unresponsive to surroundings.	Drinking excessively or for extended periods. Aggression when water is present. Dark, thick or strong-smelling urine. Abnormal faeces.
Exhaustion	Unresponsive to surroundings. Inability to stand or move. Collapse.	Lethargy, dull demeanour. Leaning or resting head. Reluctance to move or stand.
Body Condition Score (BCS) (animals may be at risk of other health problems)	Poor/emaciated: Ribs, hips, backbone prominent. Skin stretched tightly over bones. Horses with a BCS $\leq$ 1 and a BCS=5 are unfit for transport	Very fat: Fat deposits on top of neck. Ribs and pelvis buried. Back broad and flat. Deep gutter along spine.

CONDITIONS	ABMs to declare horses UNFIT FOR TRANSPORT	FURTHER ASSESSMENT REQUIRED
Infectious disease	High rectal temperature (above 38.5°C/101.3°F). Fitting, paralysis or collapse.	Swellings, lumps or abscesses. Repeated coughing. Discharge from any orifice. Sensitivity to light, touch or noise. Abnormal faeces.
Colic	Repeated rolling, lying down, looking at abdomen. Pain.	Restlessness. Groaning. Profuse sweating. Abnormal posture or head and neck position.
Swelling, inflammation or abscess	Significant swelling, heat and/or redness. Pain. Ruptured abscess. Inability to move. Lameness.	Abscess that has not ruptured. Mild lameness. Reluctance to move.
Hernia	Signs of pain, poor general health or colic.	Large hernia. Evidence of lesions or infection. Transport likely to aggravate condition.
Visually impaired	Total blindness.	Discharge from eye/s. Abnormal eye/s. Material present on the eye.
Dangerous behaviour	Behaviour that presents a risk, such as rearing, bucking, kicking, biting or striking out	If in any doubt about an animal's behaviour
Unbroken Equidae	Journeys over eight hours for animals that cannot be led by a halter/head collar without causing avoidable excitement, pain or suffering. BUT score can be used to differentiate these animals (Menchetti et al., 2021).	If in any doubt about whether an animal is broken/handled or not.

3.4. Loading/unloading

3.4.1. Current Practice

For the purpose of this Scientific Opinion, loading starts when the first horse to be transported is moved from the pen or stable at the place of departure (a racetrack, assembly centre or horse farm) into the means of transport and ends when the last horse is loaded and up the ramp is closing. A handled horse is led towards the transport vehicle, which is either a truck or a trailer. The horse then walks up a loading ramp, or in the case of some trailers, steps up into the trailer itself (Figure 5).

Figure 5: Horse led by the handler into a horse truck (Photographer: Barbara Padalino, UNIBO)

Unhandled horses cannot be led, so they are self-loaded usually one after the other. In contrast, horses that have been handled will usually be loaded by a handler, one by one.

Unloading starts when the ramp is open, and the first animal exits the means of transport, and ends when the last animal exits. The horses are released from the single or group compartments within the transport vehicle. The handled horses will be led, one by one, down the ramp to the place of destination. The unhandled horses will be coaxed from the vehicle, self-unloaded one after the other (Figure 6) and driven in a group to a holding facility of some sort.

Figure 6: Unhandled horse during self-unloading (Source: Barbara Padalino, UNIBO)

3.4.2. Highly relevant welfare consequences

Loading is a fear-inducing stage in horse transport (Waran, 1993; Tateo et al., 2012). Indeed, loading was reported as one of the most stressful and frustrating phases of transport by participants in surveys on horse transport issues and practices (e.g. Riley et al., 2018; Hall et al., 2020; Dai et al., 2021). Even horses very habituated to being loaded and transported often show an increase in heart rate and cortisol after loading (Tateo et al., 2012). During loading, fear results from factors such as apprehension of entering an enclosed space, unfamiliar noises, the height of the step leading onto the ramp and the instability and incline of the ramp (Houpt and Lieb, 1993). These factors, and maybe previous negative experience, can result in inexperienced horses, and some experienced horses, exhibiting evasive behaviour and a strong reluctance to step onto the ramp. The energy expended in climbing the ramp as well as fear contribute to the elevation in heart rate (Siniscalchi et al., 2014,b). Fear of loading is difficult to avoid, but some environmental stimuli may exacerbate it. For example, loading a horse directly from a brightly lit arena into a dark trailer (Cross et al., 2008).

As a fear response, many horses show signs of aversion and avoidance behaviours during loading (Dai et al., 2019a), thus increasing stress and risking injury to the horse and handler. Rearing, pulling back, head-tossing, pawing and turning sideways may be shown by some horses. Showing avoidance towards the pressure applied from the handler, as well as pawing and kicking, may also occur (York et al., 2017). These behaviours can be negatively reinforced when loading is aborted by the handler. The incidence of transport-related horse injuries has been most extensively studied for horses moved by road using commercial companies and varies from 1.6% to 33% depending upon the population studied (Roy et al., 2015a; Miranda-de la Lama et al., 2020). In Australia, injuries associated with commercial and non-commercial equine transport averaged 22% annually over 2 years (Padalino et al., 2016a). In another Australian study of non-commercial horse transport at equestrian events, 25% of the owners reported to have experienced a transport-related injury within a 15-year period (Riley et al., 2016). Such injury often occurs during loading/unloading. Swedish horse owners reported a 12% frequency of horse injury during loading, and 5% of these handlers were injured concurrently with the horse (Yngvesson et al., 2016).

The highly relevant WCs identified for the loading/unloading stage were handling stress, heat stress, injuries, restriction of movement and sensory overstimulation. The selected ABMs for the assessment of these welfare consequences are shown in Section 3.2.2. Below, hazards (in bold), preventive (PRE), corrective and mitigating measures are described.

i) Handling stress

The hazards leading to handling stress depend on the handler, the method and equipment used for handling, the level of tameness of the horse, the previous training for loading and transport, the facilities (i.e. type of vehicle, ramp) and the management during the loading/unloading.

Horses without basic training: Unhandled horses are not habituated to wearing halters and/or to being led, nor understand the cues or signals (i.e. pressure) applied by their handlers, and may not have a relationship with humans at all. Consequently, when unhandled horses are being loaded and unloaded, they can be distressed and display the fight or flight response. In such a situation, they may try to avoid and escape the situation, and when this is not possible, they may start to show aggressive behaviour including fighting one another or kicking and biting handlers.

- PRE: A recently developed behavioural test called the BUT-test can be applied before loading animals to recognise whether a horse shows signs of being able to be tied or led by a halter without causing avoidable excitement, pain or suffering or not (Menchetti et al., 2021). For unhandled horses transported in a group, it is recommended to keep the same group, and let the horses be loaded in a group without halters and/or ropes. If possible, load the horse with a buddy.

Lack of training for loading and transport: Horses not trained to be loaded, transported and/or unloaded will experience considerably higher stress levels in comparison with previously trained horses.

- PRE: It is crucial to train horses for loading and transport to reduce the occurrence of transport related problems (York et al., 2017). Positive reinforcement training (target training), self-loading and/or habituation (Dai et al., 2019) all require time to train the horse before the real shipping. Therefore, horses should be trained and habituated to loading, being on the transport vehicle and the unloading process before any journey is undertaken and inexperienced horses benefit from being handled very calmly and allowed more time to adjust to the surroundings (as recommended by Consortium of the Animal Transport Guides Project (2017a)).

Inappropriate handling: Inexperienced and/or untrained handlers, or handlers using coercive equipment (e.g. whip) have been identified as a risk factor for transport-related behavioural problems and subsequent injuries in both horse and handler (York et al., 2017). The use of whips, bum rope and other driving instruments has been identified as a risk factor for transport-related problem behaviour and injuries (York et al., 2017). The use of positive punishment, where a stimulus is added to suppress an unwanted behaviour (i.e. pushing, whipping, slapping) (McGreevy and McLean, 2009), should be avoided because it can lead to distress, confusion and consequently to the fight or flight response (Henshall et al., 2022).

- PRE: Horses should be handled properly by trained, knowledgeable and experienced handlers with sufficient time (Padalino and Riley, 2020) to ensure a calm and correct loading of the horses. Horses should be handled avoiding positive punishment, applying whips or other external aids. Handlers should give the horse enough time to adjust and move in or out the vehicle and avoid moving in the blind spot of the horse (Rørvang et al., 2020). Appropriate handling of horses should be based on learning theory and handlers should have a good knowledge of horse behaviour and the horses' perception of the environment. Shouting and high-pitched noise from equipment should be minimised when working with horses (McGreevy et al., 2014).

Poor loading facilities: This hazard includes steep external and internal ramps, lack of ramp gates, slippery floor and different lights between inside and outside the vehicle, as these may increase the level of fear in horses and a fearful horse may become more difficult to handle, thereby increasing the frequency of avoidance and aggressive behaviour.

- PRE: Handling facilities should be adequate for loading/unloading horses, complying with the inclination of ramp requirements, non-slippery floors and switch on an artificial low light inside the vehicle.

Time pressure: Rushed loading or unloading, not giving horses enough time for adjustment/exploration can induce fear and agitation leading to a higher risk of slipping, falling and injuries.

- PRE: Fast movements and time pressure should be minimised when working with horses (McGreevy et al., 2014). Horses should be given enough time to explore. When the first horse loads, the other will typically follow.

Isolation: Although horses are normally loaded in individual stalls separated by partitions, they can be stressed when loaded onto a vehicle alone or with unfamiliar horses.

- PRE: If the horse shows signs of stress, let a buddy horse join the loading. If the horse does not want to load by itself, another horse could be loaded first and be used as demonstrator (McGreevy, 2012).

Corrective/mitigating measure of handling stress

Abandon the loading of animals showing strong aggressive and/or avoidance behaviours. Train or retrain them with appropriate training methods for loading and transport before a future journey (Cregier, 2010; Slater and Dymond, 2011). Call a handler with greater experience, handling skills and knowledge of the appropriate training principles (ISES, 2018). Use a demonstrator, a calm, well experienced horse to calm down the frightened horse (McLean and McLean, 2008).

ii) Heat stress

Numerous hazards can induce heat stress during loading/unloading of horses including:

High effective temperature: The higher the environmental temperature (taking into account dry heat and humidity levels), the higher the risk of heat stress.

- PRE: In order to prevent the risk of heat stress, loading/unloading should take place in places and/or times of the day when it is not too hot, and the vehicle should be parked in the shade. See Section 3.5.3.1 for recommendations on microclimatic conditions in transport vehicles.

Solar radiation: Direct exposure to sunlight causes the interior of a transport vehicle to heat up.

- PRE: Provide shade during loading/unloading.

Insufficient ventilation: Insufficient air changes within the vehicle while horses are being loaded can lead to an increase in humidity, thereby leading to an increase in effective temperature experienced by the horses.

- PRE: Place the vehicle in an area where ventilation is ensured or leave mechanical ventilation on.

Physical exercise before loading, i.e. horses loaded soon after a competition/race.

- PRE: Avoid exercising the horse immediately prior to a journey, or wait for the horse to cool down until normal body temperature is reached (can be verified by measuring rectal temperature).

Lack of access to water: Heat stress will be exacerbated by dehydration (Brownlow et al., 2016).

- PRE: Water should be freely accessible until the moment of loading.

Prolonged loading and unloading: When effective temperatures are high, horses waiting in the vehicle for loading or unloading to be completed are at a greater risk of heat stress.

- PRE: A journey should be well planned to avoid any delays after animals are loaded. Recommendations for the microclimatic conditions inside the vehicle is presented in

Section 3.5.3.1. Depending on the ambient conditions, this can be done by a combination of mechanical ventilation, air conditioning, parking in the shade and maintaining all ventilation openings open.

Corrective/mitigating measures for heat stress

Any horse showing signs of heat stress should be removed from the transport vehicle if already loaded, placed in the shade, given a cold shower, water to drink and a veterinarian should check the health status of the animal (Brownlow et al., 2016). After the loading process has started any animal showing signs of heat stress should not be loaded. Any horse showing signs of heat stress must be allowed to completely recover before reloading.

iii) Injuries

There are several hazards that can lead to somatic lesions in the horses during the loading/ unloading:

Level of training of the horse: In the equine industry, the level and method of training horses for transport are significantly associated with the risk of injury at loading and unloading (as reviewed by York et al., 2017).

- PRE: As previously discussed (see Section 3.3.1), training horses to be loaded is essential to prevent TRPBs and subsequent injuries (as reviewed by York et al., 2017).

Transport related behavioural problems: A horse with TRPB has higher risk of experiencing accidents as a result of uncontrolled behaviour during loading/unloading. Some breeds (thoroughbred/ thoroughbred crosses) have been identified as a risk factor of transport-related injuries (Riley et al., 2022).

- PRE: A horse showing TRPB should not be loaded and should be retrained using appropriate methods (as reviewed by York et al., 2017).

Unsuitable vehicles and facilities: The transport vehicle is the second most frequent source of traumatic injuries to horses, with injuries in the paddock or yard the most common (Darth, 2014). Limb injuries associated with the loading ramp are common (Mansmann and Woodie, 1995). These can result from projections, obstacles or auditory stimuli frightening the horses.

- PRE: Ensure that the loading/unloading is performed in a calm location, and the facilities are appropriate and well maintained without broken or protruding structures. Check that partitions in the vehicle do not have gaps with the floor. After loading, make sure the partitions are well closed to avoid escape behaviour. Use padding inside the partitions and the other vehicle structures.

Inappropriate handling: Many human factors are associated with an increased risk of injury to an equine during transport. Padalino et al. (2018c) concluded that the experience and knowledge in horse handling of people responsible for moving horses by road was the most important human factor associated with transport-related injuries in equines. The use of whips and other aids (bum rope, ropes etc.) were identified as factors increasing the occurrence of transport-related behavioural problems and injuries during loading and unloading (Padalino et al., 2017d; Padalino et al., 2018b; Hall et al. 2020; Dai et al., 2021).

- PRE: Horses should be loaded by experienced and knowledgeable people. Inappropriate handling instruments should not be used.

Sedatives or tranquilizers: Administration of sedation before loading has been identified as a risk factor, increasing the likelihood of a horse experiencing injuries during loading/unloading (Padalino et al., 2016b) as it may impair the ability of the horse to walk and balance.

- PRE: The use of sedation and other medication before transport should be minimised and only used after consulting veterinary advice.

Corrective/mitigating measure of injuries

Remove the horse from the loading procedure. Place in a suitable facility and provide appropriate veterinary treatment.

iv) Restriction of movement

The hazards leading to restriction of movement during loading and unloading are listed below:

Poor loading facilities: The ramp on which the horse gains access to the transport vehicle is a stressor to many horses, particularly if it is steep, or unstable, or makes noises when the horse steps onto it. The stress associated with the ramp at loading might lead the horse to freeze and/or refuse to load. Stress during unloading may result in some horses charging out of the vehicle on arrival at the destination.

- PRE: Ensure that the loading facility is appropriate, the ramp is not too steep (< 20 degrees Celsius) and fitted with a system such as provided by foot battens, to facilitate foothold. The ramp should have side barriers, should be stable and not make noises. The ramp can be covered with straw, sand or sawdust to prevent slipping and to reduce noise.

Presence of inappropriate tools: Bandages, hobbles and protective equipment can restrict horse movement and make walking uncomfortable (Cregier and Gimenez, 2015). Among other inappropriate tools are leading horses with a short rope or any other aid which can induce pain and extra restraint.

- PRE: Before loading, all wearables (such as bandages, hobbles, blinkers) and other tools that can restrict the movement of the horse, should be removed.

Inappropriate handling/unexperienced handler: see Section 3.3.3i [handling stress during preparation].

<u>Corrective/mitigating measures for restriction of movement</u>

If horses show signs of movement restriction, move to a better facility or upgrade the existing loading facilities (e.g. ramp, side panels). Let the horse calm down and restart proper loading, using positive reinforcement (Hendriksen et al., 2011). Maybe try with a different, more experienced, handler, take off any inappropriate wearable materials and stop using tools that may induce fear and pain.

v) Sensory overstimulation

Horses get easily agitated in unfamiliar situations and various sensory stimuli during loading and unloading can lead to sensory overstimulation. The hazards have been discussed in more detail in Section 3.3.3.

Auditory stimuli: (see Section 3.3.3).

- PRE: Shouting or loud auditory stimulation from equipment or other animals (i.e. dogs barking) should be avoided during the loading and unloading.

Visual stimuli (i.e. handler position, moving objects): (see Section 3.3.3).

- PRE: Excessive visual stimuli should be avoided in the loading/unloading areas. Handlers should be appropriately trained in the flight zones and how to approach horses. Light contrasts and shadows should be avoided or limited as much as possible. Lighting of the inner part of the animal compartment in the vehicle, and the loading/unloading facilities, should be provided. According to the Consortium of the Animal Transport Guides Project (2017a), adequate light in this area will ease the entry of the horses into the vehicle. Ensure that handling area and inside of vehicle have similar light conditions. Avoid bright sunlight or strong artificial light in the loading area.

Unfamiliar olfactory stimuli: (see Section 3.3.3).

- PRE: Olfactory stimuli that are unfamiliar to the horses should be avoided in the loading/ unloading areas.

<u>Corrective/mitigating measure of sensory overstimulation</u>

If a horse is reluctant to load/unload and shows signs of sensory overstimulation, the area should be checked to see if any sensory stressors are present, such as frightening auditory stimuli, unfamiliar/ aversive olfactory stimuli or visual disturbances, and remove them if so.

3.5. Transit stage

3.5.1. Current practice

In this Scientific Opinion, the transit stage starts when the ramp has been closed and ends when the first animal unloads. There is a diverse range of vehicles used to transport horses, from small trailers to specialised trucks (see for example Figure 7). In Europe, trailers can be for a single horse but far more commonplace are double trailers where usually the two stalls are side by side with the horses forward facing. There are also some trailers where the horses are transported laterally to the direction of movement of the vehicle. Trucks vary in size from small two-horse trucks to larger ones capable of carrying 8 or 10 horses, or more. In Europe, mostly the stalls within these are configured in a way so that the horses are transported forward facing. In Ireland and the UK, however, horses are mostly transported perpendicular to the direction of the vehicle movement.

Figure 7: Commercial truck used for horses. The vehicle is passively ventilated. Source: Barbara Padalino, UNIBO

3.5.2. Highly relevant welfare consequences

Welfare of horses during the transit stage depends on several factors such as journey duration, space allowance, ambient conditions (temperature, humidity, vibrations and noise), vehicle design and driving conditions.

The welfare consequences selected as highly relevant during the transit stage are: gastro-enteric disorders, heat stress, injuries, motion stress, prolonged hunger, prolonged thirst, respiratory disorders, resting problems, restriction of movement and sensory overstimulation. The selected ABMs for the assessment of these WCs are shown in Section 3.2.2. Below, hazards (in bold), preventive (PRE), corrective and mitigating measures are described.

i) Gastro-enteric disorders

Epidemiological studies have suggested that transport can be associated with the development of colic in horses, and may be associated with colitis, gastrointestinal impaction, displacement or obstruction (Archer and Proudman, 2006; Hillyer et al., 2002; Stewart et al., 1995) and gastro-enteric disorders are frequently cited by horse owners as an adverse consequence of transport (Padalino et al., 2016a,b). Altered intestinal motility has been suggested as contributing to colic (Blikslager, 2019), and might be related to movements in mucosal transport of water and nutrients, or to changes in the gastrointestinal microbiome (Perry et al., 2018; Schoster et al., 2016).

Time off feed and water: Journeys of 12 h without feed and water can lead to development of stomach ulcers (Padalino et al., 2020) and also predispose for colic.

— PRE: To avoid the hazard from time off feed, palatable feed should be freely accessible during the transit stage. This can be done by positioning hay on the floor or below wither height to motivate horses to lower their head and assume a natural feeding posture.

Unfamiliar feed: If unfamiliar feed is provided to horses, and especially with a high proportion of concentrate, digestive problems or colic may develop (Sadet-Bourgeteau et al., 2017).

— PRE: During the transit stage, horses should be given the type of feed that they are accustomed to. Too much concentrated feed should be avoided. Feed should also be given together with access to water (Freeman, 2021).

Pre-existing condition/fitness for transport: Horses may develop enterocolitis due to *Salmonella* spp. if they are already carrying the pathogens which, due to transport stress, may increase in proportion within the gut flora and lead to clinical and often fatal salmonellosis (McClintock and Begg, 1990).

— PRE: Fitness for transport should be checked properly. Especially when the planned journey is long, it may be advantageous that a veterinarian checks the horse (Padalino et al., 2015).

Corrective/mitigating measures for gastro-enteric disorders

If gastro-enteric disorders are suspected in transit, the contingency plan should step into action, it is recommended to call a veterinarian, and that the horses are treated according to prognosis.

ii) Heat stress

The main hazards for heat stress during the transit stage are listed below. More detailed information on the quantitative assessment of the microclimatic conditions inside the transport vehicle can be found in Section 3.5.3.1.

Poor microclimatic conditions inside the vehicle: Temperature, humidity and ventilation inside the vehicle are critical aspects of any journey. Links have been reported between the level of ventilation and the occurrence of heat stress and shipping fever (Yousuke and Masaaki, 2019). Purswell et al. (2006) reported that horse trailers are under-ventilated at all vehicle speeds from 13 to 90 km/h.

— PRE: As described in Section 3.5.3.1, horses are recommended to be transported within their thermal comfort zone. In addition, avoid parking the vehicle under direct sun, and open the ventilation slats to a maximum during stationary periods. Keep the vehicle engine running to allow forced ventilation or air conditioning to operate.

Physical exercise required to keep balance: The muscular effort required to keep balance generates heat, which can lead to an increase in the body temperature. Consequently, if the horses do not have enough space to spread their legs facilitating balancing, the drivers have poor driving skills or the route is full of minor roads and cornering, the risk of heat stroke is increased (as discussed by Padalino and Riley (2022a) in a recent textbook chapter).

— PRE: Sufficient space as described in detail in Section 3.5.3.2 should be provided to facilitate maintenance of balance during the transit stage, correct positioning inside the vehicle and smooth driving should be ensured.

Limited space allowance/high animal density: A high animal density increases the effective temperature inside the vehicle, increases the metabolic heat load and limits evaporation by reducing air movement around the body of the animals in there.

— PRE: Ensure animals have at least the minimum space allowance as detailed in Section 3.5.3.2.

Time off water: When temperatures are high, dehydration is an important hazard for heat stroke in horses, dehydrated horses are at a higher risk of experiencing heat stroke (Marlin, 2008).

— PRE: Based on the results of an online survey conducted in Australia with 797 responses, offering water and hay *ad libitum* until loading was found to be associated with a lower likelihood of reporting cases of heat stroke (Padalino et al., 2016b). When horses were watered and fed with the possibility to lower their head during a journey of 22 h, they did not show signs of heat stress (Padalino, unpublished data). Out of more than 300 horses transported to a slaughterhouse following current Council Regulation (EC) No 1/2005[1] (watering every 8 h) only horses transported outside the thermoneutral zone were showing signs of sweating at unloading (Messori et al., 2016).

Corrective/mitigating measures for heat stress

Horses showing signs of heat stroke in transit should be unloaded as soon as possible, and placed in the shade, given water and a cold shower and a veterinarian should be called (Brownlow et al., 2016).

iii) Injuries

There are several hazards that can lead to somatic lesions in the horses during the transit stage.

Lack of space: Horses need to balance in transit and to balance properly they need space. See detailed examination of the need for space during horse transport in Section 3.5.3.2.

- PRE: Horses should be transported with the space allowance suggested in Section 3.5.3.2.

Lack of basic training: When unhandled horses are transported, tethering and isolation in a small space increases the risk of distress. Therefore, during escape attempts, they may get injured.

- PRE: The BUT test can be applied before loading animals to recognise whether a horse shows signs of being able to be tied or led by a halter without causing avoidable excitement, pain or suffering (called 'broken' in EC 1/2005) or not (Menchetti et al., 2021; Dalla Costa et al., 2021). Horses showing signs of not being able to do so, should be transported loose in a small group of familiar conspecifics.

Level of horse training for transport: In the equine industry, the level and method of training horses for transport can be associated with the risk of injury during the journey.

- PRE: As previously discussed for handling stress during preparation of horses for transport (Section 3.3.3), habituation to transport (Houpt, 1982; Houpt and Lieb, 1993) is essential to prevent TRPBs and subsequent injury.

Transport-related behavioural problems: A horse with TRPB has higher risk experiencing accidents as a result of uncontrolled behaviour during the transit stage.

- PRE: As mentioned in Section 3.3.3i, a horse showing TRPB should not be loaded and should be retrained before initiation of a new journey using appropriate methods (Riley et al., 2022).

Lack of bedding/antislippery floor: If urine is not properly absorbed by bedding, and the floor is not covered by antislip mats, the floor may become slippery during the transit stage, thereby increasing the risk of loss of balance and consequent injuries (Hall et al., 2020).

- PRE: Ensure a suitable floor surface is maintained, and add fresh bedding if the floor is slippery.

Unsuitable vehicles and facilities: The transport vehicle is the second most frequent source of traumatic injuries to horses, with injuries in the paddock or yard the most common (Darth, 2014). Trailers are less stable than trucks, and they have been identified as a risk factor for transport-related injuries in transit (Hall et al., 2020). Vehicle characteristics, such as the presence of chest bar, stallion guard, improper partitions and sharp and pointed objects inside the vehicle have been associated with a higher risk of transport-related injuries in transit (Cregier and Gimenez, 2015).

- PRE: Ensure that the vehicle has passed an inspection check before loading the animals, and that the vehicle is compliant with the current legislation and well maintained without broken or protruding structures. Check that partitions in the vehicle are correct for the load. After loading, make sure the partitions are well closed to avoid escape behaviour. Use padding inside the partitions and the other vehicle structure.

Driving skills and knowledge in recognising stress: Many human factors are associated with an increased risk of injury to horses during transport. Padalino et al. (2018c) concluded that the experience and knowledge in recognising signs of stress by the people responsible for moving horses by road and the driving skills (i.e. type of driving licence) are important factors associated with transport-related injuries in horses. Driving skills are crucial for balancing in horses and other animals (Cockram et al., 2004; Colborne et al., 2021).

- PRE: Horses should be transported by people with specialised horse driving experience and knowledge. People moving horses should be educated at recognising stress in horses, and how to drive a vehicle transporting live animals. High speed, sharp cornering and abrupt braking should be avoided.

Use of sedatives or tranquilizers: Administration of sedation before loading was identified as a risk factor increasing the risk of horse injuries in transit based on an online Australian survey (Padalino et al., 2016b).

- PRE: Sedatives may impair the ability of a horse to balance in transit, consequently the use of sedation and other medication before transport should be minimised and administered only after veterinary advise.

Transported with unfamiliar conspecific: The presence of an unfamiliar horse has been reported as the cause of injuries to other horses in transit (Riley et al., 2022). Even when transported singly, if a horse is close to an unfamiliar horse, aggressive behaviour might be shown, and horses may kick against structures of the vehicle and get injured, specifically if not protected. While it can be difficult to identify potentially aggressive animals before transport, a major cause of injury is the fighting of unfamiliar horses transported loose to slaughterhouses over relatively long distances (Weeks et al., 2012).

- PRE: Dominant and aggressive horses must be separated, stallions must be transported in a single bay and away from other stallions, and horses in a single bay should not stand next to unfamiliar horses. Knowles et al. (2010) showed differences in the level of aggression between groups of four vs. eight semi-feral ponies. In their study, Knowles et al. (2010) also observed signs of separation anxiety when a pony was transported in a single stall. It is often recommended to let familiar horses (so-called buddies) be transported together (McGreevy, 2004). A larger group size, as is commonly seen in North and South America, may cause a higher injury rate (Roy et al., 2015a; Miranda-de la Lama et al., 2020).

Lack of fitness for transport: Fitness for transport has been identified as a risk factor for injuries during transport (Riley et al., 2022).

- PRE: Before loading is initiated, horses should be assessed for fitness for transport.

Type of road and vehicular motion: The type of road has been identified as a risk factor potentially affecting the level of vibrations and the ability of a horse to balance (Riley et al., 2022).

- PRE: Plan the journey to minimise the part on bad pavements and/or windy roads.

Positioning inside the vehicle: The best orientation for horses during a journey is still a matter of debate. The position inside the vehicle can affect the ability of the horse to balance (see Section 3.5.3.2 for details).

Corrective/mitigating measures of injuries

An emergency plan should be put in place when a horse gets injured in transit. The horse should be unloaded as soon as possible, placed in a suitable facility and appropriate veterinary treatment provided.

iv) Motion stress

There is no evidence that horses experience motion sickness.

During transport, animals are exposed to vertical, lateral and horizontal vibrations. Unpaved roads or roads with strong wind currents transmit a more significant amount of vibrations. Motion stress may lead to fatigue over time. The more tired and unsteady the animals become, the more likely they are to slip and fall, which can result in injury.

During transport, animals experience stress and/or fatigue due to the forces exerted as a result of acceleration, braking, stopping, cornering, gear changing, vibrations and uneven road surface. Vibration is the movement of a body about its reference position, and occurs because of an excitation force that causes motion. Vibration has been shown to alter animal behaviour and induce physiological changes as well as to cause effects at the cellular and molecular level. For these reasons, vibrations have a considerable potential to alter animal welfare status (Reynolds et al., 2019). Vibratory movement (Colborne et al., 2021) has a direction (generally in three planes) (Figure 8), a magnitude (how far) and a velocity (how quickly – what rate).

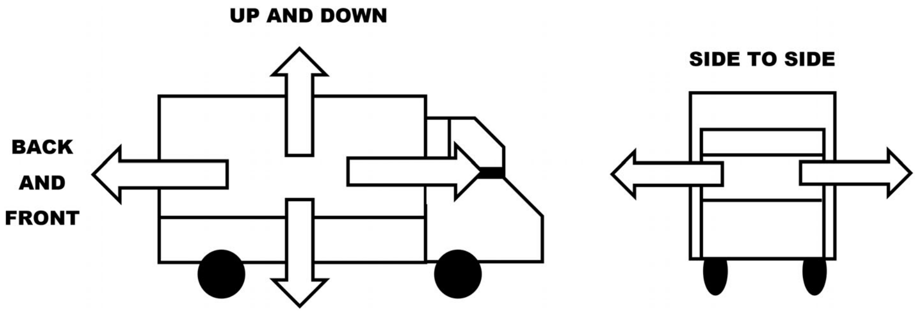


Figure 8: Schematic drawing showing the three planes of vibratory movements animals are exposed to during transport by road. The arrows show movements along the direction of the vehicle, whereas the horses can be facing in several directions during the transit stage. Adapted from Humane Slaughter Association (2022)

Motion stress is regarded as a highly relevant WC in the transit stage. Prevalence is high, as motion stress is likely to affect all animals in a moving vehicle. Duration depends on journey duration and onset of vehicle motion. Severity depends on the driving conditions, road condition, positioning of the horse inside the vehicle and vehicle design and will most likely increase over time as animals become fatigued. An extreme case of motion stress, with a low prevalence but a very high severity, is vehicle accidents. These are not covered specifically in this Scientific Opinion, but may have severe consequences in terms of animal welfare. See Section 3.5.3.3A for additional information on motion stress and how the WC evolves during journeys. The main hazards leading to this WC are:

Inadequate vehicle suspension: Excessive exposure to vibrations has been shown to result in motion stress, and increased skin damage scores due to slips and falls while the vehicle is moving. The truck suspension system can affect the welfare of horses in the form of increased frequency of balance-related behaviours, TRPBs and injuries (Dai et al., 2021).

- PRE: Good suspension in vehicles can to some extent prevent vibrations.

Rough driving: Rough driving, which comprises high acceleration and noticeable braking, or routes with minor roads and many turns, e.g. in mountain areas, may cause instability, toppling, sliding and excessive corrective muscular action, resulting in bruising, muscular fatigue, fear and general injuries to the animals (Driessen et al., 2022).

- PRE: Driving should be done carefully. When possible, better roads should be used to reduce the motion stress.

Space allowance: Horses have the need to spread their legs apart to keep balance while in transit, and providing limited space has been associated with motion stress and injuries (Cregier and Gimenez, 2015; Padalino and Raidal, 2020).

- PRE: Provide enough space to allow horses to perform balancing behaviour, as indicated in Section 3.5.3.2.

Orientation in the vehicle: See explanation of this hazard as well as the preventive measure in Section 3.5.2iii discussing the WC injuries.

Slippery floor/lack of bedding: Horses may struggle to balance if the floor is slippery or inadequate bedding is present (Riley et al., 2018).

- PRE: Vehicles should be equipped with antislip mats and adequate bedding before loading horses. When possible, during stationary periods, faeces and urine should be removed and new bedding added.

Rolling and pitching of truck: Whilst the vehicle is moving, all horses are to some extent exposed to motion stress. The stress is increased during acceleration events (including braking and accelerating) and turns. Rural roads are a mixture of paved, unpaved and surfaced roads. The latter two increase the transmission of vibration to the animals compared to larger roads and, when waterlogged, can cause the vehicle to lose stability and the animals can lose their balance.

PRE: Among the preventive measures for this hazard are planning the journeys on motorways, and driven by experienced and skilled drivers. In addition, vehicle vibration can be reduced through the choice of suspension system.

Corrective/mitigating measures for motion stress

If severe motion stress is observed in the animals, stop the vehicle, unload the horse in a safe place and let the horse calm down.

v) Prolonged hunger

If horses are not fed during the transit stage, the WC prolonged hunger is regarded as highly relevant. Depending on factors such as time off feed before the journey starts, horses may not be hungry during the initial phase of the journey, but hunger will develop over time if enough feed is not eaten or no feed is provided. The duration of the WC depends on journey duration and availability and accessibility of feed while transported, and severity is expected to increase with increasing duration, as the need for feed becomes increasingly problematic for the animals. Prolonged hunger may lead to exhaustion and a weakened condition. See Section 3.5.3.3 for a more detailed examination of hunger during the transit stage. The main hazards, preventive and corrective/mitigating measures are:

Time off feed: Long feed deprivation can result in hunger, over time leading to weight loss and impaired bodily functions. Lack of knowledge of the operator is the main origin of this hazard, when horses are left without access to feed (on-farm and/or during transport) for too long. It is being discussed whether horses should be fasted before transport, but this may increase the stomach pH and risk of equine gastric ulcer syndrome.

— PRE: Feed should be provided to horses for *ad libitum* intake before loading and then at regular intervals, as discussed in Section 3.5.3.3G. It is better, though, if hay is available during the transit stage. Hay should be damped in water and positioned low (ideally on the floor) to minimise the risk of respiratory disorders (Allano et al., 2016; Marlin, 2004).

Reduced intake of feed: Horses tend to reduce intake of feed during the transit stage, as they are less willing to eat in unfamiliar and stressful surroundings and from unfamiliar sources (Kay and Hall, 2009). When horses are transported in adequate conditions and are familiar with transport, they will eat (about 5 kg of hay and 1 kg concentrate during a 12-h journey) during transit (Padalino, 2022 (unpublished data); Bannai et al., 2021; Anon, 2014). Unlike most other transported livestock species, equines can be fed forage without taking them off the vehicle (WHW, 2022).

PRE: This hazard cannot be fully prevented during the transit stage, not even by giving access to feed. The only preventive measure, then, is to provide free access to feed before loading, and to limit journey duration, so that the WC prolonged hunger will not develop. If journey breaks are involved, horses could be fed during these.

Corrective/mitigating measure for prolonged hunger

The most effective measure to correct hunger is to offer feed after stopping the vehicle.

vi) Prolonged thirst

The WC prolonged thirst is regarded as highly relevant in the transit stage, if horses do not have access to water. Prevalence may be high, if water is not provided to the animals or if they for some reason (e.g. due to lack of familiarity with drinking devices or neophobia) are not able to obtain enough water. Depending on factors such as time off water before journey start and/or microclimatic conditions, horses may not be thirsty during the initial phase of the transit stage, but thirst will develop over time, if they are not able to drink as much as they need. The duration of thirst depends on time off water, and severity is expected to increase with increasing journey duration and heat, as the need for water becomes more problematic for the animals. Prolonged thirst may lead to dehydration, discomfort and suffering. Additional information on this WC is included in Section 3.5.3.3G. The main hazards, preventive and corrective/mitigating measures are:

Time off water: The interval until initiation of the WC, prolonged thirst, depends on time off water.

— PRE: Horses should be well-watered before the start of the journey, if not, they already start with a deficit. In order to prevent the WC prolonged thirst, horses should have access to water at regular intervals during the journey, e.g. every 8 h (Friend, 2000). Enough drinking stops should be planned in advance, as well as places where water supplies can be replenished.

Reduced intake of water: Horses tend to reduce intake of water during the journey, because they are less willing to drink in unfamiliar and stressful surroundings and from unfamiliar sources (Kay and Hall, 2009). While some horses are reluctant to drink during transport, horses that are familiar with transport, and transported in adequate conditions have been reported to drink in transit (Padalino (unpublished data); Bannai et al., 2021; Anon, 2014). Stull and Rodiek (2000) reported that horses consumed 23 $\pm$ 6 L of water during a 24-h journey; when watered five times at regular intervals with familiar water (i.e. water from a familiar water source, as different taste may affect the drinking behaviour (Houpt, 2018)), and the majority of the water was drunk after the first 12 h. Gibbs and Friend (2000) found that a group of four to six horses transported to slaughter could be watered on the vehicle when provided with troughs on both sides of the pen and having 0.4 meter of trough per horse. Thus, unlike some other transported livestock species, horses can be watered without taking them off the vehicle (WHW, 2022).

- PRE: To mitigate prolonged thirst, use a vehicle with suitable drinking devices and take along enough water in the water tank (45 litres/horse per 24 h). Fill up the tank with clean, fresh water prior to each stage of transport (as recommended by the Consortium of the Animal Transport Guides Project (2017a)).

Inadequate/not working/unclean drinking devices: Dehydration during the transit stage may occur if the drinking devices are not designed and/or positioned in an appropriate way to allow use, or if horses are inexperienced with the use of the drinking devices, or if the driver has turned the water off.

- PRE: Ensure the drinking devices work properly and that horses know how to use them. Otherwise, stop and water the animal at regular intervals, in order for them to nor develop the WC prolonged thirst (Iacono et al. 2007a; Anon, 2014).

Too high effective temperature: During hot weather, horses will sweat and need to consume more water. Water deprivation can result in dehydration that can lead to difficulties in coping with other hazards such as high temperatures.

- PRE: Ensure horses are transported in their thermal comfort zone (see Section 3.5.3.1). In hot weather take measures to reduce heat stress.

Diseases or specific physiological states: Certain diseases can lead to higher risk of dehydration, e.g. if one of the symptoms is diarrhoea. Horses in certain physiological states will also require more water, e.g. lactating mares with a foal at hoof.

- PRE: Specific diseases should be detected at the fitness for transport assessment (Section 3.3.4). For other known conditions increasing water requirement, it is recommended to offer water for *ad libitum* intake during the journey.

Consumption of certain feedstuffs: Feed with a high salt content or very dry feed will require the horse to consume more water and increase the risk of dehydration.

- PRE: Take on board familiar feed, which is not too dry and not with a high salt content.

Corrective/mitigating measures for prolonged thirst

If a horse shows signs of moderate or severe dehydration, the horse should be treated, preferably by a veterinarian. Oral electrolytes and water can be given via a nasogastric tube to mild or moderately dehydrated horses, or intravenously if dehydration is severe. Feed and fresh water should be offered to the horses at arrival. Monitoring the hydration status by capillary refill time (see Section 3.2.2) and weight loss (using a weight tape or scales) is recommended following transport to enable appropriate rehydration strategies to be implemented where required (Padalino et al., 2016a).

vii) Respiratory disorders

Transport pneumonia, also called shipping fever or pleuropneumonia, is commonly associated with long distance transport of horses, and is an area that has been studied in some detail previously (Leadon et al., 1989; Oikawa et al., 1994; Austin et al., 1995; Raidal 1995; Raidal et al., 1996; Raidal et al. 1997; Oikawa et al., 2004; Oikawa et al., 2005; Copas, 2011). In an online survey conducted in

Australia Padalino et al. (2016b) reported that 797 respondents, reported an incidence of transport pneumonia of 9.2% which was similar to a Japanese study including journeys of 1,000–1,300 km (Oikawa and Jones, 2000).

The hazards which have been associated with respiratory disorders during the transit stage are:

Inability to lower the head and neck below the height of withers: In a study involving 12 horses, transported as two consignments of six horses, for 8 h in individual stalls measuring 0.8 × 2.3 m and sideways positioned, the time spent with the head in a low position (below wither height) was recorded during 25 min per hour of the journey. Horses did not have access to feed or water. During the journey the horses spent less than 300 s/25 min (20%) with their head lowered, whereas this behaviour was observed for more than 600 sec/25 min (40%) during a stationary break in the journey and for ~ 1,200 s/25 min (80%) when the horses were observed in the home environment. Significant negative correlations were found between the time spent with the head lowered and parameters measured after the journey such as tracheal wash bacterial count ($\log_{10}$ CFU/mL), the tracheal mucus score and inflammation score (Padalino et al., 2018a). In non-transport conditions, earlier results showed that horses that spend more than 12 h confined in a way where they cannot lower their head have higher risk to develop pneumonia (Raidal et al., 1995).

- PRE: Ideally, do not tie horses during transport. If they are tied, the length of the rope should allow horses to lower their heads below wither height in order to balance and clear their airways.

Low space allowance: Horses transported at low space allowance are limited in their ability to lower their head. Moreover, if they cannot spread their legs and are agitated, they tend to stand in an alert position (with the head up).

- PRE: Horses should be allowed to lower their head to floor level during transport and have enough space to spread their legs apart for balancing.

Journey duration: Journey duration has been identified as the major risk factor for all transport-related health problems, including respiratory problems. Journeys conducted in single stalls with short tethered horses, lasting more than 20 h increased the likelihood of the horses developing respiratory disorders upon arrival (Padalino et al., 2015).

- PRE: Respect the recommendation on journey duration provided in the quantitative Section 3.5.3.3.

Dehydration: Dehydration status can range from mild to severe after transport and is largely dependent on the transport conditions (e.g. microclimatic conditions) and journey duration. Dehydration can also cascade into more serious metabolic conditions and predisposes animals to pneumonia.

- PRE: Provide water for *ad libitum* intake or in regular rest stops in enough quantity (see Section 3.5.3.3).

Airway disease already present before transport (horses not fit for transport): In a study involving 12 horses, transported for 8 h in two consignments, associations were found between having airways subclinically inflamed before transport, and the tracheal mucus score upon arrival (Padalino et al., 2018a).

- PRE: Fitness for transport must always be correctly assessed. In principle, before long journeys, respiratory endoscopy is recommended to avoid transporting animals with subclinical respiratory diseases. The prejourney use of immunostimulants seems to be beneficial in reducing shipping fever after transport (Nestved, 1996; Akai et al., 2008; Endo et al., 2017).

High effective temperature: Exposure to high effective temperature is a hazard for dehydration, and may, thus, indirectly, also favour respiratory disorders.

- PRE: Keep microclimatic conditions inside the vehicle within the thermal comfort zone of horses (Section 3.5.3.1).

Mixing horses from different sources: Horses coming from different farms may have subclinical diseases and be vectors of some pathogen. Thus, the risk of spreading pathogens among the horses is higher when they are mixed (Muscat et al., 2018).

- PRE: Avoid mixing animals from different farms in the same vehicle.

Corrective/mitigating measures for respiratory disorders

It is important to have an emergency plan, and call a veterinarian as soon as possible, if horses show signs of respiratory disorders, as early diagnosis is essential. Shipping fever, if left untreated, can lead to severe pneumonia which can be life-threatening.

viii) Resting problems

At some stage during transport, horses get fatigued, because they have to continuously adapt to the movement of the vehicle. Horses usually rest in a standing posture on three legs, but this will be difficult when they have to balance the whole time. Tripod standing posture has been reported only in horses transported facing backwards in relation to the direction of driving, or loose in a large box stall (Padalino et al., 2012; Padalino (unpublished data)). REM-sleep has only been documented in horses in sternocostal or lateral decubitus (when lying sternally or laterally), but horses will lie down only in a familiar environment and in a quiet situation (Fullagar et al., 2015). In most vehicles, horses do not have enough space or are tied too short or do not have good bedding for foothold, so they cannot properly rest. In contrast to other species, they almost never lie down in transit. Lateral decubitus of a horse in transit has, however, been reported by Padalino (unpublished data) in horses transported loose in a large box stall (6 m^2).

The hazards that can lead to resting problems during journey are the following:

Rough driving: See Section 3.5.2iv.

- PRE: Avoid rough driving. Try to take highways and drive carefully (maintain a constant speed if possible and decelerate slowly).

Inappropriate vehicle design, insufficient bedding material: The use of inappropriate vehicles, too small stalls or lack of suitable bedding material can all prevent the horses from standing in a tripod posture. If partitions are wrongly positioned, too close to the horse, horses also have more problems keeping balance due to lack of space, and thus cannot rest standing (see Section 3.5.3.2)

- PRE: Ensure the truck has suitable design and bedding. Wood shavings and several kinds of chopped straw (dust free, and not likely to cause intestinal obstruction when eaten) are suitable bedding materials. The Consortium of the Animal Transport Guides Project (2017a) recommended a layer thickness of 1 cm per 100 km for wood shavings, and that bedding is cleaned or refreshed at least every 24 h. Reduce stocking density/increase space allowance so all horses can stand comfortably with legs spread apart and balance properly.

Short tethering: When horses are tied too short, they cannot lower their head, which is essential to assume the standing resting posture.

- PRE: Ideally, do not tie horses. If they are tied, the length of the rope should allow horses to lower their heads sufficiently in order to balance, and thus assume the standing resting posture.

Corrective/mitigating measures for resting problems

If severe resting problems are observed during the journey, the vehicle should stop and horses be unloaded to give the opportunity to rest.

ix) Restriction of movement

Horses are transported in single stalls, which are usually very limited in space. This can cause them to bump against the sides, or bump their head, which can cause injuries and pain. When allowed too little space, horses also have difficulties in performing the movements required for balancing. The main hazards leading to this WC during transport are listed below.

Tied too short: If horses are tied too short, it restricts their capacity for movement.

- PRE: Ideally, do not tie horses. If they are tied, they should be tied in single and the rope length should allow horses to perform the movements they are motivated to do (to balance and clear their airways).

Insufficient space allowance: More details on the space required by the horses during the transit stage can be found in Section 3.5.3.2.

Corrective/mitigating measures for restriction of movement

If severe restriction of movement is observed during the transit stage, the vehicle should stop and horses be unloaded to give them the opportunity to move.

x) Sensory overstimulation

The hazards that increase the risk of sensory overstimulation are listed below:

Sound/noise: During journeys loud auditory input can originate mainly from traffic, sirens or from rough roads inducing vibration to the structure of the vehicle. Sounds of more than 80–85 dB are deleterious to horse welfare, and induce an increase in heart rate which has been interpreted as fear (AlZubi et al., 2016).

- PRE: If horses are not habituated to being transported, it can help to drive at quiet hours when traffic is low or take roads with not too much traffic but still easy to drive on. Chains and all possible noisy materials inside the vehicle should be avoided.

Fumes and gases: Improper design of the vehicle, or of the compartments within it, leads to the risk of introduction of exhaust fumes into the animal compartments. In addition, improper ventilation leads to overexposure of noxious gas produced by the animals and their excreta, such as NH_3 followed by clear signs such as watering eyes, nasal discharge, coughing, retching and ocular/vision disorders (Leadon, 2015).

- PRE: Use a correctly designed vehicle with a ventilation system that avoids introduction of exhaust fumes into the space destined to the animals.

Lighting: Sudden change in lighting (high contrast with bright and shaded areas) during the transit stage can have a negative effect on horse welfare and cause fear (Timney and Macuda, 2001).

- PRE: Low levels of, and consistent, lighting should be provided throughout the transit stage.

Corrective/mitigating measures for sensory overstimulation

If severely stressed horses are observed during the transit stage, the vehicle should stop and horses be unloaded to recover in a quiet place. Deficiencies in vehicle function should be repaired.

3.5.3. Quantitative examination of thresholds to protect horse welfare during the journey stage

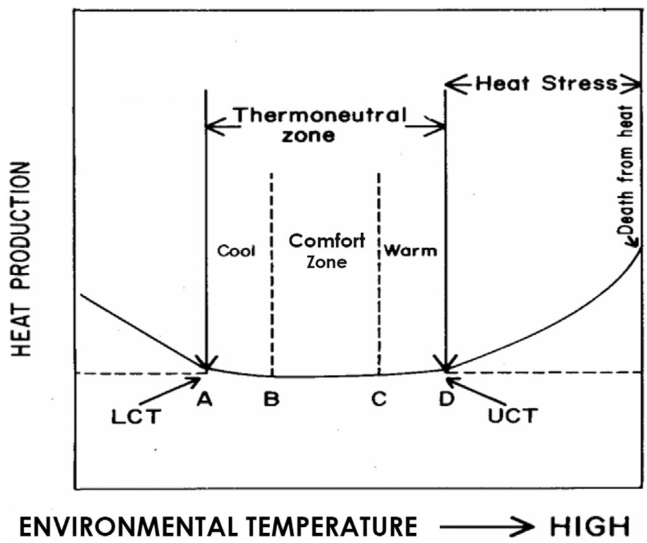
3.5.3.1. Thresholds for microclimatic conditions

A) Introduction

From an evolutionary perspective, horses have been successful at adapting to different environments and, with intervention from man, have colonised diverse environments. Thus, some breeds cope better in hot or cold conditions, and horses can adapt to new ambient conditions within ~ 2 weeks (Cymbaluk and Christison, 1990). As mammalian homeotherms, horses regulate and maintain their core body temperature through physiological and behavioural processes (Holcomb et al., 2013; Mrowka and Reuter, 2016). Mechanisms used by horses to dissipate heat and avoid an increase in core body temperature, include evaporation, conduction, radiation and convection (Hodgson, 2014). Evaporation is the primary process used for heat dissipation, and sweating has an important role in horses. During high temperatures, surplus heat is transferred to the skin by vasodilation increasing blood flow to skin capillaries, followed by transfer to the external environment (Kohn and Hinchcliff, 1995).

B) Background

Thermoregulation is the physiological process allowing the balance between heat production and heat loss mechanisms. The approach taken in this Scientific Opinion to recommend microclimatic conditions during horse transport is based on the thermoregulatory concepts and model as described by EFSA (2004) (Figure 9), and originally formulated by Mount (1974). The figure covers a range of environmental temperatures from cold to very hot. Since cold stress was not chosen as a highly relevant welfare consequence for horses, this assessment of thresholds for microclimatic conditions focusses on temperatures higher than B as indicated on Figure 9.



LCT/A: Lower critical temperature (LCT), UCT/D: Upper critical temperature; B: Lower limit of thermal comfort zone; C: Upper limit of thermal comfort zone.

Figure 9: Schematic representation of thermal zones as a function of the environmental temperature (Adapted from EFSA, 2004)

The following concepts from the figures need to be introduced:

Thermoneutral zone (TNZ): As reviewed by Bracke et al. (2020), the TNZ covers the range of environmental temperatures within which metabolic rate and heat production are constant and independent of the ambient temperature. The zone is limited by the lower critical temperature (LCT) (marked as A in Figure 9) and the upper critical temperature (UCT) (marked as D in Figure 9). Many factors influence the TNZ of an individual animal including the size, body condition score, breed, level of nutrition, agitation level and environmental factors such as air velocity around the animal, but also motor activity (e.g. maintaining balance during transport) (Bracke et al., 2020).

Thermal Comfort zone (TCZ): According to Silanikove (2000) subdividing the TNZ into a zone of thermal well-being is the most suitable way to describe the relation between an animal and its environment from the viewpoint of animal welfare. Based on studies in humans (e.g. Schlader et al., 2011), Kingma et al. (2014) described the TCZ as defined in terms of perception, qualifying as the state of mind that expresses satisfaction with the thermal environment. Translated into animal welfare, Silanikove described the TCZ (denoted comfort zone in Figure 9) as the environmental temperature interval, where the energetic and physiological efforts of thermoregulation are minimal, and the animal is in the preferred or chosen thermal environment. In the figure above, the upper limit of the TCZ is marked by C, where an animal will activate evaporative physiological thermoregulation processes, mainly sweating in horses, and may start to display thermoregulatory behaviour. The TCZ is sometimes called the safe zone as it is referred in EFSA Scientific Opinion on the welfare of animals in containers during transport (EFSA AHAW Panel, 2022b).

Upper Critical Temperature (UCT): As outlined in EFSA (2004), there are several definitions of UCT. UCT describes the point above which an animal must significantly increase the use of physiological mechanisms to prevent a rise in body temperature above normal. For example, evaporative heat loss and metabolic rate increase (Silanikove, 2000). In the TNZ, evaporation is per definition kept to a minimum, whereas increased evaporative heat loss through the skin and/or respiratory tract occurs when the organism is challenged with higher ambient temperatures. At high ambient temperatures, heat transfer by conductive, convective and radiant changes are less effective, because of the reduction of the required minimal thermal gradient between skin and air temperature (Renaudeau et al., 2012).

According to the definition of WCs (EFSA AHAW Panel, 2022a), the term 'heat stress' is defined as: '*A situation where an animal experiences stress and/or negative affective state(s) such as discomfort and/or distress when exposed to a high effective temperature*'. This definition differs to some extent from other proposed definitions of heat stress, e.g. focusing on lack of ability to cope or on performance loss.

The scientific literature underpinning the model shown in Figure 9 is based on studies involving a certain level of feed intake under stable or resting conditions. As reviewed by Bracke et al. (2020), care should, thus, be taken when extrapolating findings obtained from experiments in conventional barns to transport conditions. During transport, horses are often exposed to factors that may act as stressors and/or limit their possibility to thermoregulate, as they would have done in non-transported control conditions. In contrast to the conditions provided to horses under basic thermoregulatory studies, transport may include deprivation of feed and water, includes exposure to vibration and motion forces causing muscle work which is comparable with light exercise (Codazza et al., 1974; Giovagnoli et al., 2002), low space allowances and highly variable ventilation rates**.** Consequently, if a negative impact on animal welfare from the microclimatic conditions during journeys is to be fully prevented, animals should be transported in their TCZ. This means that the WC, heat stress, defined by the accompanying stress and/or negative affective states, may start when an animal is no longer in the TCZ, and the risk and severity of heat stress, is likely high when animals reach UCT. Once this point is reached, the rate of evaporative heat loss starts to increase exponentially, meaning that signs of heat stress increase steeply in an effort to stop the rise in core body temperature above normal.

The warm zone in Figure 9, also sometimes called the alert zone (EFSA AHAW Panel, 2022b), between temperatures C and D, is not as such included in the TCZ. However, even though heat stress cannot be fully excluded when animals are exposed to conditions as between C-D, the risk and the severity of heat stress is likely not high in this interval. This approach is based on the definition of the animal welfare consequence heat stress, addressing a situation where an animal experiences stress and/or negative affective states such as discomfort and/or distress.

C) Heat stress and welfare during transport of horses

As reviewed by Rashamol et al. (2019), not only the ambient temperature, but also other environmental conditions influence heat load placed on animals. Examples of these are: the relative humidity (RH), the solar radiation including long- and short-wave radiation, the heat and moisture generated by the animals, the heat loss from the vehicle, vertical height, placement of compartment partitions along the longitudinal axis of the vehicle, the vehicle type, the type of ventilation shutters and many more. These will all influence the microclimatic conditions experienced by horses and should, in theory, all be taken into account when microclimatic conditions of horses during transport are evaluated. However, due to the complexity of such tasks, as well as the strong evidence for the effect of humidity on heat stress, at least the combined effects of temperature and humidity should be taken into account when animal welfare during transport is evaluated.

The water vapour content of the air is important because it impacts the rate of evaporative heat loss through the skin and respiratory tract (Hodgson, 2014). When the ambient temperature is above the animal’s TCZ, a high level of humidity in the air will reduce evaporative heat loss and therefore result in increased risk of heat stress. In this case, the routes of conduction, convection and radiation for heat exchange are reduced, and the only remaining route of heat loss is through evaporative routes, which require a vapour pressure gradient and thus dictates that relative humidity is a major factor controlling rate of evaporative loss (Collier et al., 2019).

Generally, air water vapour content is assessed by relative humidity (RH), which is a measure of the percentage saturation of the air with water vapour at a specific temperature in relation to the maximum water vapour that the air could potentially contain at that temperature. However, RH is temperature dependent and thus the same RH at different temperatures may equate to very different water vapour contents. Therefore, although sensors recording temperature have been used in road transport of animals in the past, it would be a significant refinement to use improved sensors taking account of humidity effects.

In the context of animal transport, ventilation functions to replace the metabolic heat and moisture produced by the livestock in the vehicle with air of a certain temperature and humidity from outside the vehicle. Ventilation also serves to mix and redistribute internal air to attempt to make the internal thermal microenvironment more homogeneous. In addition, concentrations of different gases (O_2, CO_2, NH_3) can be modulated. The effect on individual animals depends on the rate of air change and the flow around the bodies of the animals. In this way, the temperature and humidity (and all other microclimatic conditions) in the vehicle can, in theory, be kept only slightly elevated compared to the level of those outside the vehicle, but only if ventilation is very efficient.

For all passively ventilated vehicles when the vehicle is stationary (for example during mandatory driver breaks) there is no driving force for ventilation other than buoyancy or free convection or external factors such as cross winds. The problems when stationary will be exacerbated in vehicles operating with restricted air inlets with minimal gaps for air to enter, exit and circulate within the load. Smaller vehicles are often under-ventilated, but fans can be applied on the roof and on the side of the vehicles to increase air ventilation (Purswell et al., 2006). Wind speed in passively ventilated horse vehicles can drop to close to zero when stationary (Padalino et al., 2018a).

Even vehicles fitted with fans to aid ventilation often have the airflow dictated by two principles. The stack-effect causes heated air to rise and colder air to descend and is the dominant means in stationary vehicles. When moving, the stack effect continues to operate, particularly in areas of the load with low ventilation but is overlaid by the air flows which operate around and within a vehicle in motion. Both of these drivers of air flow may be influenced by external factors such as wind.

In addition, the flow of air around the heads and bodies of each animal plays a significant thermoregulatory role facilitating the loss of body heat via convection and conduction. However, it appears that no studies have been carried out describing the airflow within a transport vehicle for horses.

There is a ventilation rate (by mechanical or passive ventilation) that, for a certain range of environmental temperatures, in theory, reduces the effective temperature within the transport vehicle to the same level as that outside the vehicle. This rate will depend on many factors such as the temperature and humidity of the air coming into the vehicle, the heat and moisture and heated generated by the animals, solar radiation and heat loss from the vehicle. This ventilation rate is unknown for horse transport, as far as we know, as the relevant studies have not been found.

D) Thermal heat indices

Several indices have been developed to predict stressful microclimatic conditions that take into consideration multiple weather-related factors and allow execution of abatement strategies. The majority of these have been based on ambient temperature and relative humidity. One, temperature-humidity index (THI) (as originally described by Thom (1959)), has been taken up by the livestock industry as a weather safety index to monitor and reduce heat-stress-related production losses. The fact that the use of THI thresholds is mainly focused on avoiding production losses, such as in-transit mortality, means that it is not necessarily aligned with animal welfare, as defined by affective states. THI thresholds have, however, never been developed for horses during transport.

Other methods have been developed for horses in a non-transport context, for example a heat index, the FEI Comfort Index (Marlin et al., 2018), that uses dry temperature and RH and is designed to provide guidance on whether or not it is safe to ride or exercise horses.

The Wet-bulb Globe Temperature (WBGT) is another method used for measuring effective temperature for horses (Schroter and Marlin, 1995). The WBGT takes account of the absolute temperature, humidity and radiation, and is calculated using the following equation: WBGT (°C) = (0.7 × Tw) + (0.2 × Tg) + (0.1 × T); where: T = dry air Temperature in °C; Tg = Globe Thermometer Temperature (in °C) (this is measured by a thermometer placed in a special black globe to estimate solar radiation) and; Tw = Wet-bulb Temperature (in °C). The Federation Equestre Internationale (FEI) uses the WBGT to regulate horse competitions; values > 28°C require precautions, e.g. misting fans, to reduce thermal stress, and values > 33°C are not compatible with competition (Jeffcott, 1996; Marlin et al., 2018).

The wet-bulb temperature is another form of heat measurement that has been used in transport of livestock by sea. This method takes account both heat and humidity. The wet-bulb temperature can be calculated using dry air temperature and RH using the following formula:

$$Twb = T \times \arctan[0.151977 \times (RH\% + 8.313659)^{(1/2)}] + \arctan(T + RH\%) - \arctan(RH\% - 1.676331) + 0.00391838 \times (RH\%)^{(3/2)} \times \arctan(0.023101 \times RH\%) - 4.686035$$

where T = dry bulb temperature (in °C) and RH is the relative humidity in percent. The arctan function is the inverse of the tangent function. The wet-bulb temperature is measured in °C.

No thermal indices have been developed and validated for use in horses during transport.

E) Identification of environmental conditions to protect horses from heat stress during transport

Increased heart rate, respiratory rate and sweating were described (Padalino et al., 2019) at temperatures above 25°C and, from a behavioural perspective, horses will also seek shade at 25°C (Hartmann et al., 2015) although Honstein and Monty (1977) did not see any such behaviour. Marlin (2008) suggested 25°C as the upper critical temperature in horses, and Cymbaluk and

Christison (1990), referring to Honstein and Monty (1977), said that the UCT was between 25°C and 30°C based on rates of sweating.

Honstein and Monty (1977) measured the physiological responses to variations in environmental heat at rest and in a group of hydrated horses acclimatised to the natural hot and dry conditions of Arizona in the United States. They found that rectal temperatures were higher during the summer when compared to the winter season but remained stable throughout the day. Even when afternoon temperatures during the summer peaked at almost 42°C, rectal temperature remained stable with only an average increase of 0.4°C when compared with early morning temperatures. Heart rate and respiratory rate did not change significantly with exposure to the higher ambient temperatures of summer. However, sweating rate increased markedly with higher ambient temperatures (Figure 10). The rate of sweating increased from 12 g of sweat/m^2 of skin surface at 6.5°C to a maximum of 267 g of sweat/m^2 at 39.7°C.

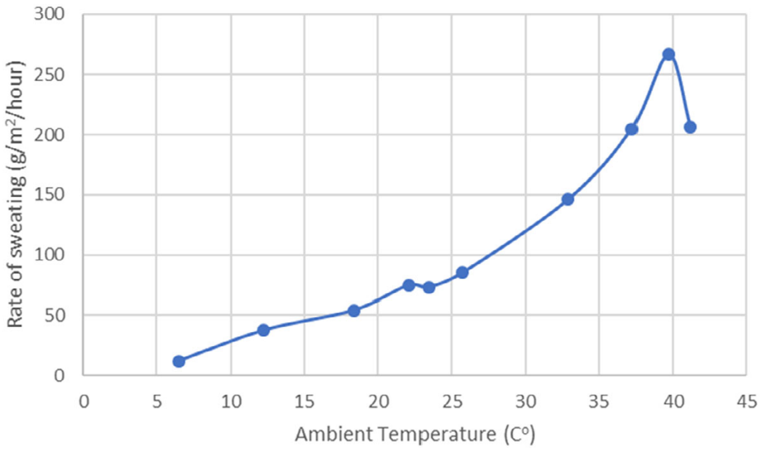


Figure 10: The average rate of change of sweating (g/m^2 per hour) for changes in ambient temperature (°C) in a group of hydrated horses acclimatised to the natural hot and dry conditions of Arizona in the United States (Graph using data from Honstein and Monty, 1977)

In a study by Morgan et al. (1997), five horses were acclimatised to an indoor temperature of 15–20°C and an outside temperature of –5°C to 5°C in a paddock. During the study, the horses were exposed to the following temperature levels, with RH in brackets: –3°C (50%); 6°C (55%); 15°C (55%); 20°C (45%); 30°C (40%) and 37°C (40%) as measured inside a climatic chamber. Each horse participated once at each temperature level, and the order was randomised. In each experiment, the horse was kept in the climatic chamber for one and a half hours. The study showed that the evaporative heat loss increases from the baseline at ~ 20°C (Figure 11) (Morgan et al., 1997). The study also showed that the rate of total heat loss increased at ~ 25°C (Figure 12).

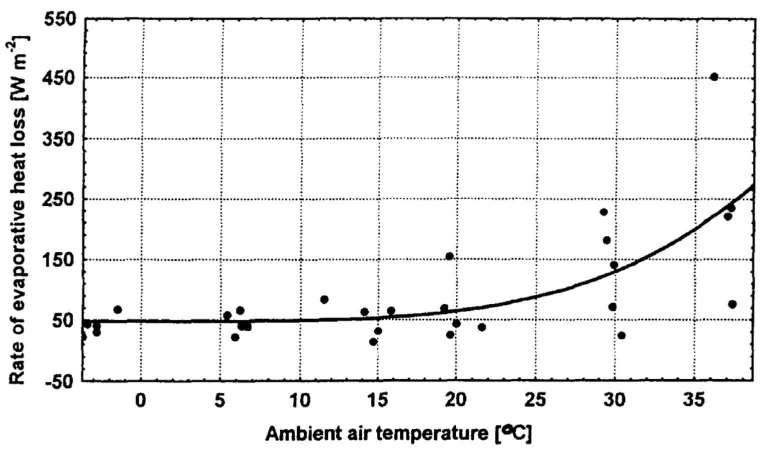
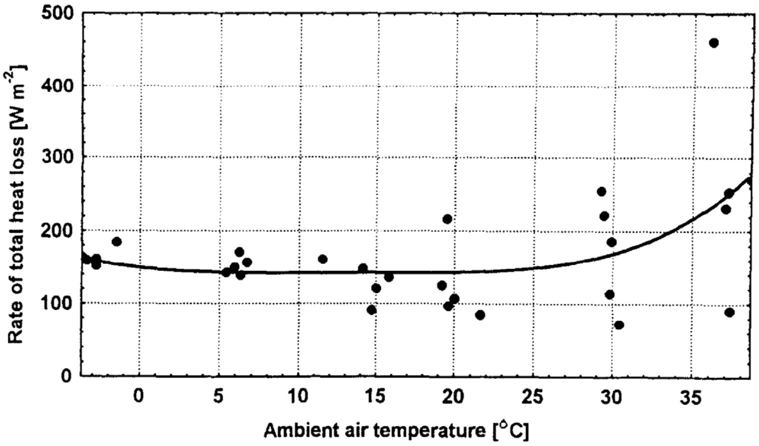


Figure 11: The rate of evaporative heat loss as a function of ambient temperature from five horses, acclimatised to an indoor temperature of 15–20°C and an outside temperature of -5°C to 5°C in a paddock). Source: Morgan et al. (1997)

The horses were exposed to the following temperature levels, with relative humidity in brackets: –3°C (50%); 6°C (55%); 15°C (55%); 20°C (45%); 30°C (40%) and 37°C (40%) as measured during a 1.5-h stay in a climatic chamber. The temperature inside the climate chamber is shown on the X-axis, and the rate of total heat loss (Wm^{-2})



The horses were exposed to the following temperature levels, with relative humidity in brackets: –3°C (50%); 6°C (55%); 15°C (55%); 20°C (45%); 30°C (40%) and 37°C (40%) as measured during a 1.5-h stay in a climatic chamber. The temperature inside the climate chamber is shown on the X-axis, and the rate of total heat loss (Wm^{-2})

Figure 12: The rate of total heat loss as a function of ambient temperature from five horses, acclimatised to an indoor temperature of 15–20°C and an outside temperature of –5°C to 5°C in a paddock. Source: Morgan et al. (1997)

Summary of microclimatic conditions: During transport, horses can be exposed to factors that may act as stressors and/or limit their possibility to thermoregulate, as they would have done in non-transported conditions. Examples of these are deprivation of feed and water, exposure to vibration and other motion forces, low space allowance and highly variable ventilation rates. Consequently, if a negative impact on animal welfare from the microclimatic conditions during journeys is to be fully prevented, horses should be transported in their thermal comfort zone. This means that, during transport of horses, the welfare consequence, heat stress, may start when they are no longer in their thermal comfort zone, and the risk and severity of heat stress is likely high when the thermal conditions reach the upper critical temperature.

Not only the temperature but also other environmental conditions such as relative humidity, thermal radiation, temperature of surrounding surfaces and wind speed play a role. These will all influence the microclimatic conditions experienced by horses and should, in theory, be taken into account when microclimatic conditions of horses during transport are evaluated.

As defined above, the upper limit of the thermal comfort zone (point C) is the environmental temperature at which an animal will activate evaporative physiological thermoregulation processes, mainly sweating in horses, and may start to display thermoregulatory behaviour. Based on the commencement of evaporative heat loss and sweating rates, the upper threshold of the thermal comfort zone (point C) is suggested to be ~ 20°C.

The upper critical temperature describes the temperature above which an animal must significantly increase the use of physiological mechanisms to prevent a rise in body temperature above normal i.e. any increase in metabolic rate or heat production or a significant increase in the rate of sweating. Based on the initiation of heat production above baseline levels and the increase in the rate of sweating, the upper critical temperature is estimated to be ~ 25°C (quantified at a relative humidity of 50%). This will vary somewhat depending on the individual horse, the breed and the degree of acclimatisation to heat.

The FEI index and WBGT-index have been used in relation to horse sports, but have not been validated in transport conditions. Whether they can be used under transport conditions is not known.

Air conditioning is currently used to some extent and potentially presents an opportunity to facilitate the transport of horses when the external environmental conditions are too extreme; however, research is required before its use can be recommended.

3.5.3.2. Threshold of space requirements during journeys

A) Current practice

Currently most horses must be transported in individual stalls (see Section 3.3.2). Generally, an individual stall in a trailer or small truck is 0.76–0.91 m in width, 1.8–2.1 m in length, and provides 1.40–1.95 m^2/horse (Stull, 1999). In a commercial transport vehicle with horses positioned sideways to the direction of the movement of the vehicle, the individual stalls are generally 0.70–0.80 m in width and 2.48 m in length (Padalino, unpublished data).

According to current legislation, unhandled horses are allowed to be transported only on journeys shorter than 8 h and must be kept loose in a group of maximum four horses, with a minimum of 1.75 m^2/horse Council Regulation (EC) No 1/2005[1]. In Canadian commercial transport vehicles transporting loose horses, the code of practice specifies a minimum space allowance of 1.2 m^2 per 500 kg horse (CARC, 2001). In Australia, a minimum allowance of 1.2 m^2/ adult horse is required, and 1.75 m^2/adult horse is required for long journeys in the EU (Reg 1/2005). However, many countries do not have regulations or operate under imprecise guidelines from World Organisation of Animal Health (WOAH, 2011) that do not address the behavioural needs of the horse (i.e. horses require sufficient space to adopt a balanced position appropriate to the climate) (Woods and Messori, 2014).

Mares with foals at foot are transported in the same stall but there are no specific conditions laid out in EC 1/2005 in terms of minimum space requirements.

In this Scientific Opinion, the space required for individual horses and horses transported in groups will be explored separately.

B) Introduction and methodology

Due to the limited research available, the multiple factors that can influence how horses respond to space during transport, the variability in types of horse and in journey conditions, it is considered preferable to provide minimum rather than target or recommended space allowances for different types of horses. The evidence of WCs when inadequate space is available, is stronger than that available for determining optimal conditions. The minimum space requirement will vary with the type of horse, what the space is needed for, for how long, and the vehicle and journey characteristics. It is, thus, a complex issue to provide a minimum recommended space allowance during transport that will be applicable within all situations. If the minimum recommended space allowance is set too low for a particular situation, it will likely increase the risk of adverse WCs.

In the assessment of minimum space requirements for horses during transport, the following approach will be used:

During transport, horses require a minimum space allowance that will accommodate (i) their physical size and allow them to ii) adjust their posture in response to acceleration and other events, (iii) lower and raise their heads (iv) eat and drink if feed and water are provided in the vehicle, (v) rest in a standing position, (vi) urinate and defaecate. Recommendations for a minimum space allowance will be set by the first limiting factor that reduces the ability of the horse to undertake one of these biologic functions i.e. whichever of the above requirements needs the most space.

The section also discusses compartment height (vertical space), as another dimension of space.

In addition to the six biological functions of space listed above, a seventh one, relevant for horses transported in single stall as well as in groups, is the ability to thermoregulate. The minimal space allowance that can protect the welfare of horses during transport, will be influenced by the environmental conditions, i.e. temperature and humidity inside the vehicle the effectiveness of the ventilation system (while the vehicle is in motion and is stationary) and by the ability of the animals to thermoregulate effectively. Decreasing space allowance can increase the number of horses in a given compartment or vehicle, and thus the amount of metabolic heat and moisture that they produce. Unless this extra metabolic heat and moisture can be effectively removed by ventilation, it can be detrimental at warmer temperatures and high humidity, and predispose the animals to WCs such as heat stress, involving discomfort and potentially leading to distress.

On hot and humid days, increasing space allowance reduces the risk of heat stress. Estimating the actual influence of the increase in space allowance on the microclimatic conditions inside vehicles transporting animals requires detailed modelling and precise data, not only on the heat and water vapour produced by the animals, but also on the heat loss from the vehicle, the ventilation and the dynamic nature of these interactions. No experimental studies on the effect of changes in space allowance on measures of heat stress in horses during transport have been found.

In cold conditions, especially horses kept in groups during transport require sufficient space to be able to move away from cold areas, such as air movement at ventilation inlets. Otherwise, they may experience the WC cold stress, involving discomfort and potentially distress.

Additional information on space allowance and microclimatic conditions (temperature, humidity and ventilation) during road journeys of horses can be found in Section 3.5.3.1.

It may be advantageous to provide additional space. However, in the horse literature, no research was identified in horses to provide specific quantitative information on appropriate space allowances in relation to thermal conditions.

C) Horizontal space for horses in a single stall

i) Space to accommodate physical size

For horses transported individually, the spatial dimensions of the stall holding the horse during transport are important for the animal welfare – in the vertical as well as the horizontal plane – and lack of space may lead to several WCs such as restriction of movement, injury, respiratory problems, resting problems and heat stress.

Horses come in a wide variety of sizes, as demonstrated by Brooks et al. (2010), and their shapes may vary even if they have the same bodyweight. The standard horse of 500 kg can be brachymorphous (e.g. draught horses are tall and wide, the width of the chest is about 60 cm), dolicomorphous (e.g. thoroughbreds are tall and narrow, the width of the chest is about 40–45 cm) or mesomorphous (e.g. quarter horses are more proportioned in their height and width, the width of their chest being about 42–50 cm) (Sly, 2018). It is also important to highlight that the widest point of the horse may be the width of the hips (i.e. distance from the left to the right point of hip) or the abdomen and not the width of the chest. For instance, the average width of the chest of Lipizzan horses, which are mesomorphous horses (height at the withers about 155 cm) was estimated to be about 45 cm while the width of the hips was about 52 cm (Zechner et al., 2001). Moreover, young horses tend to be narrower than adult ones, since they grow first in height and then in width. Recently, a new approach to take zoometric measurements of an animal's body based on digital three-dimensional modelling has been suggested to be useful (Pérez-Ruiz et al., 2020). Moreover, other specific factors can affect how much space some animals require. Heavily pregnant mares require more space than non-pregnant ones. Mares with foals need more space, since the foal should be able to lie down during the journey and suckle in transit.

Grandin (2019) suggested that an average size horse (height at withers 158 cm, weight 550 kg) should have ~ 90 cm in width and 2.4 m in length and space for height of standing. When participants were asked about space requirement during the European Transport Guide project, they suggested that a space of between 10 and 20 cm between the horse and the partitions are needed on both sides (Messori et al., 2016). Also, Westen et al. (2010), cited in EFSA AHAW Panel (2011), 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 should be a certain width wider than the horse and a certain length longer than the horse when standing in a natural posture.

ii) Space required to adjust posture in response to acceleration and other events

Horses transported in a single stalls in a horse trailer may show loss of balance and scrambling due to abrupt braking and cornering, and be agitated or fearful during the journey (Riley, 2016). These responses are influenced by the amount of space provided for the horse during the journey.

In their report of 2017, the Consortium of the Animal Transport Guides Project recommended 10–20 cm of space in front of, behind and on both sides of horses (Figure 13).

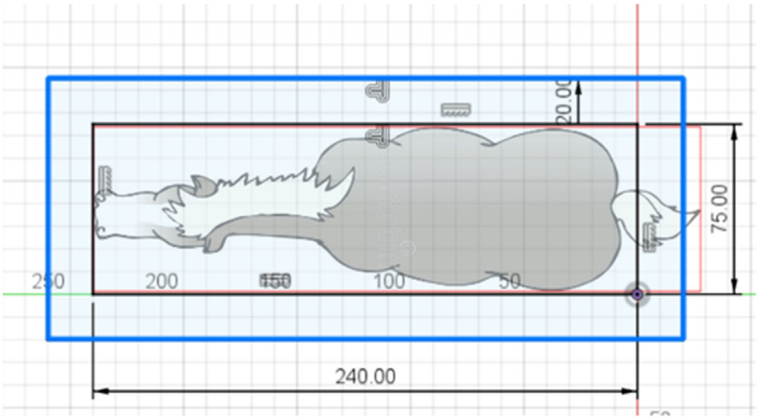


Figure 13: Schematic drawing of the space around a horse transported in a single stall. The red marking indicates the dimensions of the horse (length and width), and the blue marking indicates a situation, where the horse is provided with 20 cm on all four sides. Source: Liv Cool

According to the Consortium of the Animal Transport Guides Project (2017a), this space allowance means that horses are provided with adequate space to prevent balancing problems, injuries and damage to the vehicle. It is mentioned that some horses need more space because of their size and their tendency to 'stand wide'.

Waran et al. (2007a,b) was also mentioning injuries and TRPB as the consequences of loss of balance, and explained that the need for adequate space in horses during transport differ from species with lower centres of gravity. The horse's centre of gravity is high because they are long-legged relative to their body mass and they carry 60% of their bodyweight over their forelegs (Roberts, 1990). Increased bruising and transport-related injuries can occur due to trauma arising from loss of stability due to insufficient space to adjust posture in response to acceleration, and by individual animals losing their footing, then not being able to get up, bumping against partitions or falling and being stuck under improper partitions. If a horse does lie down or fall during transport, a much greater width is required than when standing in order to get up, as a horse puts the hind legs to one side and is unable to fold the legs underneath the body.

In order to balance, Cregier (1982) observed that horses adopt a certain body posture whilst being transported whilst facing forward, in the direction of the vehicle movement, which later has been described as a 'bracing' posture (Waran and Cuddeford, 1995). The posture can be described as the hind and forelegs are held wide apart (splayed), and the forelimbs are advanced from the usual position beneath the body (Waran, 1993). Moreover, while in transit horses showed a higher frequency of balance behaviours (leaning on rails, forward, backward and lateral movements) in comparison with confinement in a stable (Padalino et al., 2018a). It is well recognised that maintaining balance in a vehicle is difficult, as shown by increases in the activity of muscle enzymes in the blood (Codazza et al., 1974; Tateo et al., 2012), and that factors like vehicle design, type of suspension, driver ability and type of road all affect the horse's ability to balance (Riley, 2016). Horses respond to motion forces during transport by activating different balance adjusting behaviours, defined as passive, yielding or reactive sway (Roberts, 1990). Overall, these responses involve the horse attempting to take several steps in different directions in association with foot repositioning movements, as well as moving the head more upright or forward in response to acceleration, deceleration, braking and cornering (Stull, 1999).

ABMs of horse welfare during transport have been used to identify the space requirement of horses in a commercial vehicle (Padalino and Raidal, 2020). In a study involving two journeys and a total of 26 adult thoroughbred and standardbred mares, transported for 12 h in stalls of different width and length, and facing either in the direction of the vehicle movement or opposite (forwards or backwards), horses given wide stalls (1.12 m width and 1.9 m length/horse) showed fewer balance-related behaviours (leaning on the partitions, loss of balance and backward steps), in comparison with horses transported in narrower single stalls (76 cm width and 1.9 m length/horse). Horses transported in narrow single stalls showed more biting, head tossing, touching the tie cord (interpreted as a redirected behaviour) and turning the head in comparison with horses either transported in wide stalls or in non-transport confinement, i.e. in stocks (Padalino and Raidal, 2020). Since the average width of a thoroughbred has been reported to be 45 cm ((Sly, 2018), this suggests that 31 cm of extra width is inadequate as compared to 67 cm of extra width. Similarly, racehorses transported in individual stalls of 112 cm width showed fewer balance- and stress-related behaviours than horses transported in narrower stalls (76 cm) (Padalino and Raidal, 2020).

iii) Space required to lower and raise their heads

Horses use their head and neck for balance in a pendulum effect to counteract actions of the hind part of their body, and can raise or lower the head to help maintain or regain balance, and to help adjust to the speed or direction of the vehicle (Krueger et al., 2022). If a horse is tied too short, this will prevent the horse from balancing and adopting the safest and most comfortable posture when the vehicle is in motion. Stull and Rodiek (2002) found that horses are less stressed when their heads are not tied during transport. In addition, time spent with the head in a position lower than the height of the withers was found to be inversely correlated with the score for tracheal mucus accumulated during the 8-h journey, thereby increasing the risk of transport pneumonia (Padalino et al., 2018a). Therefore, in order to reduce the risk of respiratory disease, especially transport pneumonia, horses benefit from being able to lower the head at will during journeys. This requires the provision of sufficient length in front of and behind the horse, the length being with reference to the total length of the horse measured from the tail to the nose when the neck is parallel to the ground.

iv) Space required to eat and drink, if feed and water are provided in the vehicle

If a journey is sufficiently long, at some stage horses need to drink and feed. Horses do not usually drink while the vehicles are moving, but many will drink during rest stops. Provision of water, preferably every 4.5 h, and, if necessary, feed at least every 8 h was mentioned by Consortium of the Animal Transport Guides Project (2017a) in their final report. In a preliminary study of provision of water to horses during commercial transport in North America, Iacono et al. (2007b) described the behaviour of 71 horses distributed on seven journeys. Horses were watered at least once during 1-h stationary periods after different intervals of driving (earliest water provision after 8 h) and showed drinking. However, within vehicles, between 10% and 71% of the horses did not drink during the first 1-hour break.

When horses are transported with on-board watering systems, the vehicle needs to be equipped with drinkers (one for each stall) or a water trough or buckets. Currently, extra space for drinkers is not required, and is probably not needed, since they are usually placed in the empty space in front of the chest of the horse.

Horses will often be offered forage in the form of hay or 'haylage' in a bag. A hay net requires space, of about half a metre, and should be positioned at the knee level of the horse, to minimise the risk of respiratory disease (Allano et al., 2016).

Figure 14: Low hay net (the bottom edge of the hay net is level with the midpoint of the cannon bone) on the left, and hay net positioned high (the bottom edge of the hay net is level with the horse's elbow) on the right picture. Hay can also be provided on the floor of the vehicle. Source: Raspa et al. (2021)

In the high-placed hay net position (Figure 14 right), the back and neck postures as well the mandibular angle are different compared to those exhibited by horses feeding from the ground or floor. In the low-placed hay net position (Figure 14 left), the back posture more closely resembles that exhibited while feeding from the ground, but the neck posture and mandibular angle are different (Raspa et al., 2021).

v) Space to rest in a standing tripodal position

With the exception of foals, horses seldom lie down to rest during journeys. Horses transported freely in a very large space (6 m^2/horse), in a high-quality vehicle with good suspension and driven by an experienced driver on a motorway, have been seen to lie down for short periods, though (Padalino, ATA-2022, unpublished data). However, the lack of space and ongoing movement of a regular transport vehicle most likely accounts for the reluctance of horses to adopt sternal recumbency during journeys.

Horses can also achieve rest in a tripodal position, which does not appear to involve a particularly wide stance, but requires that the horse is free to lower the head below the height of the withers. Padalino et al. (2012) compared the behaviour of 12 horses transported tied (without food and water) for 3 h in three different transport positions (facing forward, facing backwards and sideways) but the behaviour was only observed during journeys where horses were transported facing backwards in relation to the direction of the vehicle.

vi) Space required to urinate

Horses (including mares, geldings and stallions) adopt a broad-based stance with their hind legs extended out behind the body while urinating. In order to perform this excretory behaviour, horses require additional space as compared to the normal standing posture. If sufficient space is not available to the horse to facilitate urination, a horse may be reluctant to urinate, potentially leading to discomfort and health problems. Horses urinate approximately six times daily and male horses in particular need to be able to stand in the urination posture, which seems difficult to do comfortably while the vehicle is in motion (Weeks et al., 2012).

Summary regarding minimum horizontal space needs for horses in individual stalls: Regarding the space allowance – lateral space as well as space in front/back of the horse – there is not sufficient data in the literature upon which to reach a definitive conclusion with respect to the minimum space required to meet the biological needs during transport in a single stall. The available evidence, however, suggests that horses will benefit from additional space with respect to the length and the width of their body.

Lateral space is necessary for spreading the legs to balance and adopt the excretory posture. It has earlier been recommended to provide 10–20 cm of free space on both sides of horses (irrespective of horse size) in order to allow the animals to balance during transport and avoid injuries. Recent evidence suggests that ~ 30 cm of free space on each side of the horse allowed better balance possibilities than ~ 15 cm.

Front/back-space is necessary for lowering the head for balancing, resting and clearing of airways, with additional space possibly required for the positioning of feeders and drinkers in vehicles. Consideration of the biomechanics involved in changing head posture suggests that a free space of 20 cm both in front and behind the horse (a total of 40 cm) when standing with the neck parallel to the ground will allow the animal to lower the head for balancing, resting and clearing of airways. If a hay net is placed in front of the horse, ~ 50 cm of extra space (in addition to the 20 cm) is needed.

D) Horizontal space for unhandled horses transported in a group

The amount of space allocated to animals transported in a group can be expressed in two ways: as stocking density or space allowance. The stocking or loading density refers to the live weight of animals within a specified area of floor space. The space allowance can be quantified as the floor area per animal (m^2), but the relevant live weight must be specified. The relationship between space requirements and live weight is not linear over the potential live weights of horses transported within the EU (Warriss, 1998). Therefore, in other livestock species that are transported in groups, space allowances are estimated using the allometric equation for space allowance:

space allowance (m^2/animal) $A = k \times W^{2/3}$

where k is a constant and W represents live weight in kilograms (Petherick and Phillips, 2009). However, the use of the allometric equation has not been developed or validated for horses and so cannot be used in this Scientific Opinion.

During transport, unhandled horses require a minimum space allowance to allow them to (i) adjust their posture in response to acceleration and other events, (ii) mitigate group stress and injuries as a consequence of aggression, (iii) eat and drink if feed and water are provided in the vehicle, (iv) rest in a standing position. A minimum space allowance needs to be set by the first limiting factor that reduces the ability of the animal to undertake one of the above biological functions, i.e. whichever of the above requirements needs the most space.

If horses in this category are transported for a maximum of 8 h, as under current Council Regulation (EC) No 1/2005[1] then the (i) ability to balance and (ii) to mitigate group stress are the most important factors to consider. However, if the horses are transported for more than 8 h, space will also be needed to eat, drink and rest.

The studies underlying the assessment may involve journeys of more than the currently allowed 8 h.

i) Space required to adjust posture in response to acceleration and other events as well as mitigate group stress

Limited studies are available to compare space required to adjust posture in response to acceleration and other events, and findings are difficult to compare. In a North American survey, Roy et al. (2015b) did not find an association between the risk of injury and estimated stocking density (median 349 kg/m^2, Q_1 = 326 and Q_3 = 362) in horses transported from 12 to 36 h. The stocking density was, however, much higher than that currently allowed in the EU.

In their preliminary study, Iacono et al. (2007b) transported horses in North America for journeys of 9 and 20 h, comparing three different stocking densities (397 $\pm$ 7 kg/m^2, 348 $\pm$ 5 kg/m^2 and 221 $\pm$ 8 kg/m^2 per compartment) in groups of unrestrained horses. The authors demonstrated an increased risk of horses falling during transport in the highest density group. The authors observed no significant differences in aggression in relation to stocking density. The cause of aggression seemed to be related to certain individuals, rather than the influence of high density. Calabrese and Friend (2009) observed the same, that the level of aggression was strongly influenced by one or two horses causing disruption of the group, and not to the same extent by factors such as space.

In another North American study, Collins et al. (2000) transported 3 groups of a total of 30 mares and 29 geldings during a 25-minute journey created to involve accelerations, braking and turning corners (including sharp turns). The horses were transported at either 1.28 m^2/horse (groups of 14) or 2.23 m^2/horse (groups of 8). Assuming an average horse weight of 500 kg, these space allowances would be equivalent to 390 kg/m^2 and 224 kg/m^2, respectively. The authors reported a higher proportion of horses falling and getting injured at the high stocking density, and concluded, based on behavioural observations, that it was more difficult for the horses transported at the high stocking density (however, potentially confounded with group size) to get up after a fall.

Knowles et al. (2010) transported 145 semi-feral ponies for 1 h, distributed on 18 journeys. The ponies were kept either alone, or in groups of four or eight during the journey. The study involved different stocking densities ranging from 139 to 316 kg/m^2. Ponies transported at low stocking density showed decreased aggression and fewer collisions during transit as well as decreased levels of plasma cortisol and CK-activity post-transport, as compared to the ponies transported at high stocking density. In groups of four ponies, fewer slips, falls, stumbles and collisions were observed during transit as compared to ponies transported in groups of 8. The authors concluded that a stocking density of ~ 200 kg/m^2 is a suitable maximum stocking density.

Summary regarding minimum horizontal space allowance for grouped horses: Unhandled horses benefit in terms of reduced aggression, stress and falls from being transported loose in groups. Few studies have examined group size, but small groups (of 4) were found to be advantageous as compared to larger groups (of 8).

In high density conditions, it is likely that horses have more problems balancing, are more likely to fall and get injured and are more exposed to aggressive conspecifics, and they may also have more difficulties getting up after a fall.

The limited available evidence (implying significant uncertainty) suggests that a stocking density of no greater than 200 kg/m^2 leads to improved welfare as compared to higher stocking densities.

E) Orientation in the vehicle

The best orientation for horses during a journey is a matter of debate. The orientation of the horse with reference to the direction of movement of the vehicle has recently been demonstrated to play a role in the level of stress experienced by horses. Currently, horses are primarily transported while facing forward, some facing perpendicular or sideways and some facing backwards. When facing forward, the forces of severe braking make horses step forward to preserve balance, and this brings the head of the animal closer to the bulkhead (Riley, 2016), with the possibility of collision. Positioning horses sideways with respect to the direction of movement of the vehicle may restrict the ability of the horses to lower their heads, particularly for large horses, because there are restrictions on the maximum width of a vehicle (2.48 metres), and thereby lead to more difficulties balancing.

Facing backwards in relation to the direction of movement of the vehicle has been recommended in a number of studies (Cregier 1982; Clark et al., 1993; Smith et al., 1994). Facing backwards has been reported to be associated with fewer impacts against the slides and front/back of the trailer, less frequent loss of balance, fewer stress-related behaviours and less balance movements, whereas horses positioned sideways have been reported to lose their balance and touch the stall rails more frequently (Cregier, 1981; Clark et al., 1993; Cregier and Gimenez, 2015). Horses have been shown to exhibit reduced stress and fatigue, and to balance more easily when facing backwards (Cregier and Gimenez, 2015). Padalino and Raidal (2020) found that backwards facing horses demonstrated fewer backwards, forwards and lateral movements and fewer balance-related behaviours. Smith et al. (1994) found that horses transported untethered preferred to face backwards, and Kusunose and Torikai (1996) confirmed this preference, demonstrating that yearlings developed a preference for backwards facing during their first journey. However, Knowles et al. (2010) did not observe any preference for either facing forwards or backwards in their study of untethered ponies.

F) Vertical space

In addition to the horizontal space in a vehicle compartment, the vertical space should be considered. In the EU, a general height limit for vehicles is 4 metres.

Low vertical space can be associated with (1) reduced ventilation; (2) lack of ability to move around; and (3) lack of space for natural movements, which should be prevented in order to avoid WCs such as heat stress and restriction of movement.

The need for vertical space depends on several factors such as the type of ventilation, size of the animals, ambient climatic conditions and type of vehicle and ventilation openings. Vehicles transporting horses may have varying types and sizes of ventilation openings. The height of compartments within which horses are transported influences their ability to adopt a comfortable unimpeded posture and may lead to injuries if the height is too low. In addition, it is necessary for adequate temperature regulation and removal of noxious gases that the height of the compartment is sufficient for effective ventilation to occur (SCAHAW, 2002). Finally, at least for horses transported loose, the compartment height may affect the manoeuvrability of the animals as well as the ability to locate resources such as preferred orientation, feed and water, but at the same time, unrestricted compartment height may facilitate unwanted (and potentially dangerous) behaviours such as mounting.

Earlier recommendations stated that the compartment height must be well above the head of the tallest animals when standing with their head in a natural position (SCAHAW, 2002; TRAW, 2009). However, the natural position was not specified.

For good ventilation and freedom of movement, a minimum internal height of the compartment has been recommended as the height of the withers of the tallest animal in a compartment + 75 cm (SCAHAW, 2002; Consortium of the Animal Transport Guides Project, 2017a; and DG SANTE (2019) in the Transport guide for extreme temperature). It has, however, not been possible to find studies validating this recommendation.

3.5.3.3. Threshold for journey times

There is not a linear relationship between the duration of a journey and stress or animal welfare (Faucitano and Lambooij, 2019; Padalino and Riley, 2022a,b). Moreover, the journey time, per se, is rarely the root cause of poor welfare, while transport conditions are the major factors which may lead to poor health and welfare (Nielsen et al., 2011). However, for horses, there is evidence that horses transported for long durations may be more at risk of fatigue, dehydration and respiratory disorders likely due to the additive effects of hazards such as fasting duration, ambient conditions, space allowance, restriction of neck movements than to transport time or distance per se (Padalino and Riley, 2022a).

Journeys involve several potential stressors that can negatively affect animal welfare, leading to WCs. The impact of transport in terms of animal welfare is influenced by the experience of the animals with transport, their physical condition, the type of vehicle, the type of road, driving skills, transport conditions and the nature and duration of the journey (Consortium of the Animal Transport Guides Project, 2017a). Inappropriate handling and transport can be associated with overt injuries, stress responses, immunosuppression and metabolic disturbances. 'Long journeys in poor transport conditions have been mooted as especially impacting on the general status of the animals, because of the longer duration of the above-mentioned stressors. The driver (and attendants) have sole responsibility for the welfare of the animals on the road, and thus play a crucial role during this stage of the transport. They not only operate the vehicle, but also monitor and take care for the animals and deal with emergencies if these occur' (Consortium of the Animal Transport Guides Project, 2017a). When considering the implications of journey duration, it is important to consider the influence of each of the potential stressors that can affect animal welfare.

Although quantitative limits often are included in legislation, there is no scientific consensus on: (a) the basis to be used to identify maximum journey durations; (b) what maximum journey durations should be specified; (c) what other factors should be considered when specifying a maximum journey duration; or (d) whether the current emphasis on using the intervals required to provide feed, water and rest is always the most appropriate way of specifying a limit on journey duration (Cockram, 2007).

In order to describe how the level of welfare develops over time during journeys, based on the above examination of the highly relevant WCs associated with the transit stage, the available research has been examined to identify what factors associated with transport have the potential to either increase or decrease the risk of WCs as a journey continues.

Cockram (2007) proposed that a rationale for a scientific justification of journey durations could be made based on one or more of the following criteria:

a) there are aspects of welfare that are negatively affected after a specific journey duration, and thus stopping a journey before this occurs would help to minimise these effects;
b) transported animals are exposed to continuous or periodic WC(s), and restricting journey duration would minimise the duration of this exposure;
c) there are many risk factors associated with a specific form of transport that have the potential to negatively affect aspects of welfare; therefore, the risk that these will occur will increase the longer that the journey takes to complete.

In this Scientific Opinion, the work carried out has been based on the approach suggested by Cockram (2007), involving the categorisation of each of the highly relevant WCs for the transit stage into these three categories.

There are, however, a number of factors limiting this work: The available experiments have often been done using conditions that conform to what is considered good or best practice. As the quality of these journey conditions are likely to be high, and only fit and healthy animals were used, the results of many of these studies may not have identified major WCs associated with commercial journeys (as discussed by Cockram (2007, 2019) across animal species).

When considering journey times, horses do, however, differ from other livestock species in various respects. They can be trained for loading and transport, and some animals may be transported many times in their life. This means that not only the transport conditions, but also the past experience of the animals will influence the effects of transport on horse welfare. Furthermore, because current EU legislation dictates that horses are transported only in individual stalls (handled horses) or groups of no greater than four animals (unhandled horses), their transport conditions will be different and possibilities for human intervention easier (e.g. feeding, watering which is compulsory at least every 8 h under current EU legislation) as compared to those of the large groups normal for other species.

Multifactorial studies of commercial situations that apply epidemiological approaches to identify risk factors affecting specific outcomes, such as mortality or clinical deterioration, can sometimes identify a potential relationship between a WC as indicated by an ABM and journey duration (Cockram, 2007), but often focus on rather extreme welfare end points (such as serious health impairment) and not on the protection of animals from WCs. In this Scientific Opinion, signs of activation of coping mechanisms are taken as an indication of the presence of a hazard potentially leading to the corresponding WC.

Among the highly relevant WCs during the transit stage, the following are considered relevant for this work, and dealt with in further detail below: motion stress, sensory overstimulation, restriction of movement, resting problems, prolonged hunger, prolonged thirst, gastro-enteric disorders, respiratory disorders and injuries. In addition, pain and/or discomfort associated with pre-existing or newly caused health conditions has been included. Below, the relation between journey duration and these WCs is examined, ordered to align with the three different categories suggested by Cockram (2007): continuous or semi-continuous WCs, progressively developing WCs and the more sporadic health conditions.

The scenarios considered in this section refer to the transport of animals within the EU and take into account the recommendation made on microclimatic conditions (Section 3.5.3.1) and space allowance (Section 3.5.3.2). Across studies underlying this assessment, it is important to consider in their interpretation whether the animals had limited access to food and water, and more restricted space allowance than permitted in EU legislation.

A) The welfare consequences motion stress and sensory overstimulation

These WCs are present for the whole period of the journey, or at least periodically (repeated intermittent).

Transport is a stressful event for horses as evidenced by many papers that report increases in physiological indicators of stress during the transit phase. For example, increasing plasma cortisol was observed in experienced horses, which were cross-tied in single stalls (0.76 × 1.67 m), throughout a single 24-h journey, made during hot summer conditions with access to hay ad libitum in transit, and regular watering every 4–5 h (Stull and Rodiek, 2000; Figure 15). Fifteen horses were housed individually during two consecutive days (day 1 and 2) in individual stalls during the month of August. The third day, horses were transported during 24 h cross-tied in individual stalls (1.27 m^2/animal) and provided alfalfa hay all journey. Six horses faced in the direction of the movement of the vehicle, three were parallel to the length of the vehicle and nine were transported facing backwards. After the journey, horses were unloaded into individual stalls for recovery. Blood samples were collected at 8:00, 11:00, 14:00; 20:00 and 02:00 every day. One additional sample was collected at 7:00 before the journey.

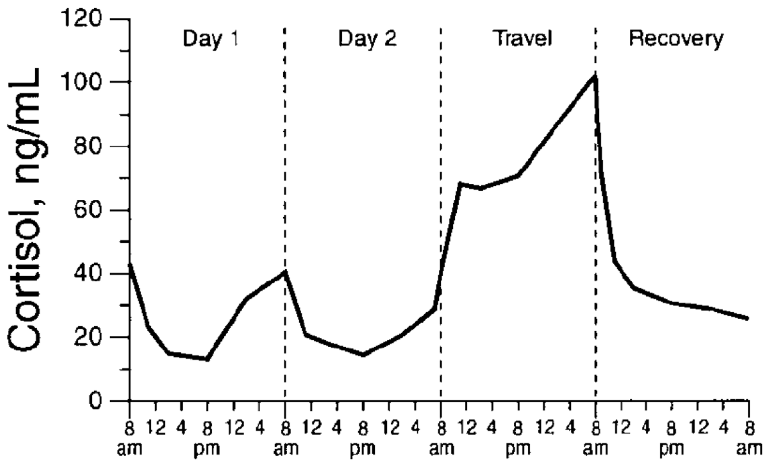


Figure 15: The effect of a 24-h journey under hot conditions on the plasma concentration of cortisol (Stull and Rodiek, 2000)

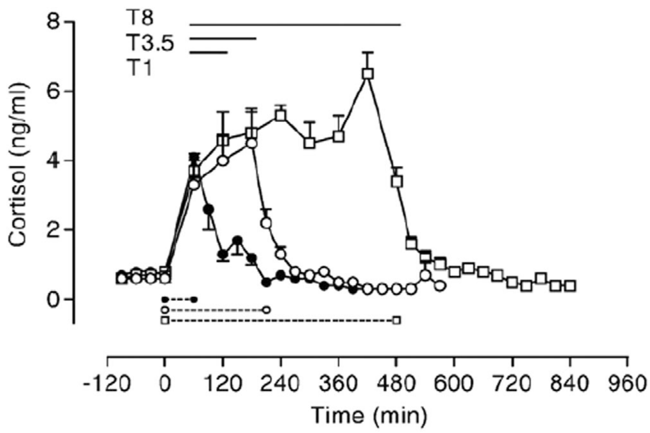
Significant increases in plasma cortisol compared to untransported controls were also detected at mid-journey and end of journey in mares after 12 h of transport (Baucus et al., 1990a). Mares were transported in groups of six in a trailer, tied in diagonal position, while control animals were placed in unfamiliar pens with hay and water. Similar increases were detected also after short journeys (1 and 3 h) when horses were kept in single stalls and without access to feed and water in comparison with no-transport conditions (Tateo et al., 2012).

In slaughter horses transported for 24 h (continuously and in two periods of 12 h), the cortisol concentration was higher than in control resting horses. Samples were taken after the 24-h journey and every 12 h in the split journeys, revealing an increase from the first 12-h journey (Stull et al., 2008).

Padalino et al. (2018a) studied the effects of 8-hour journey in 12 experienced horses, in two separate consignments, transported without access to feed and water. The horses were kept in a single stall (of 1.2 m^2/horse) and were maintained sideways in relation to the direction of the vehicle. The authors identified a significant increase in plasma cortisol levels, heart rate and respiratory rate at unloading, in comparison with preloading parameters. The frequency of stress-related behaviours increased most markedly in the first hour of transport and tended to decrease as the journey progressed. In contrast to this, the occurrence of balance-related behaviours was greater in the last hour of the journey.

In six horses habituated to being transported loose in a large box stall (6 m^2) with and without access to hay on the floor, plasma cortisol (measured every 3 h during the 12-h journey) increased after 3 and 6 h in comparison with the prejourney level, but not thereafter (Padalino, 2022, unpublished data). Whether the decrease later in the journey resulted from habituation to transport or depletion of readily available cortisol due to a negative feedback mechanism cannot be determined.

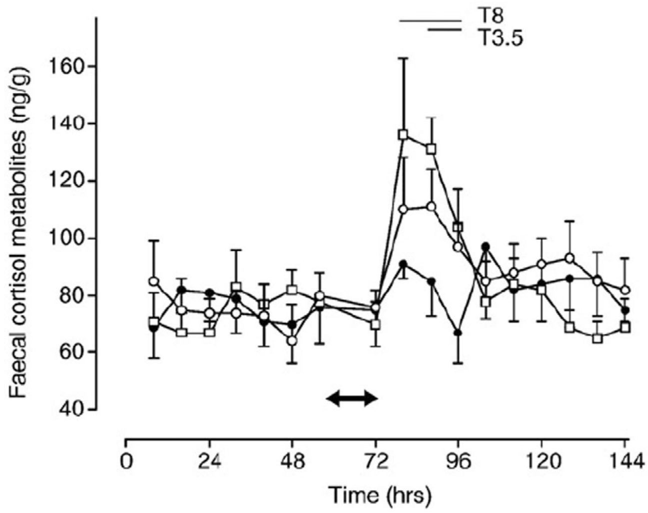
In addition to these data on plasma cortisol, salivary cortisol has also been used to evaluate stress in horses during transport. Schmidt et al. (2010a) studied horses without recent experience of transport during three journey durations (1 h, 3.5 h and 8 h). The horses were kept in forward-facing individual stalls of unspecified size, and no food or water was provided. Salivary cortisol was measured every hour during the journey and every 30 min for 6 h after the journey. In all journey durations, the maximum levels of cortisol were detected towards the end of each journey (at 1 h in 1-h journey, at 3 h in 3.5-hour journey and 7 h in the 8-hour journey) (Figures 16 and 17). No changes in the road conditions that might cause this were mentioned by the authors, as they stated that all journeys were carried out in two-lane national roads in flat land. After unloading, values returned to baseline within 2 h.



The horses were kept in forward-facing individual stalls of unspecified size, and no food or water was provided. Dotted lines parallel to x-axis indicate journey duration.

Figure 16: Saliva cortisol concentrations (ng/mL) collected in horses before, during and after road transport for 1, 3.5 and 8 h (n = 8 per group) in horses without recent experience of transport. Source: Schmidt et al. (2010a)

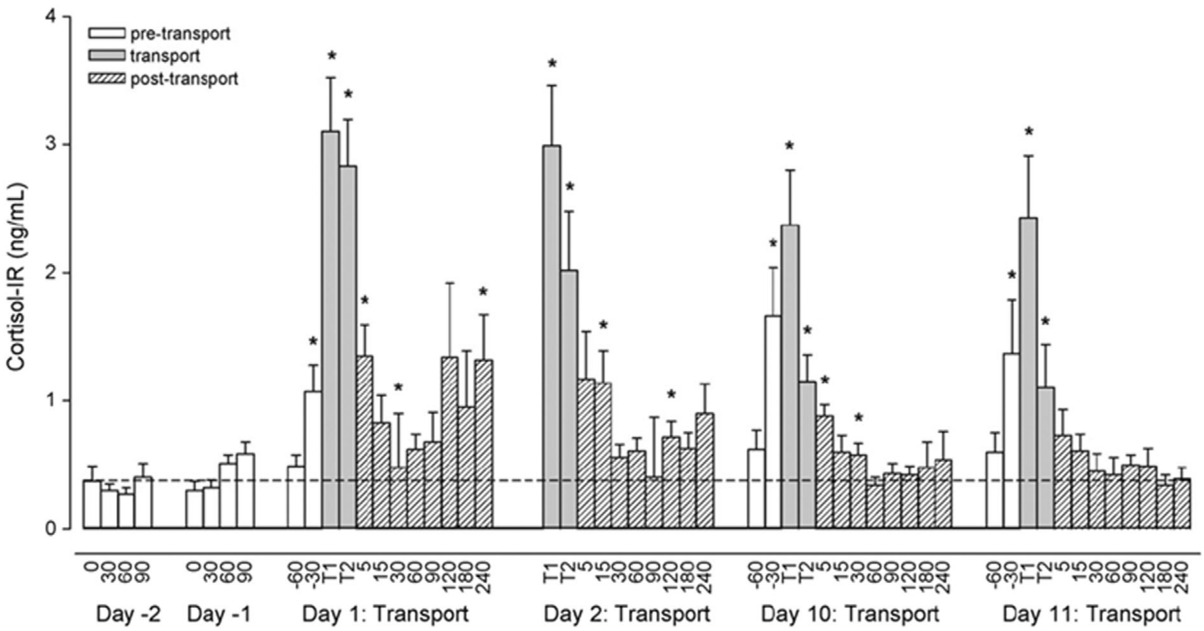
While plasma and salivary cortisol concentrations are directly correlated with acute changes in cortisol release, the cortisol metabolites in faeces rise 24 h after increase in cortisol in blood, and are thus a better reflection of prolonged stress (Palme and Möstl, 1997; Merl et al., 2000). In the study by Schmidt et al. (2010a), involving horses without experience with transport, significant differences in the concentration of cortisol metabolites in faeces on the day after transport were also detected in the horses transported for 3.5 or 8 h, in comparison with the basal levels (average 78 ng/g). The highest levels were detected in horses transported 8 h (136 $\pm$ 27 ng/g), followed by the group transported 3.5 h (111 $\pm$ 13 ng/g).



The horses were kept in forward-facing individual stalls of unspecified size, and no food or water was provided. The arrow indicates day of transport.

Figure 17: Faecal cortisol metabolite concentrations before, during and after 1, 3.5 and 8 h of road transport (n = 8 per group), in inexperienced horses. Source: Schmidt et al. (2010a)

Schmidt et al. (2010b) studied seven competition horses, well accustomed to transport, during two occasions of a two-day (1,370 km) journey separated by 8 days. Specifically, the horses were transported for 24 h on days 1 and 11, and for 20 h on days 2 and 10. During the journeys, horses were not fed but received water during stops at 4- to 5-h intervals. The horses were kept in stalls of 75 × 205 cm, oriented at 75° to the direction of the movement of the vehicle. In all journeys, the horses showed a significant increase in salivary cortisol during transport, and in faecal cortisol on the following day. Specifically, cortisol in saliva increased significantly from the basal levels starting 30 min before the first journey and reaching a peak in the mid-term journey measurement in all journeys (see Figure 18). For all journeys, the levels were significantly elevated at the end of the journey, and in three of the four journeys, significantly increased levels were still detected 4 h, 2 h and 30 min after the journey (days 1, 2 and 10, respectively). It cannot be determined which stressors associated with the journey were responsible for the cortisol increase and thus may have involved more than one WC.



The x-axis indicate minutes during two occasions of a 2-day (1,370 km) journey separated by 8 days. The horses were transported for 24 h on days 1 and 11, and for 20 h on days 2 and 10. During the journeys, horses were not fed but received water during stops at 4- to 5-h intervals. The horses were kept in stalls of 75 × 205 cm, oriented at 75° to the direction of the movement of the vehicle.

Figure 18: Saliva cortisol concentrations (ng/mL) before, during and after transport of seven experienced competition horses. Source: Schmidt et al., 2010b

Changes in ABMs of heart rate can also be used as an indication of transport stress. However, like cortisol, heart rate may be affected by many factors including psychological stress and muscular exertion thereby challenging the interpretation. Piccione et al. (2013a,b) studied the influence of transport on heart rate analysing two different distances (110 and 225 km) at different times of the day, including secondary roads and change of speed. The conclusion of the authors was that heart rate is too variable an ABM that is strongly affected by environmental and other conditions, to allow interpretation in terms of transport stress without tight control of these elements.

Waran (1993) compared the heart rate of horses in similar conditions in a stationary vehicle or a vehicle in motion, and showed that the heart rates in horses in the moving vehicle were higher. Although these studies were done over a short period of time (30 min) and horses had a companion during the time confined, the results show that motion stress does occur. Schmidt et al. (2010a) reported a significant increase in the heart rate (Figure 19) which was maintained throughout the three journey durations that they studied and, in the case of 3.5-hour journey, the increase persisted for another 7 h after transport. Additional changes in beat-to-beat interval and other heart rate variability parameters indicative of stress were also reported during the journey.

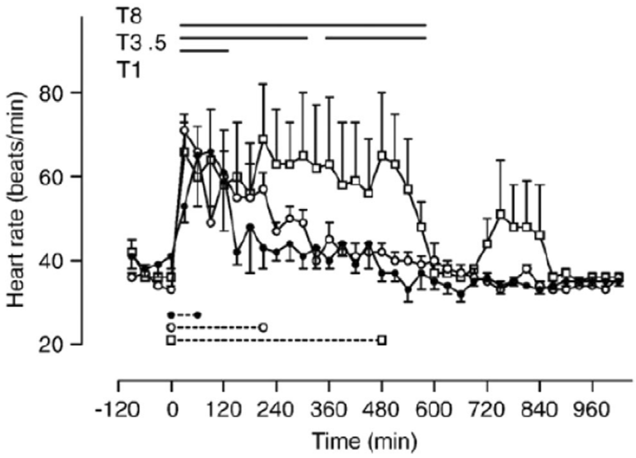


Figure 19: Heart rate of horses during transport for three different journey lengths: 1 (black circle), 3.5 (open circle) or 8 h (open square) (n = 8) in inexperienced horses kept in forward-facing individual stalls of unspecified size, and no food or water was provided. (Schmidt et al., 2010a)

In a recent study, Ohmura and Hiraga (2022) demonstrated that restraint inside a transport vehicle and being cross-tied in stalls of unspecified size, was sufficient to affect heart rate, even when the horses were not transported. Heart rate was also increased in a moving vehicle, relative to a vehicle which was stationary but with the engine running, indicating the additional importance of motion stress. Parasympathetic nervous activity decreased during transport, and to a lesser extent during time in a stationary vehicle, in comparison to a resting period in a stall. The authors concluded that transport stress affects autonomic nervous activity during the transit stage, due to accumulated effects of stressful conditions that included not only vibration and noise during transport but also restraint in the transport vehicle.

Research on heart rate variability has been done in horses, including high and low frequency power and their ratios, suggesting that, if done correctly, this could be a good indicator of the stress levels during transport (Ohmura et al., 2006). Heart rate variability has subsequently been found to be higher (indicative of better welfare) in horses transported freely in a large stall in comparison with horses transported in a single stall, and to be higher in horses fed ad libitum during transport in comparison with horses transported without feed (Padalino, 2022, unpublished data).

In summary, regarding these two welfare consequences, transport may be a continually stressful experience, with the extent of the measured stress responses depending greatly on the journey conditions.

B) The welfare consequence resting problems

Resting problems may occur throughout the duration of the journey, but their effects are likely to be cumulative, potentially leading to fatigue and, eventually, exhaustion. They result from the needs of the animal to continuously make postural adjustments to compensate for vibration and acceleration changes, which preclude sustained adoption of a normal resting posture (see Section 3.5.3.2). Horses may be more active at the onset of a journey, moving about more per unit time during shorter (1 h) journeys than longer (3 h) ones, with more forward and backward movements (Tateo et al., 2012). However, road conditions will play an important role. Padalino et al. (2018a) reported that the frequency of balance-related behaviours decreased slightly after the start of an 8-hour journey, then fluctuated from 2 to 7 h before increasing significantly again. It was suggested that this increase at the last hour could be due to the minor roads crossed to arrive at the destination.

The effects of balance-related activity may be measured in changes in blood biochemistry indicative of muscular activity. Codazza et al. (1974) compared serum enzyme and metabolite changes in racehorses after a 300-km journey with those after cantering for a distance of 1.5 km. When considering creatine phosphokinase (CPK), lactic dehydrogenase (LDH) and lactate levels, the authors suggested occurrence of similar muscular function during transport to that from prolonged work.

Padalino et al. (2018a) found a significant increase in creatinine kinase and lactate concentrations in blood after 8 h of transport (conducted in fasting conditions, in narrow single stalls (1.2 m^2/horse) with sideways orientation) which could be indicative of muscle fatigue as a result of the journey. These parameters did not return to normal values 1 day after the journey, but actually peaked at that time, possibly because the horses went straight into single box stalls without the recommended post-transport practices (i.e. walking for at least 30 min).

Similar results were obtained by Stull and Rodiek (2000), when transporting 15 horses on a single journey of 24 h (cross-tied in forward and rear-facing single stalls of $1.3m^2$, with access to hay and watering every 4–5 h) during a summer period in California. As seen in Figure 20, the lactate and CK levels started increasing three hours after the beginning of the journey, when the first sample was taken and increased rapidly in the following 12 h, indicating increased muscular activity.

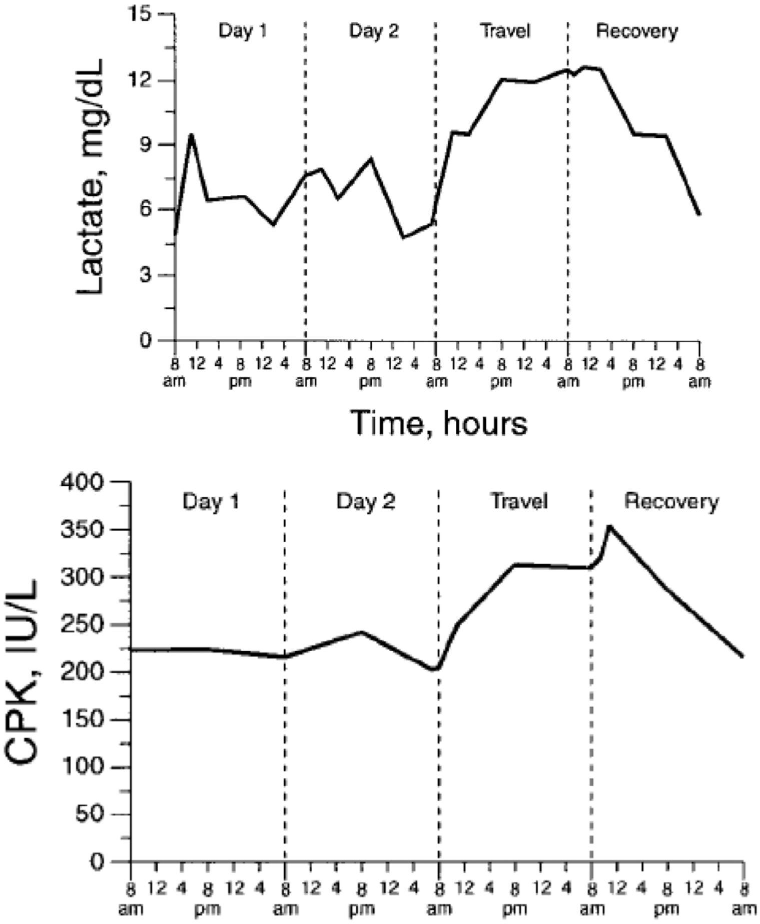


Figure 20: The effect on muscle fatigue indicators (mean value for plasma lactate (mg/dL) and creatine phosphokinase (CPK), IU/L) of a 24-h journey in summer conditions in California. Source: Stull and Rodiek, 2000

Cumulative fatigue may give rise to other physiological consequences indicative of transport stress. Measurement of the outcomes for 16 show-jumping horses on the same 4-day journey, indicated a detrimental effect on several clinical (rectal temperature, heart rate, respiratory rate), immunological (lymphocyte proliferation test), haematological (neutrophilia, hyperglobulinaemia) and oxidative (plasma total antioxidant status) responses, indicating an acute phase response impairing the cell-mediated immune response (Padalino et al., 2017b). The journey time totalled 94 h with 51 h in transit, consisting of the following stages: 6-h journey – 12-h rest – 24-h journey – 12-h rest – 9-h journey – 19-h rest – 12-h journey. Horses were transported tied in individual stalls of unspecified size and orientation.

Excessive oxidant production or insufficient antioxidant capacity can lead to oxidative stress which has an important role in the development of numerous health problems including muscle, neurological and lower airway diseases (Kirschvink et al., 2008). Horses transported for 12 h in individual stalls with access to hay showed significant changes in oxidative stress parameters, including oxidants such as malonedialdehyde (MDA) and antioxidants such as superoxidase dismutase (SOD), together with increased respiratory and heart rates (Onmaz et al., 2011). The increase of MDA and decrease of SOD after 12 h in transit could be associated with the effort required during transport and the increased formation of free radicals due to the stress associated with the transport process. Wessely-Szponder et al. (2015) quantified MAD at slaughter in horses transported for 1 and for 12 h (in conditions pertaining to current EU legislation). The study involved fillies and older mares, but no information is given on the prejourney conditions, thereby limiting the interpretation of the results.

Journeys of 8 h had an impact on the concentration of antioxidative enzymes (glutathione reductase after transport and glutathione peroxidase 24 h after transport), probably due to their use in neutralisation of reactive oxygen species (Niedźwiedź et al., 2012). Furthermore, when analysing the plasma Total Antioxidant Status (PTAS), which is used to determine the levels of antioxidant enzymes and non-enzymatic compounds, Niedzwiedz et al. (2013) demonstrated a significant increase after 8-hour journey.

Other authors measured the concentration of numerous oxidants (including reactive oxidant metabolites, advanced oxidation protein product, ceruloplasmin and hydrogen peroxidase in the breath) and antioxidants such as total antioxidant status in blood and saliva of 12 horses after 8 h of transport (Padalino et al., 2017c). Transport caused an increase in the mucus scores, and the oxidants and antioxidants evaluated, with most of them peaking at the time of unloading (in comparison with preloading, 12 and 24 h after the journey). However, the redox homeostasis was maintained all along the study.

C) Effect on immune function

Whilst impaired immune function is not, in itself, a WC listed by EFSA (EFSA AHAW Panel, 2022a,b), it predisposes animals to other health-related WCs. Because of the sensitivity of measures of the immune response to a range of different stressors, it has often been used experimentally as an iceberg ABM for welfare impairment (see below). The acute phase response is a core part of the innate immune system and provides a non-specific early defence system that is activated in a wide range of circumstances including trauma, infection, inflammation and stress. The acute phase response is a complex systemic reaction involving physical barriers, and molecular activation such as the acute phase proteins (APP). The importance of the different APP varies between animal species, with Serum Amyloid A (SAA), Haptoglobin (Hp) and Fibrinogen (Fg) the ones mostly affected in horses (Cray et al., 2009). The stress associated with physical and psychological stimuli has been associated with decreased immunocompetence and increased susceptibility of animals to infectious and other diseases (Kelley, 1980; Griffin, 1989; Gross and Siegel, 1997; Peterson et al., 1991). This sensitivity of the immune system to external stimuli has resulted in the use of immune function tests as a bioassay to provide information considered relevant to animal welfare. However, as discussed by Vedhara et al. (1999), it is necessary to consider the clinical significance of any altered immunity, and whether the magnitude of any stress-associated immune change is sufficient to alter immunocompetence.

A significant increase in Hp, SAA and white blood cell (WBC) count was reported in 10 experienced horses after 4 h of transport, in conditions conforming to Council Regulation (EC) No 1/2005[1] on a mixture of road surfaces, with a faster increase of Hp than of SAA (Casella, 2012).

Changes in leucocytes, neutrophils and consequently, total white blood cells count have been frequently documented in horses after transport. Raidal et al. (1997) reported significant neutrophilia after 12 h of transport of cross-tied horses without feed, that persisted 36 h after the end of the journey. The phagocytosis function of the peripheral neutrophils was also impaired until 36 h after transport. However, leucocyte levels increased but not significantly after the journey. Similarly, Padalino et al. (2017b) showed impaired lymphocyte proliferation in horses after a very long journey (94 h in total with ~ 51 h on the road and 43 h at control posts).

Significant increase in neutrophils was also detected in slaughter horses transported for 24 h (either continuously or in two 12-hour periods separated by a rest of 12 h) in comparison with non-transported animals. In those animals, transported in densities from 1.2 to 1.3 m^2/animal (depending on the compartment of the trailer), the ratio neutrophils:lymphocytes (N:L) was also significantly higher than in non-transported animals and higher than that considered normal in healthy animals (Stull et al., 2008). Very similar results were obtained in Stull and Rodiek (2000), where WBC count and N:L ratio increased significantly after 3 h of a 24-h journey, reaching the peak at the end of the journey (WBC) and 4 h after the journey (N:L ratio). These findings were confirmed by Rizzo et al. (2017) where, after a 145-km journey, the horses presented significant increase of the levels of neutrophils, leucocytes and total WBCs.

Recently, the influence of transport on the immune response and its specific capacity to fight specific pathogens was studied. Bannai et al. (2021) studied six horses, on two separate vehicles, transported for 12 h in large stalls ($6m^2$) and offered water every 3 h. The authors identified a negative impact on the virus neutralisation capacity against equine herpesvirus type 1 (EHV-1) and type 4 (EHV-4) after the 12-hour journey.

D) The welfare consequence respiratory disorders

As mentioned above, the risk of horses developing respiratory disorders are known to be multifactorial. Oikawa et al. (2005) described how 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.

Many studies, as exemplified in previous sections, have explored the influence of transport on the immune response and physiological parameters of transported horses, demonstrating an important role even in journeys as short as 1.5–2 h (Miller et al., 2021), and potentially in the development of transport pleuropneumonia, often called 'shipping fever'. Raidal et al. (1997) monitored six horses over a 12-hour journey in which they were confined within individual stalls and their heads were restrained by ropes to the side of the vehicle at a height of ~ 1 m. During the journey the vehicle was stationary for one hour, but horses were not unloaded or offered food or water at any time. Productive cough and changes in auscultation (increased airway noise and gurgles) were observed in five of the six horses, although none of them developed pulmonary disease afterwards. These clinical changes were accompanied by increased volume of mucus in all the horses with an increased number of cells.

Padalino et al. (2018a) transported 12 horses for 8 h on two occasions (same vehicle and same driver, with six horses each). All the horses, which had previous experience of transport, were clinically normal before the journey. When unloaded, six horses presented audible coarse airway sounds to auscultation, and three of them coughed during the examinations. These two symptoms persisted until day 5 after the journey. These animals presented increased tracheal mucus, trachea inflammation and increased percentage of neutrophils. The concentration of bacteria in the tracheal wash was higher in these affected animals all along the study (from preloading until the end of the experiment) and the predominant bacterial isolate was a *Pasteureullaceae*. There was a negative correlation between the time the horses spent with the head at the level of withers or lower and the factors previously mentioned (increased tracheal mucus, bacterial concentration and inflammation scores).

Several authors have measured the frequency of respiratory diseases in horses after transport, which affected from 0.66% of 1,650 horses transported 4,000 km (Padalino et al., 2015) to 9.7% based on owners' opinion collected through a survey in Australia (Padalino et al., 2016a). Miranda de la Lama (2021) found in a cross-sectional study that 12% of the horses (n = 2,648) transported between Mexico and USA and vice versa (9 and 16 h on average, respectively) presented nasal discharge potentially indicative of respiratory disorders on arrival at an abattoir. When the animals were grouped in clusters based on the clinical examination, 66% of the horses with nasal discharge were placed in a cluster that were transported from 6 to 12 h.

As mentioned, respiratory disorders associated with transport pleuropneumonia are a complex entity, as this is a multifactorial problem. A long time in a head-raised position (Padalino et al., 2018a; Raidal et al., 1996), cross-tying (Raidal et al., 1996; Stull and Rodiek, 2000), environmental factors such as noxious gases and dust concentration (Oikawa et al., 1995, 2005), presence of subclinical respiratory diseases (Padalino et al., 2018a), orientation during transport (Waran, 1993) and journey duration, have all been demonstrated to play a role in the development of these problems.

Austin et al. (1995) analysed the clinical records and potential exposure to risk factors of horses with pleuropneumonia in a case control study (45 cases and 180 controls) in Australia. The results indicated that animals that had been transported for more than 805 km (~ 10 h) in the previous week were at higher risk of developing pleuropneumonia.

Oikawa and Kusunose (1995) transported 29 horses for 36 h in two groups. Horses had access to hay during transit and water during stops (every 4 h of road transport). The horses were transported cross-tied in single stalls. Signs of respiratory disease (fever, coughing, nasal discharge and lethargy during transport, as previously described in Oikawa et al. (1995), were checked in the animals every 4–5 h. Of the transported horses, 44% presented signs of respiratory disease at the end of the experiment. The first clinical signs of respiratory disease started as early as 14 h and increased over time with a marked increase between 21 and 24 h of journey.

Other authors studied the influence of long journeys (42 h) on the bronchoalveolar fluid (BALF). Hobo et al. (1997) identified significant differences in BALF of transported horses (transport conditions unspecified), specifically a decrease in fluid recovered, an important increase in the number of nucleated cells (mostly neutrophils), increase protein concentration and decrease of phosphorus concentration.

The effect of journey duration on the risk of respiratory disorders is likely to be strongly influenced by journey conditions, and particularly the possibility to lower the head and clear the airways. In recent experiments conducted in Japan, where horses were transported loose (n = 4) or in a single stall (n = 12) but with the ability to lower the head, with access to water and feed during the journey or every 4 h, no sign of respiratory disorders, no tracheal inflammation score and inflammatory cells in the bronchoalveolar lavage fluid (BALF) was found even after 22 h of transport in comparison with horses transported for the same distance without the ability to lower their head (n = 6) (Padalino, 2022, unpublished data).

E) The welfare consequence prolonged thirst

As reviewed in the Code of Practice for the Care and Handling of Farm Animals: Transportation (2018) from Canada, during a journey, water is gradually lost through the skin, respiratory tract and via urine and faeces (Van den Berg et al., 1998). Without access to drinking water during a journey there is a risk of dehydration. Roy et al. (2015b) found a significant association in Canadian slaughter horses between increased journey duration (ranging from 6 to 36 h) conducted without access to water, and the plasma total protein concentration measured at the time of slaughter. These concentrations were greater than those reported for non-transported horses and indicative of dehydration (Kingston, 2014). Stull (1999) found greater increases in total serum protein concentration of group-housed slaughter horses (1.1–1.5 m^2/horse) after US commercial journeys conducted without feed and water when comparing 27–30 h with shorter journeys (< 23 h) and greater increases after 16–23 h than after < 6 h.

Non-transported horses (about 500 kg), previously offered hay and water and then kept at temperatures of 12–33°C without feed and water for 72 h, experienced 6, 9 and 11% live weight losses after 24, 48 and 72 h, respectively. The plasma total protein concentration was raised after 24 h and plasma osmolality was raised after 48 h (Carlson et al., 1979). Progressive dehydration due to lack of access to drinking water during a journey would be faster in warm conditions because of the increased evaporative heat loss that occurs in horses at temperatures of ≥ 20°C (Morgan, 1998; Jose-Cunilleras, 2014)'. Horses transported for 24 h under hot summer conditions, and provided with water during stops every 4–5 h for 40 min, consumed on average 22.7 L of water during the journey, with 91% of this consumed during the second half of the journey (last 12 h). The authors reported increased total protein concentration in blood compared to the preloading values, with peak concentration reached after 12 h of transport, and increased haematocrit concentration peaking at the end of the journey (Stull and Rodiek, 2000).

With transporting grouped horses under hot conditions, Friend (2000) showed that transport for 24 h without water induced severe dehydration, while blood biomarkers of dehydration (osmolality, plasma protein and serum protein) had already exceeded clinical reference values after 8 h. This was used as the interval to water animals in a study of Iacono et al. (2007b), with slaughter horses transported in groups of different stocking density for 18–20 h. The authors demonstrated that all horses drank in the first 20 min when offered water during a rest stop after 8 h of journey. Although they had 27% lower total plasma protein concentration at the end of the journey than horses in the same vehicle which were not watered, this difference did not reach statistical significance. Horses transported for 8 h in individual stalls in the TNZ but without access to feed and water showed mild, but significant, increases in albumin, total protein, capillary refill time and decreased body weight, all consistent with mild dehydration (Padalino et al., 2018a).

Tateo et al. (2012) took blood samples from experienced horses transported in single stalls for 1 or 3 h, being transported within the TCZ but without food and water. At the time of unloading, horses after both journey durations showed an increase in haematocrit and blood total protein (6.78–6.82 g/dL), this being indicative of mild dehydration. After the longer journey, the horses engaged in more drinking bouts than did horses after the short journey, particularly in the first 2 h post-transport. The latency to drink was significantly shorter than for horses deprived of feed and water for the same period of time, but not subjected to transport.

When water is offered, horses may be reluctant to drink in a moving vehicle (McGreevy, 2004). In a cross-over study design using six experienced thoroughbred mares, horses failed to drink during transit (sideways orientation in stalls of unspecified size), although familiar water was always readily available (van den Berg et al., 1998). Based on bodyweight, the transported horses were on average 3% dehydrated at the end of the 8-hour transit, and they drank more in the following 6-h recovery period than not-transported controls. The majority of horses will drink when the vehicle is stationary, but some may not. When slaughter horses were offered 1 h of access to water during stops at intervals of 6–12 h during journeys of 16–28 h, the number of horses drinking ranged from 86% to 100% and their intake was estimated to be low (Iacono et al., 2007a). A low intake may be exacerbated by water from an unfamiliar source with a different taste (Mars et al., 1992).

F) The welfare consequences prolonged hunger and gastro-enteric disorders

Horses at pasture or in housing eat frequently and are not observed to fast voluntarily for more than three to five hours (Baumgartner et al. 2020).

As horses can digest and absorb soluble carbohydrates, the plasma glucose concentration represents the balance between absorption, gluconeogenesis and utilisation of glucose. Digestion of fibrous carbohydrates occurs in the cecum and colon where they are converted into volatile fatty acids that can be absorbed and act as a longer-term energy source (Evans, 1971). During fasting, body energy reserves are mobilised, the blood glucose concentration falls, the plasma free fatty acid concentration rises due to lipolysis of adipose tissues and the plasma urea concentration rises due to increased catabolism of body protein (Christensen et al., 1997). These metabolic changes were apparent within 12 h, when first measured, and marked by 24 h. Although hypoglycaemia can occur in exhausted and fasted horses (Christensen et al., 1997), stress associated with transport can cause liver gluconeogenesis and increased blood glucose concentration (Stull and Rodiek, 2002).

When offered feed during a journey, horses transported in a single stall and in forward-facing orientation fed less in a moving vehicle than when they were standing in a stationary vehicle, which suggests that the movement of the vehicle inhibits feeding (Waran and Cuddeford, 1995). Racehorses transported by road for 6 h, in a single stall and in forward-facing orientation, where feed was offered, still experienced an average 2.5% reduction in their body weight (Waran, 1993). However, in another study using six experienced thoroughbred mares, horses showed only a non-significant reduction in hay intake during an 8-h transport period (sideways orientation in stalls of unspecified size) in comparison with their intake in control stabled conditions. Water from a familiar source was available in both situations (van den Berg et al., 1998). Similarly, when studying six horses, on two separate vehicles, transported for 12 h in large stalls ($6m^2$) and offered water every 3 h during a 15-min break, all ate their ration and no signs of dehydration were found at unloading (Padalino, personal communication, regarding the study of Bannai et al., 2021).

In an analysis of reports of transport related health problems identified by drivers and horse owners for 180 journeys of an Australian company transporting horses between Perth and Sydney (~ 4,000 km), gastro-enteric problems were the most common category (Padalino et al., 2015). Of the 1,650 horses in the sample, four showed signs of colic at rest stops with another three showing signs of colic post-journey. All cases were interpreted as impaction colic; two resolved without treatment (requiring only monitoring) and five were treated medically. Enterocolitis was identified in one horse during transport, and in two horses post transport, with all horses requiring hospitalisation.

A separate online survey of equine industry personnel, asked to report on transport-related health problems experienced by their horses in the previous 2 years, indicated the incidence of transport-related gastro-enteric health problems to be 20% for diarrhoea and 10.3% for colic (Padalino et al., 2016b). There are several factors involved in transport of horses that can contribute to this risk; specifically, the stress of transport, as previously discussed, that increases the cortisol release and the changes in feeding regime (potential pretransport fasting plus time off feed during transport). Padalino et al. (2020) investigated the appearance of gastric ulcers and gastric pH in horses transported for 12 h (880 km) after different periods of pretransport fasting. Increased gastric pH was seen two hours into the journey and remained elevated for the extent of the journey and increases in gastric squamous ulcer scores were seen immediately after transport (no effect on glandular mucosa). However, due to the design of the study, it is difficult to interpret the results in terms of journey duration, as the fasting conditions and feeding management prior to and during transport are relevant for the prevention/control of gastric ulcers associated with transport.

G) Summary on journey duration

Regardless of how optimal the conditions of the journey provided are, horses can potentially be exposed to several hazards during transport that might, either on their own or in combination, result in impaired animal welfare. The amount of time the animals are exposed to these hazards is dependent on the journey duration. Any aversive effects of resting problems, or reduced availability of, or restricted access to water and feed are likely to increase with journey duration if the horses are not fed, watered and rested and could interact with other factors, such as temperature, that might also change during a journey.

Above, the available research has been appraised to identify relationships between journey duration and highly relevant welfare consequences. The information is summarised below. Based on estimates of risk, prevalence and severity of the welfare consequences, a table (Table 19) has been created to show the estimated journey duration after which the welfare consequences are expected to be present. The assessment for journey duration takes as a starting point that recommendations on microclimatic conditions and space allowance are followed.

Summary – motion stress and sensory overstimulation: As soon as a vehicle starts moving, and during all time when it is moving, all horses are to some extent exposed to motion stress and often also, at least periodically (repeated intermittent), to sensory overstimulation. As a consequence of the vehicle motion, animals experience stress potentially leading to fatigue and negative affective states such as fear and distress, due to the forces exerted as a result of acceleration, braking, stopping, cornering, gear changing, vibrations and uneven road surface. During a journey, horses experience continuous muscular activity to maintain balance. The extent of this depends on factors such as road conditions and driving style, but also space and orientation in the vehicle and ability to move head and neck freely. Motion stress is regarded as a highly relevant welfare consequence in the transit stage. Prevalence is high, as motion stress is likely to affect all animals in a moving vehicle. The duration of the welfare consequence depends on journey duration and onset of vehicle motion. Motion stress will vary over time depending on the journey conditions, but the severity of its effects will most likely increase over time and may eventually lead to fatigue. Based on the constant presence of motion stress- and the semi-continuous nature of sensory overstimulation, it is not possible to estimate a temporal cut-off for onset of this welfare consequence after initiation of the transit stage.

Summary – prolonged hunger: The welfare consequence prolonged hunger is regarded as highly relevant in the transit stage. Prevalence is expected to be high if horses are not fed during journeys. Depending on factors such as time off feed before journey start, horses may not be hungry during the initial phase of the transit stage, but hunger will develop over time if feed is not freely accessible. The duration of prolonged hunger depends on journey duration and the ability to eat in transit, and if this is not possible, severity is expected to increase with increasing duration, as the need for feed becomes problematic for the animals. Prolonged hunger may lead to exhaustion and a weakened condition. The available data does not allow a detailed determination of the interval between journey start and initiation of prolonged hunger. Based on the available knowledge from studies of transport of horses, mobilisation of body energy reserves can occur after about 12 h and these changes might be associated with the initiation of hunger. Horses will eat if feed is available on the vehicle in transit, and especially during a rest stop, but the amount consumed during a journey may be less than in non-transport conditions. However, feeding on the vehicle during regular rest stops can preclude the detrimental consequences of prolonged hunger.

Summary – gastro-enteric disorders: The welfare consequence gastro-enteric disorders is regarded as highly relevant in the transit stage. Gastro-enteric problems can be present in horses after transport. There are, however, several factors other than journey duration that can contribute to this risk; specifically, the stress of transport and the changes in feeding regime (potential pretransport fasting plus time off feed during transport). Transport without feed for 12 h increases the risk of gastric ulceration. In addition, pathological changes have been seen after as little as 4 h of transport, when measured 5 days later.

Summary – prolonged thirst: The welfare consequence prolonged thirst is regarded as highly relevant in the transit stage. Prevalence may be high, if water is not provided to the animals or they, for some reason (such as lack of familiarity, neophobia or fear of other animals) are not able to drink enough water. Although some horses may not drink while the vehicle is in motion, many of them will drink if water is available on the vehicle during a journey break, but the volume consumed may be less than in non-transport conditions. This is particularly so if the water is from a non-familiar source. Depending on factors such as time off water before journey start and/or microclimatic conditions before and during the journey, horses may not be thirsty during the initial phase of the transit stage, but thirst will develop over time if water is not accessed. The duration of prolonged thirst depends on accessibility of water and journey duration, and severity is expected to increase with increasing duration, as the need for water becomes problematic for the animals. Prolonged thirst may lead to dehydration, discomfort and suffering. The available data does not allow a detailed determination of the interval between journey start and initiation of thirst. Haematological changes indicative of mild dehydration have been observed after journeys of 1 and 3 h without water, when being transported in the thermal comfort zone. Behavioural indicators of increased thirst as a result of transport are apparent after a 3-h journey.

Summary – resting problems: Resting problems are regarded as a highly relevant welfare consequence in the transit stage. Prevalence is expected to be high, as horses will not lie down, and because the needs of the animal to continuously make postural adjustments to compensate for vibration and acceleration changes, preclude sustained adoption of a normal resting posture. Resting problems may occur throughout the duration of the journey, but their effects are likely to be cumulative, potentially leading to fatigue and, eventually, exhaustion. Duration depends on journey duration, and severity is expected to increase with increasing duration, but is also affected by transport conditions such as type of road. Due to lack of knowledge, it is not possible to estimate a temporal cut-off for onset of this welfare consequence after initiation of the transit stage.

Summary – respiratory disorders: The welfare consequence respiratory disorders is regarded as highly relevant in the transit stage. Horses are particularly prone to respiratory disorders as a result of transport stress, with clinical signs apparent in many studies after journeys of 8–14 h, especially when space and tying conditions prevent the possibility to lower the head and clear the airways.

Other summarising considerations: In addition to the welfare consequences summarised above, the risk of animals experiencing pain and/or discomfort, as well as the severity of it, will also increase with journey time. This may happen if animals had a pre-existing, but non-identified, painful condition. Even though this should not happen, it is not always possible to identify pathological conditions while animals are on-farm, that could subsequently affect their ability to respond to transport.

In addition, animals which did not show a health condition before the journey may get injured during the journey due to, e.g. collisions or aggressive behaviour, and the pain and discomfort from such conditions will continue, and likely worsen, until the animal can be unloaded. In this weakened state, horses may be less able to cope with the challenges associated with transport, and their condition can potentially to deteriorate with time and journey duration.

The pain and/or discomfort from both types of the above-mentioned health conditions are not expected to be prevalent, but for the affected animals, the consequences may be severe, and will often develop over time. The duration of these negative affective states will depend on journey duration, as they cannot be terminated until the journey is stopped (or sometimes not until post-transport healing). During a journey, such health conditions may lead to suffering. It is, however, not possible to establish a temporal cut-off for when pain and/or discomfort may start.

In addition to the welfare consequences from health conditions, ABMs indicative of impaired immune function are reported in some studies, involving different conditions, even after journeys as short as one hour.

The table below summarises the estimated interval from journey initiation and until presence of the welfare consequences.

Table 19: Potential welfare consequences developed during the transit stage with the estimated time to start and expected development over journey time

Type of Welfare consequence	Welfare consequence	Number of hours[(2)] until the estimated start of the welfare consequence	Expected development over time
Continuous or semi-continuous	Motion stress	Motion stress continuous throughout the transit stage	Severity will increase over time leading to fatigue
	Sensory overstimulation	Sensory overstimulation repeated intermittent	Can lead to fear and distress
	Effect on immune function[(1)]	Changes of acute phase proteins, white blood cell counts were reported from 3- to 4-h journey, and antibody neutralising capacity reduced after 12-h journeys	Can lead to higher susceptibility to infectious diseases.
	Resting problems	Continuous throughout the transit stage. Signs of oxidative stress reported from 8-hour journey.	Severity will increase over time leading to fatigue
Progressively developing	Respiratory disorders	Signs of respiratory disorder in tracheal wash after 8 h and clinical disease after 10- to 14-h journeys.	Despite multiple factors being involved, the risk and severity of respiratory disorders will increase over time.
	Gastro-enteric disorders	An increase in gastric pH is seen after 2 h with initial signs of gastric ulceration after journeys of 12 h in unfed horses.	The risk and severity of gastro-enteric disorders will increase over-time.
	Prolonged thirst	Behavioural indicators of thirst first reported after 3 h and biomarkers of dehydration after as little as 1–3 h in some studies. Very variable between studies depending on temperature conditions.	Severity will increase with time leading to dehydration
	Prolonged hunger	Biomarkers of tissue mobilisation reported after 12 h in some studies.	Severity will increase with time leading to weakening and exhaustion
Sporadic	Pain and/or discomfort from injuries	Most injuries occur early in a journey.	If present severity will increase with time leading to suffering

(1): Immune system and oxidative stress are not WC from the list developed by EFSA (2022). Changes in these ABMs may, however, affect the occurrence of negative affective states in animals, and thus their welfare.
(2): The conditions of transport in the studies included in this table vary and may not necessarily be aligned with the recommendations on heat and space allowance. For further detail on the conditions, please see text above (Section 3.5.3.3).

3.6. Journey breaks

At some stage during a journey, horses will need feed, water and rest in order to avoid WCs. Horses may eat while in transit, but especially drinking and resting are expressed more in a stationary vehicle or unloading of the animals (Weeks et al., 2012; Iacono et al., 2007a,b).

Journey breaks can be done in two ways: (1) by providing the animals with feed, water and rest while the truck is stationary; and (2) by unloading the animals and providing them with feed, water and rest there (at a control post). Below, the potential WCs associated with the different possibilities are discussed.

3.6.1. Provision of water and/or feed on the vehicle while stationary

When animals are offered opportunities to feed, drink and rest on a stationary vehicle, the conditions on the vehicle and the time provided must be appropriate to allow the animals proper time for these activities. For example, Gibbs and Friend (2000) proposed that the provision of drinking water on-board a vehicle is a potential means of reducing the risk of dehydration.

It should be mentioned, however, that when vehicles are kept stationary in direct sunlight and without mechanical ventilation or air conditioning, for example during a rest stop without unloading the animals, the effective temperature inside the vehicle can increase quickly and considerably.

Thus, even though journey breaks while the animals are maintained on-board the stationary vehicle has merit, at least for horses transported singly, it still cannot prevent WCs such as heat stress, resting problems and restriction of movement, unless horses are transported under conditions that are very different from the typical practice of today. WCs such as prolonged hunger and prolonged thirst can, however, likely be mitigated by providing feed and water during the rest periods on the stationary vehicle.

3.6.2. Control posts

Control posts (CPs) are specialised livestock facilities, (mostly private) where animals can be offered a journey break after reaching the maximum journey duration. Currently, a stay in a CP has to be 24 h before the journey can be continued.

CPs are used exclusively for receiving, feeding, watering, resting, housing, caring for and dispatching transient animals. The operators of CPs are obliged to ensure that the animals receive the necessary care, feed and water, and before animals leave a CP, an official veterinarian must verify that they are fit to continue their journey (Kilchsperger et al., 2010). Also, CPs typically offer facilities for vehicles, drivers and competent authorities.

In this Scientific Opinion, the section on CP includes all kinds of actions and management of the animals, which take place during the interval from when the animals have been unloaded from the vehicle, and until reloading is started to continue the onward journey. Loading and unloading are covered in Section 3.4. For management, planning and logistics issues, readers are advised to consult the recommendations of the EU Transport Guidelines (Consortium of the Animal Transport Guides Project, 2017a).

Two EU projects were funded by DG Sante of the European Commission to renovate and promote high quality CPs in the EU and to develop an EU wide animal transport certification system. The first project 'Renovation and promoting high quality control posts in the European Union' was concluded in September 2013, while the second initiative 'Development of an EU wide animal transport certification system and renovation of control posts in the European Union' was concluded in 2015. Both projects showed that many CPs had problems to be financially profitable and needed rebuilding or renovation to reach high quality standards. To the best of our knowledge, newer data on CP standards are not available. In this Scientific Opinion, the recommendations from the projects are referred to as Porcelluzzi (2013), but it is important to bear in mind that this is not a reference to a scientific study.

Porcelluzzi (2013) stated that a resting period in a CP is the most appropriate solution to the challenges of long journeys in terms of animal welfare, because the animals are supposed to be getting adequate rest, feed and water, according to their needs, and to access comfortable bedding areas, as well as feeding and watering resources. Based on this, the authors of the report concluded that the use of CPs is an efficient means to improve animal welfare. However, as mentioned by Padalino et al. (2018d), neither this conclusion nor the opposite is supported by scientific data, as currently very limited studies on horse welfare at CPs in the EU are available.

3.6.2.1. Current practice

CPs have to be approved by competent authorities as well as authorised for specific species (EC 1255/97). In January 2022, there were CPs for horses on the EU approved list in 10 EU MS, with most CPs for horses in Germany (6), France (9) and Italy (8) (DG SANTE, 2021). Some control posts indicate further provisions such as '*individual boxes for horses available*', '*individual tethered horses*', or have '*places for group housing*'. Most CPs do not specify these details. Except for the UK, no list of approved CPs outside the EU exists, which has been described as a concern for competent authorities, as they may have difficulty to verify whether the place outside the EU, where animals are planned to stop to be unloaded, has suitable conditions for ensuring welfare.

According to EC 1255/97, the fitness of transported animals must be checked by CP staff at unloading, and at least once every 12 h during the stay at the CP. In addition, the official veterinarian must check that the animals are fit for transport before they resume their journey. The official veterinarian shall decide, case by case, whether animals are fit enough to continue the journey, should be treated or if emergency killing should be performed.

3.6.2.2. Highly relevant welfare consequences

Arriving at a CP, horses have to be unloaded. The potential WCs and ABMs for unloading/loading can be found in Sections 3.2.2 and 3.4.2. A stay at a CP will potentially increasing stress due to the additional handling (loading/unloading), exposure to a new environment and the risk of disease associated with exposure to pathogens (Tarrant and Grandin, 2000).

The welfare consequences are very much dependent on the management (e.g. cleaning and disinfection procedures, knowledge of animal welfare, availability of reservation system), the housing conditions (type of stable, ventilation, bedding, etc.), the equipment (e.g. ramps to (un)load, drinkers) and training of the staff at the CPs.

Irrespective of the duration of the stay, a stay in a CP may involve several hazards for animal welfare.

The flow of animals at a CP is a major issue with respect to the spread of infectious diseases in and outside the EU (Sluyter, 2001), with potential collateral consequences for animal welfare. 'Risks are due to the mixing in the same place of animals of different origin, not only because of the simultaneous presence of the animals in the Control Post, but also due to poor cleaning and disinfection procedures between successive consignments. The European regulation establishes rules and procedures, applying to a list of diseases. However, the Control Post owner and staff, transporters and the official veterinarian in charge should also be aware of the possibility that non-listed diseases may spread and should be therefore well informed and trained so as to be able to detect non-listed diseases, as well as symptoms or changes in the behaviour of the animals that could indicate health problems' (Consortium of the Animal Transport Guides Project, 2017b). Disease is always a challenge for animal welfare (as reviewed by Broom, 2006) and can lead to animals needing treatment or even turning unfit for transport during a journey. During a journey, changes in the clinical condition of animals are a significant risk to their welfare (Padalino et al., 2015). However, limited scientifically published data exist that document changes in the clinical condition of horses during and/or after journey breaks at CPs. Hence, it is not possible to assess this risk.

Whether the WCs of the use of CPs is increased by successive stays (during very long journeys where horses are unloaded for rest more than once) have not been examined but cannot be excluded.

The highly relevant WCs identified for the CP stage are sensory overstimulation, handling stress, injuries, resting problems, respiratory disorders and gastro-enteric disorders. Definitions of these WCs can be found in Table 4 and ABMs for the listed welfare consequences can be found in Section 3.2.2. Detailed description of the hazards, preventive and corrective/mitigating measures for handling stress and injuries can be found in Section 3.3. Below, the hazards (in bold), preventive (PRE), corrective and mitigating measures for the additional hazards and WCs selected as highly relevant for horses at this stage are described.

i) Sensory overstimulation

Horses easily get agitated in unfamiliar situations and with various sensory stimuli such as unfamiliar auditory stimuli, flickering or blinding lights and unfamiliar olfactory stimuli (Rørvang et al., 2020). According to the quality control post handbook (Porcelluzzi, 2013), sensorial stress is an important potential WC in CPs.

Novel stimuli: At a CP, there are novel stimuli that the animal may have never experienced. A sudden novel stimulus such as light reflections, unfamiliar auditory input, unfamiliar olfactory input, e.g. from disinfectants, can induce stress and fear in horses.

- PRE: The stable environment, in which the animals are kept in the CP, should be controlled in order to minimise unfamiliar and sudden stimuli. Before (un)loading, the area should be checked to avoid sensorial stressors being present. The stable should be without disturbing auditory stimuli with appropriate lighting (no light reflections that can frighten the animals), with appropriate flooring for minimising the risk of slipping, enough space and bedding to rest and well ventilated.

<u>Corrective/mitigating measures for sensory overstimulation</u>

If sensory overstimulation is suspected in the CP, the area should be checked to see if any sensory stressors are present, and remove them if so.

ii) Resting problems

If resting problems appear in a CP, it can have severe consequences for the welfare of the animals. The hazards for this WC in a CP are:

Floors quality and bedding: the type of floor, quantity and quality of the bedding affect the resting of the horses in the CP.

- PRE: In the quality control post handbook (Porcelluzzi, 2013), it is recommended that floors are non-slippery, cleanable and sufficiently drained. Soft, non-slippery bedding is important. The animal transport guidelines (Consortium of the Animal Transport Guides Project, 2017a) recommend wood shavings (free from splinters and dust, and not made from hardwood) and several kinds of chopped straw (dust free, and not likely to cause intestinal obstruction when eaten) are suitable bedding materials. A layer thickness of 1 cm per 100 km is proposed for wood shavings.

Insufficient space allowance: Horses need space to be able to rest, lie down and change posture.

- PRE: It is recommended that the facility is large enough and comfortable enough to allow horses to lie down and rest. The stalls in the CP must be large enough for the horse to be able to drink, feed and rest. Generally, a stall for an average (500 kg) horse is recommended to be at least 3.0 $\times$ 3.0 m (Checchi and Casazza, 2013).

Mixing with unfamiliar horses: When horses arrive to CP they may be mixed with unfamiliar horses. This can happen whether they are single stall transported, or transported in a group. Newly mixed horses may show aggression towards each other and limit the possibility of other animals to rest.

- PRE: It is recommended that horses that are singly stalled during transport are housed in single stables. Horses that are being transported in a group, should be housed with that group and only that group.

<u>Corrective/mitigating measures for resting problems</u>

If resting problems are suspected in the CP, a thorough inspection should be done to evaluate the potential hazards and, when possible, eliminate them.

iii) Gastro-enteric disorders

Transport of horses can be associated with the development of colic in horses, and may be associated with colitis, gastrointestinal impaction, displacement or obstruction (Archer et al., 2006; Hillyer et al., 2002; Stewart et al., 1995). Among the factors contributing to this risk are the stress of transport and the changes in feeding regime (potential pretransport fasting plus time off feed during transport).

Time off feed: Horses may have been off feed for at least 8 h when they arrive at a CP, and so are likely hungry. Long journeys without food can lead to the development of stomach ulcers (Padalino and Raidal, 2020) and can also predispose horses to colic.

- PRE: In the quality control post handbook (Porcelluzzi, 2013), it was recommended that the minimum quantity of feed provided should correspond to the amount of feed required for the body maintenance, and that feed must be of homogenous quality to avoid competition.

Unfamiliar feed: If feed is given, which the horse is not familiar with, especially with a high proportion of concentrate, digestive problems or colic may develop (Sadet-Bourgeteau et al., 2017).

- PRE: At the CP, horses should be given the type of feed that they are accustomed to using clean feeding equipment that they are familiar with. Too much concentrated feed should be avoided. Feed should be given together with access to water (Freeman 2021).

<u>Corrective/mitigating measures for gastro-enteric problems</u>

If gastro-enteric problems are suspected during the stay at the CP, animals should be inspected by a veterinarian who will decide the appropriate course of action.

iv) Respiratory disorders

Transport pneumonia, also named shipping fever or pleuropneumonia, is commonly associated with long-distance transport of horses (Section 3.5.3.3D). In view of the high frequency of shipping fever in horses transported long distances, all horses should be checked at the CP to make sure they do not have symptoms.

A dusty environment: Dust, which irritates the lining of the respiratory tract and may also carry deleterious microorganisms and spores, may come from poor quality bedding.

- PRE: The CP should have bedding which does not give a lot of dust.

Corrective/mitigating measure of respiratory problems

If respiratory problems are detected during the stay in the CP, horses should be treated by a veterinarian.

3.6.2.3. Journey break – summarising considerations

At some stage during a journey, horses will need feed, water and rest in order to avoid being exposed to hazards leading to WCs such as prolonged hunger, prolonged thirst and resting problems. Horses may eat while in transit, but drinking and resting, in particular, require a stationary vehicle or unloading of the animals (Weeks et al., 2012; Iacono et al., 2007a,b).

When animals are offered opportunities to feed, drink and rest on a stationary vehicle, the conditions on the vehicle and the time provided must be enough to make sure that the animals have time to, and possibility to, feed drink and rest.

Theoretically, unloading horses into a stable or pen allows the animals to have access to resting, watering and feeding areas in order to mitigate the WCs of transport. However, stopping a journey to unload the animals to provide a period of rest, feed and water involves a number of hazards relevant for animal welfare such as the risk of stress, injury and infectious diseases. Some of these risks can be mitigated by offering high quality conditions to the animals, whereas others, such as the novelty of the surroundings, the extra unloading and reloading, cannot be avoided. Thus, care must be taken to minimise the risk of hazards and to facilitate proper rest and effective access to feed and water.

If a stay in a control post or similar should be beneficial for the welfare of horses during transport, any journey break needs to be long enough for each animal to eat, drink and rest. Assuming the recommendations made on microclimatic conditions (Section 3.5.3.1), space allowance (Section 3.5.3.2) and maximum journey times (Section 3.5.3.3) and given the limited amount of data on recovery periods, a period of 12–24 h would seem to be enough to allow for feeding, drinking and recovery from fatigue. Setting the required period will, however, depend on the journey experienced previously. It will also depend on the state of the animals. The duration of a rest at a control post, allowing welfare consequences from the transit stage to be mitigated, constitutes a gap in knowledge.

Whether the WCs of the use of CPs are increased or decreased by successive stays (during very long journeys where horses are unloaded for rest more than once) have not been examined.

3.7. Specific scenario: Long journeys to slaughterhouses

The specific scenario relevant to horses that EFSA was asked by the European Commission to consider was the transport of horses on long journeys to slaughterhouses. Based on the current legislation, specifying that a long journey is 8 h or more, this assessment takes as a starting point that the horses are transported for at least 8 h. In addition, this section only considers issues that are specific for slaughter horses in addition to those already dealt with above.

A) Current practice

Globally millions of horses are slaughtered for meat each year (Fletcher et al., 2022). Significant differences are found in the consumption of horse meat in Europe. In some countries the eating of horse meat is not a common practice (e.g. UK and Romania), in others (e.g. Italy, France and Belgium) horse meat is part of the diet. Horses destined for slaughter can be produced locally or sourced from other countries (Leadon, 2012). An estimated 254,000 horses were slaughtered in the EU for meat in 2015 (FAOSTAT, 2022) but the number has decreased to about 167,000 in 2020 (FAOSTAT, 2022). Italy slaughters the most horses in the EU with 22,575 annually, followed by Poland slaughtering 21,231 (FAOSTAT, 2022). Those numbers may increase in the future due to recent efforts to promote horsemeat as a more environmentally friendly and healthier alternative to bovine meat (Belaunzaran et al., 2015).

The European Commission has a number of categories of Equidae. **Equine animals not intended for slaughter**, which are all kept equine animals not intended to be moved to a slaughterhouse, irrespective of their status as excluded or not excluded from slaughter for human consumption. **Equine animals intended for slaughter**, which are all kept equine animals intended to be moved to a slaughterhouse. The implementation of these rules may differ between Member States. Horses may be bred for meat production or a horse can switch category after being a sport horse (Uldahl et al., 2019). The number of these horses has been shown to increase in Europe in times of economic recession.

The total number of horses transported from one EU MS directly to slaughter in a different MS decreased from 17,000 in 2019 to 10,000 in 2021 (TRACES, 2022). Italy was the biggest recipient of slaughter horses from other MSs (85% the total EU), receiving animals mostly from Poland, France and Spain, in that order (TRACES, 2022). In addition, ~ 4,000 horses were imported to Italy during those years from other MSs. Some of these might have been meat horses transported to Italy but not directly to slaughter. Based on a survey of EU MS carried out by EFSA in 2021 (unpublished), the vast majority of MSs do not record the moves of horses within MSs, including the movement of horses to slaughter.

B) Concerns specific to horses transported on long journeys to a slaughterhouse

The concerns for horses being transported by road to slaughter are essentially the same as those for any horse being transported by road. Fletcher et al. (2022) recently performed a meta-analysis of the literature on equid welfare at slaughter in the world and concluded that the majority of the studies were performed in high-income countries and focused on welfare of horses during transport, but only few of them were on horses transported to slaughter. These few studies were performed in North America, South America and Europe.

In Canada, Roy et al.(2015a) studied in detail 100 horses from 40 loads and found that 33% of them had soft tissue injuries identified by visual assessment. Pretransport assessment was not performed, so those injuries could not be directly attributed to the transport conditions.

In South America, Miranda de-la Lama et al. (2021) assessed the welfare of horses at arrival in slaughterhouses in North Mexico of 2,648 horses transported in 121 loads. Most of the loads (77%) were coming from USA (average journey duration of 16 h) and the remaining ones from Mexico (average journey duration of 9 h). The health problems most frequently observed were ocular discharge (23%), nasal discharge (12%), skin wounds (11%) and lameness (9%). No significant differences were found between the North American and the Mexican loads.

In Europe, according to EFSA (2011), horses destined for slaughter within the EU were often in a poor state of health before transport, were generally transported under poor conditions and experienced numerous abuses of their welfare. These animals tend to be of lower value and less is invested in ensuring that conditions of transport are satisfactory. The proportion of horses deemed unfit for transport at the point of origin was a big issue and this problem increased as a result of the journey. The main symptoms of unfitness were those associated with respiratory disorders and these were greatly exacerbated by transport.

In addition to the fitness for transport, in his review, Leadon (2012) also reported the following specific concerns to the transport of horses to slaughter: the type of vehicles used, resting periods, feeding and watering while in transit and sporadic injuries and fatalities.

Messori et al. (2016) collected data of 926 horses from 51 loads at slaughterhouses in Italy. The majority were coming from Poland and the average journey duration was 24 h. Half of the horses were transported in vehicles equipped with a water supply (troughs 9.2% and mobile drinkers 39.2%). The watering tanks of the vehicle were partially empty (51%) or totally empty (49%) at arrival at the slaughterhouse. Almost 90% of the examined horses were transported in a single stall with a mean space allowance of 1.64 m^2/horse. No mortalities nor severe injuries nor non-ambulatory animals were reported. A low prevalence of severe lameness (0.1%) was observed. In addition, 11% of the horses presented signs of sweating and 7.8% signs of diarrhoea.

Padalino et al (2022, personal communication) have monitored journey conditions of 1019 horses transported in 52 loads travelling to slaughterhouses in Italy. The average duration of the transport was 37 h, including stops at control post. Horse welfare was assessed using a modified version of the check list proposed by Messori et al. (2016). No mortality was reported. The preliminary descriptive statistics of the health and welfare problems recorded are reported in the 21 below. Unhandled horses transported loose in a small group showed minimal problems, even if they had travelled over a long distance and stopped in resting facilities along the way. Horses being transported in a single stall had a significantly higher probability of displaying injuries at unloading. These injuries were mainly located on eye sockets and on the base of the tail (Table 21).

Table 20: Frequency of health issues observed when unloading horses after long journeys to slaughterhouses in Italy (Padalino 2022, personal communication)

At unloading	Complete data set (N = 1,019)		Horses in single stall (N = 613)*		Horses in a group (N = 395)*		Chi-squared Fisher exact test single vs. group
	N	Prevalence	N*	Prevalence	N	Prevalence	p-value
Lesions	155	15.21%	148	24.14%	6	1.51%	< 0.001
Nasal discharge	90	8.83%	70	11.42%	17	4.30%	< 0.001
Other discharges**	72	7.07%	61	9.95%	10	2.53%	< 0.001
Diarrhoea and abnormal faeces	29	2.85%	3	0.49%	26	6.58%	< 0.001
Lameness	27	2.65%	19	3.10%	7	1.77%	n.s.
Cough	5	0.49%	5	0.82%	0	0%	n.s.

*: The total number of horses in a single stall is 613. Four consignments out of the 32 carrying single stalled horses had also 11 grouped horses.

**: Among the types of discharges observed, only four horses had penile or vaginal discharges, and the remaining had lacrimal discharges.

3.8. Transport of horses by Roll-on-roll-off ferries

A) Current practice

A roll-on/roll-off (RO-RO) ferry or vessel means 'a sea-going vessel with facilities to enable road or rail vehicles to roll-on and roll-off the vessel' (DAERA, 2013). Thus, a roll-on/roll-off vessel is excluded from the definition of a 'livestock vessel', onto which the animals are unloaded.

'RO-RO vessels, unlike livestock vessels, do not require inspection and approval before they are used to carry animals. However, the Competent Authority may decide to carry out appropriate checks at any stage of a long journey (see 3.9) in order to check compliance of journey and the means of transport' (DAERA, 2013).

Time spent on a RO-RO ferry counts towards the journey time. However, currently if crossing by a RO-RO ferry between two geographic points within the EU cannot be completed within the permitted journey times, the crossing may still be undertaken. In such cases the animals, once disembarked, must be rested for 12 h in the immediate vicinity of the destination port, unless the final leg of the journey to destination can be completed within 2 h. In practice, this is not always practical (no place at or in the vicinity of the port to rest).

Some of the sea journeys of horses transported by roll-on-roll-off ferries can take more than 36 h (as the journey from Canary Islands to mainland Europe) (WHW, 2022). Another common RO-RO transport route is from Ireland to Cherbourg (France) with involves a sea journey of more than 16 h.

In the Northern part of the UK, a 'cassette' system has been developed by the ferry line and the livestock industry. The cassette system 'was designed to protect the welfare of animals in sea crossing from offshore islands. Once on-board, each cassette is securely lashed to the deck in accordance with International Standard ISO9367, ensuring that the container does not move while the ship is in transit. The livestock containers have several key design features to support high standards of animal welfare which include a 'hospital' pen capability, solar powered inspection lights and an integral ladder that allows inspection of the upper deck. Water is provided constantly through connection to the vessel supply, feed is also provided through integral feed racks and there is an innovative effluent storage system' (WHW, 2022).

B) Concerns specific to horses travelling on Roll-of-Roll-off ferries

The WCs, ABMs, hazards, corrective/mitigative and preventive measures detailed in Sections 3.3, 3.4, 3.5 and 3.6 above are all applicable to the transport of horses by RO-RO ferries. The following is a list of additional concerns that are particular to the RO-RO transport.

i) Heat stress and too little ventilation

Depending on the deck and the place on the deck where the vehicle is loaded, too hot or too cold conditions can occur. It is important that on the RO-RO, the animals stay within the TCZ, that animals are protected from the weather, the sea and it is crucial that there is enough ventilation. Airflow around and through the vehicle's animal compartment must be sufficient to ensure that a suitable environment is maintained within the vehicle. There must be sufficient space above the animals, when standing in a natural position, to permit adequate ventilation and airflow through the vehicle's animal compartment. Requirements for ventilation will depend upon the species and age of animal, the stocking density, the ambient temperature and the RH. Animals, even when at rest, generate heat and moisture which needs to be removed from the vehicle to avoid conditions quickly becoming unsuitable and prejudicial to animal welfare.

Vehicle exhaust fumes entering the animal compartment during loading and unloading which will quickly have an adverse and possibly lethal effect on the animals is another concern. Therefore, ideally livestock vehicles should be loaded last and unloaded first.

If a livestock vehicle is put on an enclosed deck, it must be fitted with a mechanical ventilation system, which has an alarm and an emergency power source in case of failure. The air-change capacity should normally not be less than 20 times per hour.

If an open deck is used for animal vehicles, it must provide protection from sea water and other weather elements. Vehicles stowed on open decks will generally benefit from better airflow than those in enclosed decks, but are more at risk of overheating if located in direct sunlight, particularly when little air is moving across the deck. Strong cold winds may have an adverse effect, particularly on young animals.

Attention needs to be given to multitier vehicles. For horses, no two-tiered vehicles should be used unless the top deck is empty.

If allowed on the ferry, and the vehicle needs electricity for ventilation or microclimatic control, the vehicle should be placed in a convenient place in the ship with access to electricity.

ii) Motion stress

Horses transported by sea can be susceptible to motion stress especially when the sea is rough. 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 ferry. Road vehicles and rail wagons shall be secured to the ferry before the start of the sea journey to prevent them being displaced by the motion of the vessel.

iii) Prolonged thirst

Weather disruptions often cause ferry services to be postponed or cancelled. This can result in animals having to wait for a long time at the port or having to return back to their origin without water supply.

iv) Difficulties to attend to animals in case of emergencies

In a RO-RO ferry, it will not be possible to unload animals if they need emergency care. Therefore, it will be important to make sure, before entering the ferry, that all animals are fit and healthy, well-positioned, that the temperature and ventilation are within comfort ranges, that the vehicle is well-secured and that the drinkers are working and easily reachable. Ideally, the vehicle should be placed so that the animals can be inspected and if needed animals can be unloaded in case of an emergency. In reality, this will be difficult on a RO-RO as it will be difficult to unload and separate the animals and no veterinarian is on-board. Ideally, for voyages above 3 h, a suitable means of emergency slaughter and a person trained to use it should be on board.

v) Exceeding the maximum journey time

Vehicles have to wait before they can board the ferry and before the ferry leaves. Some commonly used sea journeys, e.g. from Rosslare to Cherbourg, take 17–18 h without the waiting time before boarding and the (un)boarding time. A genuine consideration of the journey to the ferry, time taken while waiting to board the ferry and onward to the destination or control post must be taken into account.

3.9. Transport by air

It is hard to find statistics on horse shipped by air. Before 2019, the ATA suggested that ~ 30,000 horses were shipped worldwide yearly. The industry has been highly affected by the COVID pandemic. The current use of transport of horses by air constitutes a gap in knowledge.

A) Current practice

Horses arrive at airport by vehicle, and then are loaded into partitioned air stables or jet stalls (usually 294 cm (length), 234 cm (width) and 232 cm (height)) designed to accommodate one, two or three horses, side by side (Waran et al., 2007a,b). Air transport is used primarily for attending competitions, racing (Hurley et al., 2016) and for breeding purposes (e.g. shuttle stallions) (Padalino, 2015). Horses are also transported by air from Europe, mainly France, to Japan for slaughter (franceinter, online).

Air transport is regulated by Live Animals regulations (IATA). However, the International Air Transportation Association (IATA) guidelines are largely based on the experience of industry players on air safety and cargo requirements and are less focused on the welfare needs of horses, with little scientific evidence or independent validation of the welfare provisions suggested.

Horses must arrive at the airport in advance, to make sure that there is time enough to be checked, watered and loaded into the jet stalls. It is recommended that horses have been already trained for loading and transport and they must be managed by handlers with experience in horse handling. After being loaded into the jet stall, the jet stall is weighed and the cargo is organised with the aim to balance the weight load properly.

The horses wait inside the jet stall with feed until there is the permission to be loaded into the plane. However, the waiting period between the loading in the jet stall and loading into the plane can be long, and microclimatic conditions inside the jet stall may become critical, since the humidity can rise up to 90% (Padalino and Riley, 2022b).

During the loading into the plane, the jet stall is lifted and positioned, and horses are exposed to new stimuli of different modalities that could elicit sensory overstimulation (Figure 21). During the journey, in compliance with IATA regulation, horses must be regularly checked (every 3–4 h) and watered, while hay nets are available. In a study by Padalino and Riley (2022b), from Germany to Japan, that lasted 12 h, all horses ate (10 $\pm$ 0.9 kg of hay/horse) and drank (17 $\pm$ 4.7 L/horse). Some companies untie the horses, so they are free to move their neck. Landing and unloading are another phase of journeys by air, where horses are exposed to novel and possibly frightening stimuli. At arrival, horses are usually loaded into a vehicle and go to a quarantine facility.

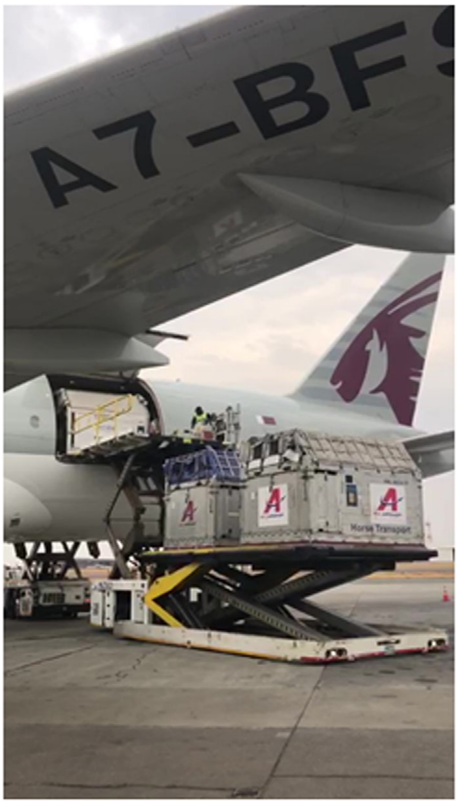


Figure 21: Jet stalls during loading of horses into a plane (source: Barbara Padalino, UNIBO)

There have been only few studies on the effects of air transport on horse health and welfare. The most stressful phases of air transport are loading, unloading, take-off and landing (Stewart et al., 2003). During the flight, horses tend to have resting heart rate values and engage in resting behaviours, indicating that they may settle better to air than road transport (Munsters et al., 2013). Quarantine regulations are generally applied after air transport to minimise biosecurity risks. However, restraint in the quarantine box stalls can cause an increase in heart rate associated with environmental stress (Ohmura et al., 2012).

B) Concerns specific to horses transported by air

i) Respiratory disorders

One of the most susceptible physiological systems to infections in horses after long-distance transport is the respiratory system (Maeda and Oikawa, 2019). A study of horses transported to Hong Kong by air found a prevalence of shipping fever of 10.8%, with at least one horse affected in 49/81 (60%) flights. Journey duration was confirmed as a risk factor and may be difficult to control in the face of flight delays and quarantine requirements (Hurley et al., 2016).

Oertly et al. (2021) also investigated the effect of long-distance transport by air; 122 Warmblood horses (28 stallions, 52 geldings and 42 mares; aged from 8 to 17 years, with a median age of 11 years) were followed from Liege (Belgium) to Miami (USA), and then to Mexico City (Mexico). After returning to Europe, 10 days later, the horses were transported to Shanghai (China). Health checks and serum amyloid A (SAA) determination were performed before and after the journey. From the 181 study cases, 31 were reported as clinically sick after transport (17%). In the sick horse group, the following clinical signs were observed: coughing (n = 15), anorexia (n = 5), nasal discharge (n = 4), swollen and painful lymph nodes (n = 3), sweating (n = 2) and distal limb swelling. Horses with elevated SAA 24 h after the study developed respiratory disorders. A cut-off of 23 μg/ml of SAA was suggested to early diagnosis of shipping fever. SAA was suggested to be more sensitive than rectal temperature to identify horses at risk of shipping fever.

Of 143 horses imported into the USA from Europe and studied by Middlebrooks et al. (2022), 30 showed signs of subclinical inflammation based on haematological abnormalities, and four horses were sick (abnormal physical parameters with or without haematological abnormalities). The four sick horses were febrile (2 horses), showed signs of submandibular lymphadenopathy (2), nasal discharge (1) and intermittent coughing (1). Among 109 healthy horses, 12 required follow-up medical appointment for reasons including ocular conditions (corneal ulcer, hypopyon; 4 horses), fever of unknown origin (2), post-vaccine related fever (2), bilateral nasal discharge and coughing (1), muscle wasting (1), urticaria (1) and colic (1). For the subclinically affected horses, follow-up medical appointments were performed in six horses for distal limb swelling (2 horses), nasal discharge (2), coughing (2), post-vaccine related fever (1) and urticaria (1). Amongst the subclinically affected horses, one experienced elevated rectal temperature of 38.9°C and another horse experienced an episode of oesophageal obstruction prior to arrival to the quarantine facility.

The effects of five journeys from Frankfurt to Tokyo, transporting a total of 89 horses, were described in a pilot study (Padalino and Riley, 2022b). Of these 89, data were collected from 24 horses (randomly sampling four or five horses per journey) (10 mares and 14 geldings, 23 Warmbloods and one Quarter Horse, aged 9.4 ± 2.8 years and weighing 575 ± 28 kg) from before to 5 days after the journey. All horses were deemed fit for transport before the journey and physiological parameters were normal. At the airport, after being transported by road for 12 h, their physiological parameters were slightly increased and 10 had watery nasal discharge. All horses loaded easily in the air stalls, tied to one side on a long lead, and were transported in cargo planes, and ate and drank in transit. At the last check before landing heart rate was in the normal range, respiratory rate was higher than normal (30±12 rpm), five horses had increased capillary refill time (4 s), 12 horses had serous bilateral nasal discharge, one had yellow bilateral nasal discharge and one serous unilateral nasal discharge. Most horses were able to cope with the transport stress; 10 showed stress-related behaviour (i.e. pawing, head tossing, tail wishing, stamping, vocalising) at the beginning and one until the middle of the journey, but no horses required sedation or treatment. Physiological parameters normalised quickly and none of the 89 horses developed shipping fever or illnesses.

ii) Injuries

In the study of Padalino and Riley (2022b) mentioned above, 3/24 of the closely monitored horses had minor injuries due to air transport. Horses often get frightened and they may get injured during scrambling.

iii) Heat stress

Preliminary data have shown that during the waiting time at the airport, the temperature and humidity inside a jet stall can increase rapidly (inside the jet stall T = 27 and H = 90% at loading in the plane) (Padalino and Riley, 2022b). During the journey, since the temperature and the humidity within the cargo are set much lower, the situation inside the jet stall improves and tends to be within the TCZ of horses (see section 3.5.3.1)

iv) Sensory overstimulation

During the different stages of transport, horses are subjects to a variety of novel stimuli of different modalities. By use of measures of heart rate variability, Ohmura et al. (2012) demonstrated that loading, taking off, landing and unloading were the most emotional stages for the horse.

v) Motion stress

Munster et al. (2013) suggested that horses cope better with transport by air than by road.

3.10. Transport of donkeys

A) Current practice

There are ~ 400,000 donkeys in Europe (FAOSTAT, 2022), kept with very different purposes across the continent. While many of them are kept as companion animals (sometimes held in rescue centres or sanctuaries), for leisure activities (zoos, riding centres) or as part of therapy associated programmes, others have production purposes, as pack animals or in milk and meat farms (Dai et al., 2016). In other parts of the world (Africa and India, among others) donkeys are commonly used to transport different goods, especially in remote areas and where vehicles are not available (Ake et al., 2013). In China, where the skin of donkeys is very valuable as traditional remedy (Ejiao) (Tatemoto et al., 2021), the farming of donkeys is changing to large scale production, involving long distance transport of animals (Jiang et al., 2021).

B) Concerns specific to donkeys

i) Social behaviour

Donkeys are gregarious animals, and as such, are negatively affected by the separation from their group. The social organisation (size and composition) of donkeys is flexible and dependent on the environmental conditions, including family groups to exclusively male groups (De Santis et al., 2021).

An important characteristic of donkeys, that differs from horses, is the frequent strong relationships developed by some pairs of donkeys (or a donkey and horse) (Murray et al., 2013). Separation of these bonded animals may result in severe stress leading to anorexia and potentially hyperlipaemia (Duncan, 2018). Being aware of these pairs is essential to avoid stress during transport, as these individuals will benefit from being managed together (Murray et al., 2013).

ii) Adaptation to microclimatic conditions

For donkeys, the following are considered normal physiological parameters (i.e. heart rate 31–53 beats/min and respiration 13–31 breaths/min) (Burden and Thiemann, 2015). However, the literature available on heat and cold stress in donkeys is scarce. Specific characteristics of donkeys such as the long ears and lower sweating rate seem to help them thermoregulate (Proops et al., 2019). When the behaviour of horses and donkeys was analysed in relation to the search for shelter under different climatic conditions, donkeys spent more time sheltering in low temperatures (under 10°C), under rain or windy conditions and sought shelter faster than horses. On the contrary, horses sought shelter more when temperatures were above 20°C (Proops et al., 2019). Based on these results, it was considered by the authors that the TNZ of donkeys could be higher than of horses, but no quantitative information was provided.

iii) Pain and fear

Although donkeys are often considered together with horses in terms of welfare and behavioural characteristics, donkeys tend to react to fear, pain or pressure in a more subtle way than horses, and to display a different repertoire of behaviours (Burden and Thiemann, 2015). The lack of obvious expression of pain in donkeys is not necessarily due to a higher tolerance of pain, but could be due to the lack of understanding of the behavioural changes in donkeys so far, as the limited research in donkeys and their hybrids is very notorious (Ashley et al., 2005). This may result in unnoticed diseases or painful conditions in donkeys which often only show apathy, social isolation or prolonged recumbency as signs of illness.

iv) Hyperlipaemia

Hyperlipaemia is more frequently observed in donkeys than in other animal species, and due to the high mortality rates of the animals affected (Burden et al., 2011), it is considered one of the big concerns for donkey diet management. In most of the cases hyperlipaemia occurs subsequent to a concurrent disease, such as dental disease, triggered by specific events that lead to negative energy balance (Burden et al., 2011). Specific risk factors that predispose to hyperlipaemia and development of an insulin resistant state include lactation, pregnancy, obesity or older age (Paraschou et al., 2018). When donkeys enter into a negative energy balance due to prolonged fasting, and exacerbated through stressors (such as transport, important changes in management or diet, loss of a companion, stress/anxiety), body fat mobilises, plasma triglyceride concentration increases and, if the situation is maintained, this fat can be infiltrated in liver, pancreas or kidneys, potentially leading to organ failure and death (Paraschou et al., 2018).

v) Research on donkey welfare during transport

During the last years, protocols for the assessment of welfare in donkeys on farm have been developed (AWIN, 2015b), including a recent app to facilitate data collection on-farm (Dalla Costa et al., 2021). However, there is still very little research available on welfare of donkeys during transport (Dai et al., 2020a).

In 1995, Forehead (1995) evaluated the effects of transport in the same six donkeys under different conditions involving journeys of 30 min and 4 h, fed and previously fasted (1 day and 3 days off-feed). Transport of the fed animals resulted in an increase of cortisol concentrations, but no differences in triglycerides, cholesterol, glucose, total protein, albumin, globulin or urea. Fastened animals showed alterations in all the above-mentioned metabolites after transport and the changes in cortisol were lower than in the fed animals. Based on the results (same animals were subjected to repeated journeys over a 12-month period), no evidence of habituation to transport was observed (Forehead et al., 1995).

Fazio et al. (2013) studied the influence of transport on hormonal changes including β-endorphin, adrenocorticotropic hormone (ACTH) and cortisol levels in six horses and six donkeys after 1 h of transport. All animals were previously habituated to transport and transported in identical conditions (individual stalls ~ $2m^2$/animal). Both donkeys and horses presented differences in the cortisol levels after transport, confirming the stress response to transport. However, only donkeys showed a difference in ACTH levels after transport in comparison with the basal levels. No significant differences in β-endorphin were found. The data confirm the existence of endocrinological differences between horses and donkeys and suggested that short journeys were more stressful for donkeys (Fazio et al., 2013).

The significant increase in plasma ACTH in donkeys after transport was confirmed by Jiang et al. (2021). Donkeys non-habituated to transport showed an increase in cortisol, ACTH and heat shock protein 90 after 21 h of transport. In addition, after transport, the gut microbiota was altered with a decrease of bacterial richness, potentially leading to decreased resistance to infectious agents.

Other authors have explored the use of other indicators to measure the changes in response to transport in donkeys. Fazio et al. (2017) confirmed that donkey thyroid response to short journeys (~ 1 hour) was similar to previous observations in horses, but not identical. Specifically, the authors confirmed the activation of the thyroid gland in both horses and donkeys, although the levels of thyroid hormone T4 in donkeys were higher than in horses (T3 higher). Ferlazzo et al. (2020) reported a specific T3 decrease as an effect of isolation, but a significant T3 increase after long-distance transport. The authors suggested that responses to different energy demands during transport in equines could be hypothesised, with T3 playing a central role in physiological stress response.

Recently, Dai et al. (2020a) suggested the potential use of salivary chromogranin A (CgA) as a marker of stress associated with transport in donkeys. They transported 19 donkeys in two journeys (65 min each) on two consecutive days and observed a significant decrease of CgA after transport.

Dai et al. (2020b) studied the effect of habituation programmes in donkeys (specifically foals following their conspecifics) comparing behavioural responses, loading time and salivary cortisol between donkeys that were never transported (control group) versus donkeys with some experience (minimum five journeys). The results showed a reduced time for loading and less stress related behaviours during loading in donkeys habituated to transport in comparison with the control group. These results contradict the previous study by Forehead (1995) indicating a lack of evidence of habituation to transport in donkeys, and agrees with the current knowledge in horses (York et al., 2017).

In summary, no scientific evidence has been found to support the treatment of donkeys in a different manner than horses during transport.

3.11. Uncertainty analysis

The uncertainty in the assessment performed for this scientific opinion was investigated in a qualitative manner following the procedure detailed in the EFSA guidance on uncertainty analysis in scientific assessments (EFSA Scientific Committee, 2018). The outcome of this scientific opinion is the identification and description of the highly relevant WCs, the related ABMs – measured in a qualitative or quantitative way – and hazards causing these WCs. Based on this identification and listing of WCs and ABMs, conclusions and recommendations are formulated allowing for different mitigation and preventing measures for the identified WCs (resource and management-based measures). As the identification and listing of the highly relevant WCs and ABMs was mainly based on expert opinion (integrating the severity, duration and occurrence of each WC) and not on a full comprehensive risk assessment, the uncertainty analysis was limited to the identification and description of the sources of uncertainty in the assessment carried out. A table describing the sources of uncertainty associated with the methodology used in the assessment is presented below (Table 21).

Table 21: Sources of uncertainty (in a non-prioritised order) associated with the assessment methodology and inputs (extensive literature search, expert's opinions) for the identification and assessment of the most relevant WCs and ABMs

Source of uncertainty	Nature or cause of the uncertainty	Impact of the uncertainty on the assessment
Literature search – Language	The search was performed exclusively in English. More studies could have been identified by including references with abstracts in languages other than English.	WCs might have been selected that in reality belonged to another category than highly relevant, and WCs that in reality were highly relevant might have missed being selected.
Literature search – Publication type	The studies considered included primary research studies identified through the extensive literature search and grey literature (fact sheets, guidelines, conference papers, EU reports, book chapters, etc.) known to the EFSA Experts, but an extensive search of the grey literature was not conducted. Therefore, there may be reports and other guidance documents on animal welfare of which the EFSA experts were not aware of.	Underestimation of the published relevant studies.
Literature search – Search strings	Although the search criteria were thoroughly discussed, some synonyms may have not been used in the search strings, and thus, less hits might have been retrieved. In addition, literature from non-transport conditions, that may still have been relevant for the assessment, may also not have been found.	The understanding of the relation between hazards and ABMs may not be complete due to having missed data.
Literature search – data sources	The search was limited to Web of Science. Although the search was complemented by internet searches and manual searches of the publicly available literature, no data were retrieved from other sources (e.g. industry, NGO or authority data). More information could have been retrieved by applying a different methodology (e.g. public call for data).	The understanding of the relation between hazards and ABMs may not be complete due to having missed data.
Literature search – inclusion and exclusion criteria	The screening phase might have led to the exclusion of certain studies that could have included relevant information.	Underestimation of the published relevant papers.
Expert group – number of experts, type of experts	This SO was carried out by a working group of 12 EFSA experts, of whom 3–5 were species-specific experts. The approaches underlying the SO is based on expertise from the whole working group, whereas the vast majority of the text within the SO has been written by the species-specific experts. Experts had to show they have no conflict of interest due to e.g. involvement with the horse industry or NGOs. This may have resulted in reduced level of technical and applied expertise	As the highly relevant welfare consequences were selected by expert opinion, the experts might have selected WCs that in reality belonged to another category than the highly relevant ones, and might have missed to select WCs that were in reality highly relevant.
Transport conditions of the studies retrieved in the extensive literature search	The transport conditions of the studies retrieved might have differed from the ones currently used in the EU, thus requiring an extrapolation exercise from the experts.	Under- or overestimation of the level of magnitude of the welfare consequences and related ABMs.

Source of uncertainty	Nature or cause of the uncertainty	Impact of the uncertainty on the assessment
Limited available knowledge on donkeys	The scientific focus on donkey transport has been limited, both in terms of available studies and available experts.	Reduced level of detail in the assessment of donkey welfare during transport.
Husbandry practices and horse breeds and categories of the studies retrieved in the extensive literature search	The studies retrieved may have involved husbandry practices and horse breeds and categories differing from EU standards. Thus, experts had to extrapolate findings to the EU relevant conditions in some cases.	Under- or overestimation of the level of magnitude of the welfare consequences and related ABMs.
Transport conditions of the studies retrieved in the extensive literature search	Transport conditions (e.g. driving style, ventilation capacity of the vehicle, external temperature) were not always specified in all the studies retrieved.	Under- or overestimation of the effects of the transport conditions on the WCs selected.
Time allocation	The time and resources allocated to this SO were limited and additional time for reflection would have facilitated a more in depth discussion of some of the aspects.	Inclusion, under- or overestimation of the level of magnitude of the WCs and related ABMs.
Lack of ABMs that are documented to be useful during transport in terms of feasibility, sensitivity or specificity	Based on the available knowledge, it was not possible to use single ABMs to assess the effect of exposure variables and transport conditions on welfare consequences.	Under- or overestimation of the level of magnitude of the WCs
Transport being a complex stressor, for which animal welfare has been studied much less than e.g. animal housing	The complexity of animal transport with the many interacting hazards, and thus, WCs means that many WCs are relevant, and thus that some can be missed in the selection of the highly relevant.	WCs that are in reality highly relevant are missed and thus underestimated.
Lack of available studies done under the recommended conditions	The number of studies available involving the conditions recommended in this SO is very limited. In addition, there are very few recent European studies. Thus, in some cases, and especially for the assessment of the journey time, experts had to extrapolate findings from studies done under different conditions.	Under- or overestimation of the level of magnitude of the welfare consequences.

4. Conclusions

The following section lists the conclusions of this Scientific Opinion. Within each transport stage, conclusions are often layered, and should be read as a whole.

4.1. General conclusions on transport of horses

- There are published protocols to assess animal welfare on farm and at slaughter, but no validated protocols are available to assess welfare of horses during transport.
- ABMs for all highly relevant welfare consequences along the transport stages are available based on expert opinion (see Section 3.2.2). Only some of these have been documented to be useful in terms of feasibility, sensitivity or specificity.
- The use of ABMs in animal transport is somewhat hampered by the reduced access to animals during the transit stage (especially when horses are transported in a group), but may be more feasible during other transport stages (e.g. loading/unloading).

- Technological developments such as artificial intelligence-based camera systems, motion sensors or belts recording physiological parameters, may increase the possibility to record and/or monitor ABMs during the entire transport process. However, such systems are still rarely used in practice.
- Many hazards have been identified (see examples below for each transport stage) during the transport of horses and the consequences of exposure to them throughout the transport stages (See Sections 3.3, 3.4, 3.5 and 3.6).
- Training for loading and transport seems to be one of the most important preventive measures to protect welfare of horses and donkeys during transport.
- Some hazards affecting the condition in which the animal begins the journey (e.g. level of hunger or thirst or health status) can only be mitigated or prevented before transport, whereas their associated consequences may appear later.
- Several sources of uncertainty were identified during the assessment, including (a) transport being a complex stressor, the consequences of which in terms of animal welfare have been studied much less than for example animal housing, especially under European conditions; (b) lack of ABMs that are documented to be useful during transport in terms of feasibility, sensitivity or specificity; (c) lack of available studies done under the recommended conditions; (d) lack of time; and (e) relatively low number of experts involved. However, the impact of uncertainty was not quantified. A list of the major sources of uncertainty can be found in Table 21.

4.2. Conclusions on preparation of horses before transport

- The preparation phase is important for the protection of the welfare of the animals throughout the journey as it may predispose animals to welfare consequences.
- At present, no published protocols to assess animal welfare during preparation for transport are available.
- If transport of animals involves complex journeys including markets, assembly centres or other temporary stops, there will in principle be preparation at several levels – before the initiation of the journey and before each reloading of the animals.
- Preparation is the first phase of transport when horses interact with humans and may lead to the following highly relevant welfare consequences: handling stress, injuries, sensory overstimulation, isolation stress and separation stress.
- During preparation, human-horse relationship and proper handling are crucial. Consequently, training of staff is a key point during the preparation of horses for transport.
- The welfare of horses benefits from horses being habituated to transport practices as early as possible (i.e. when they are foals). Habituation training and self-loading training have been proven useful to reduce transport stress and minimise the incidence of transport related problem behaviours (TRPBs) and injuries.
- A tool (a behavioural test) has been developed that can be applied before loading animals to recognise whether a horse shows signs of being able to be tied or led by a halter without causing avoidable excitement, pain or suffering or not.

A) Fitness for transport

- The assessment of fitness for transport (Section 3.3.4) before departure is of utmost importance in the protection of animal welfare. However, currently no agreed scientific definition of the concept of fitness for transport exists.
- If animals are not properly assessed, and unfit animals are loaded, it is a hazard for their welfare, predisposing them to additional welfare consequences during later transport stages, and potentially leading to negative affective states such as discomfort, pain and suffering.
- Characteristics rendering animals unfit for transport are mainly related to health impairment, but not always, as for example certain age groups or certain physiological stages lead to animals being unfit for transport. Even though some guidance for assessing fitness for transport is available, variation in the implementation of these methods exists. A list of conditions has been provided in this Opinion, of which some still require scientific validation and further study to identify the severity of the potential welfare consequences that can arise when they are present.
- At present, thresholds for ABMs as indicators of animals being unfit for transport have most often not been established or validated. Thus, knowledge about risk associated with transport

of animals with conditions potentially leading to negative affective states (e.g. emaciation, as well as the establishment of ABMs useful to identify these and their thresholds (suitable for use across professional groups), are needed. This knowledge may lead to additional conditions that will need to be added to the list provided in this Opinion.
- Successful assessment of fitness for transport requires well-educated staff including horse owners and professional groups such as veterinarians, farmers and livestock drivers, full clarity on responsibility and a clear definition of the concept of fitness for transport.
- Advanced pregnancy is associated with increased risks of welfare consequences during transport. There is consensus across different available guidelines that horses should not be transported in the last 10% of their pregnancy. However, scientific evidence to support this threshold is lacking, and the risk of reduced animal welfare may be present earlier.

4.3. Conclusions on loading and unloading of horses

- The highly relevant welfare consequences during the loading/unloading of horses are: heat stress, handling stress, injuries, sensory overstimulation and restriction of movement.
- Across the highly relevant welfare consequences, the major hazards are inappropriate handling, unsuitable facilities and high effective temperatures. Delay in loading and/or unloading means prolonged exposure to the hazards.
- In case of lack of habituation to the relevant handling, most horses are expected to experience handling stress, which may be associated with fear and may lead to distress.
- The main preventive measures are training of horses, loading with an appropriate training method, establishment and maintenance of proper facilities, and education and training of handlers.
- See Section 4.4A for conclusions on the prevention of heat stress during this stage.

4.4. Conclusions for the transit stage during road transport of horses

- The highly relevant welfare consequences for horses while on the transport vehicle are gastro-enteric disorders, heat stress, injuries, motion stress, prolonged hunger, prolonged thirst, respiratory disorders, restriction of movement, resting problems and sensory overstimulation.
- Among the major hazards for animal welfare during the transit stage are high effective temperatures, insufficient space allowance, time off feed and water, vehicle movements, restriction of neck movements and lack of possibility to rest.
- Preventive and corrective/mitigating measures for hazards and welfare consequences have been suggested (see Section 3.5). Several of the highly relevant welfare consequences of the transit stage (e.g. motion stress, resting problems, restriction of movement) cannot be fully prevented.
- There is evidence that horses drink and eat during transport when familiar water and hay are available, but also evidence that they may not achieve normal intake.
- The severity of welfare consequences during the transit stage of transport will depend on the exact conditions pertaining to an individual journey (e.g. type of vehicles, road conditions, driving skills). These hazards potentially interact. The exposure to these hazards will continue at least as long at the journey continues.

A) <u>Microclimatic conditions</u>

- Sweating is a sensitive ABM of heat stress in horses, with increases occurring prior to changes in core body temperature.
- If temperature in the transport vehicle remains below the upper limit of the thermal comfort zone, horses will most likely not experience stress or negative affective states associated with heat stress during transport.
- The welfare consequence, heat stress, may start when horses are no longer in their thermal comfort zone, and the risk and the severity of heat stress, is high when the thermal conditions reach the upper critical temperature.
- Not only the temperature in the vehicle, but also other environmental conditions influence heat load placed on horses during transport, such as humidity, thermal radiation, temperature of surrounding surfaces and wind speed. These will all influence the microclimatic conditions experienced by horses and should in theory, all be taken into account when microclimatic

conditions of horses during transport are evaluated. In addition to the dry temperature, humidity is considered the most important of these to take into consideration.

- The upper limit of the thermal comfort zone is the environmental temperature at which an animal will activate evaporative physiological thermoregulation processes, mainly sweating in horses, and may start to display thermoregulatory behaviour. Based on the commencement of evaporative heat loss and sweating rates, the upper threshold of the thermal comfort zone is suggested to be ~ 20°C, as measured at a relative humidity of 45%.
- Based on the initiation of heat production above baseline levels and the increase in the rate of sweating, the upper critical temperature is estimated to be ~ 25°C, as measured at a relative humidity of ~ 45%. This will vary somewhat depending on the individual horse, the breed and the degree of acclimatisation to heat.
- Although sensors recording dry temperature have commonly been used in transport of livestock so far, it would be a significant refinement to use improved sensors taking account of other environmental conditions influencing heat load placed on horses during transport (preferably a combination of temperature and humidity). For variations of dry temperature and relative humidity, the higher the levels of relative humidity, the lower the upper thresholds of thermal comfort zone and upper critical temperature will be, than when measured as a dry temperature only.
- Air conditioning is currently used to some extent and potentially presents an opportunity to facilitate the transport of horses when the external environmental conditions are too extreme, however research is required before its use can be recommended.

B) Space requirements

Single stall – horizontal space

- There is not sufficient data in the literature upon which to reach a definitive conclusion with respect to the minimum space required to meet the biological needs of horses during transport in a single stall.
- Horse welfare benefits from additional space with respect to the width as well as the length of a horse.
- Horses of the same body weight may vary a lot in shape (be very wide but small, or very tall and long but narrow); therefore it is more fit for purpose to indicate the minimum space needed as a distance from the body of a horse to the partitions/wall of the vehicle. Unfortunately, there is not sufficient data in the literature upon which to reach a definitive conclusion for what these minimum distances should be.
- Lateral space is necessary for spreading the legs to balance and adopt the excretory posture. Recent evidence suggests that ~ 30 cm of free space on each side of the horse allow better balance possibilities than ~ 15 cm. A combination of this evidence and expert opinion suggest that horses will benefit from at least 20 cm of free space on each side of their body.
- Front/back-space is necessary for lowering the head for balancing, resting and clearing of airways, with additional space possibly required for the positioning of feeders in vehicles. Extra space will also accommodate excretory postures. Consideration of the biomechanics involved in changing head posture in combination with expert opinion suggests a free space of 20 cm both in front and behind the horse (a total of 40 cm) when standing with the neck parallel to the ground. If a hay net is placed in front of the horse, ~ 50 cm of extra space is needed in addition to the 20 cm.

Group transport – horizontal space

- Considering that horses may have variable body weight, expressing space allowance as stocking density, such as kg/m^2, rather than space allowance (e.g. 1.75 m^2/horse), is a better way to take the differences in body size into account.
- Unhandled horses benefit in terms of reduced aggression, stress and falls from being transported loose in socially stable groups. Few studies have examined group size, but small groups (of 4) were found to be advantageous as compared to larger groups (of 8).
- In high density conditions, it is likely that horses have more problems balancing, are more exposed to aggressive conspecifics and may have more difficulties getting up after a fall.
- The limited available evidence suggests that a stocking density of no greater than 200 kg/m^2 leads to improved welfare as compared to higher stocking densities.

Vertical space

- The vertical space in a means of transport is important for horse welfare. Low vertical space is associated with reduced ventilation, lack of ability to move around and lack of space for natural movements, and should be prevented in order to avoid welfare consequences such as heat stress, injuries and restriction of movement.
- No studies have established a proper deck height for horses during transport. Earlier it has been recommended that the minimum internal height of the compartment should be the height of the withers of the tallest animal in a compartment + 75 cm. Establishment of evidence-based thresholds constitutes a gap in knowledge.

Horse orientation

- The bulk of research suggests that horse may show signs of reduced stress and fatigue, and be able to balance more easily, when transported facing backwards, although individual characteristics and other factors, such space allowance and vehicle design, may also play a role in the ability of horses to maintain balance during transport.

Head restraint

- When horses are transported under conditions where they can lower the head, the risk of respiratory disorders, especially transport pneumonia is reduced. In addition to space, this requires that horses are not too restricted by the way in which they are tied.

C) Journey duration

The conclusions regarding journey time are based on a scenario where animals are transported under the microclimatic conditions and with the minimal space allowance recommended in this Scientific Opinion (Sections 3.5.3.1 and 3.5.3.2, respectively). The implications of this are that the risk and severity of the welfare consequences heat stress and restriction of movement are much reduced and are thus given less weighting in the conclusions and recommendations on journey duration.

The remaining highly relevant welfare consequences can be classified into those that are **continuous or semi-continuous** (i.e. begin with the onset of the journey and occur continuously or intermittently throughout its duration), those are **progressive** (i.e. may not be present at the beginning of the journey but develop progressively as it continues if feed and water are not provided), and those that are **sporadic** (i.e. problems in individual animals which may be exacerbation of a pre-existing condition or may occur spontaneously at any point in the journey and whose welfare consequences will continue thereafter) These are summarised in Table 22.

Table 22: Potential welfare consequences developed during the transit stage with the estimated time to start and expected development over journey time

Type of Welfare consequence	Welfare consequence	Number of hours(2) until the estimated start of the welfare consequence	Expected development over time
Continuous or semi-continuous	Motion stress	Motion stress continuous throughout the transit stage	Severity will increase over time leading to fatigue
	Sensory overstimulation	Sensory overstimulation repeated intermittent	Can lead to fear and distress
	Effect on immune function(1)	Changes in acute phase proteins, white blood cells counts and antibody neutralising capacity were reported from 3- to 4-hour journey and antibody neutralising capacity reduced after 12-hour journeys	Can lead to higher susceptibility to infectious diseases.
	Resting problems	Continuous throughout the transit stage. Signs of oxidative stress reported from 8-hour journey.	Severity will increase over time leading to fatigue

Type of Welfare consequence	Welfare consequence	Number of hours[(2)] until the estimated start of the welfare consequence	Expected development over time
Progressively developing	Respiratory disorders	Signs of respiratory pathology in tracheal wash after 8 h and clinical disease after 10- to 14-hour journeys.	Due to multiple factors, the risk and severity of respiratory disorders will increase over time
	Gastro-enteric disorders	An increase in gastric pH is seen after 2 h with initial signs of gastric ulceration after 12 h in unfed horses.	The risk and severity of gastro-enteric disorders will increase over time
	Prolonged thirst	Behavioural indicators of thirst first reported after 3 h and biomarkers of dehydration after as little as 1–3 h in some studies. Very variable between studies depending on temperature conditions.	Severity will increase with time leading to dehydration
	Prolonged hunger	Biomarkers of tissue mobilisation reported after 12 h in some studies.	Severity will increase with time leading to weakening and exhaustion
Sporadic	Pain and/or discomfort from injuries	Most injuries occur early in a journey.	If present severity will increase with time leading to suffering

(1): Immune system and oxidative stress are not a WC from the list developed by EFSA (2022). Changes in these ABMs may, however, affect the occurrence of negative affective states in animals, and thus their welfare.
(2): The conditions of transport in the studies included in this table vary and may not necessarily be aligned with the recommendations on heat and space allowance. For further detail on the conditions, please see text above (Section 3.5.3.3).

Below, the prevalence, the severity and the duration of each of the highly relevant welfare consequences involved in this assessment are summarised:

- Motion stress and sensory overstimulation start as soon as a vehicle starts moving, and continues while the vehicle is moving, affecting all animals in the moving vehicle. Animals experience stress potentially leading to fatigue and negative affective states such as fear and distress;
- The prevalence of prolonged hunger is expected to be high if horses are not fed during journeys. The severity is expected to increase with increasing duration. Physiological changes indicative of hunger can be present after 12 h of transport.
- Gastro-enteric disorders have been reported to be frequently present in horses after transport.. Transport without feed for 12 h increases the risk and severity of gastric ulceration
- The prevalence of Prolonged thirst may be high, if water is not provided to the animals or if they, for some reason (such as lack of familiarity, neophobia or fear of other animals) are not able to drink enough water. Prolonged thirst may lead to dehydration, discomfort and suffering. Haematological changes indicative of dehydration have been observed after journeys of (1–3) h without water. Behavioural indicators of increased thirst as a result of transport can be apparent after a 3-h journey.
- The prevalence of resting problems is expected to be high, as horses will not lie down, and because of the need to continuously make postural adjustments to compensate for the motion of the vehicle. The severity is expected to increase with increasing duration, as the lack of resting becomes more problematic for the animals and may lead to fatigue and, eventually, exhaustion.
- The prevalence of respiratory disorders can be high due to multiple risk factors such as stress, insufficient space, excessively high temperatures and restrictive tying practices and can be very severe with clinical signs apparent in many studies after journeys of 10–14 h.
- The pain and/or discomfort from health conditions or injuries might be relatively rare but for the affected animals, the consequences might be severe and will worsen over time during transport and may lead to suffering;

4.5. Conclusions for journey breaks and control posts

- Per definition, breaks in journeys function to remove the animals from the hazards that they are exposed to during transit and to allow them to recover from the associated welfare consequences. With the currently available practices, it is not possible for horses to do this on a moving vehicle. Thus, for journey breaks the vehicle should be stationary or horses be unloaded.

Journey break in stationary vehicle

- Even though journey breaks while the animals are maintained on-board the stationary vehicle has merit, at least for horses transported singly, it still cannot prevent welfare consequences such as resting problems and restriction of movement, unless horses are transported under conditions that are very different from the typical practice of today. WCs such as prolonged hunger and prolonged thirst can, however, likely be mitigated by rest periods on the stationary vehicle.
- When animals are offered opportunities to feed, drink and rest on a stationary vehicle, the conditions on the vehicle and the time provided must be appropriate to allow the animals proper time for these activities.
- The provision of drinking water on-board a stationary vehicle at regular intervals is a potential means of reducing the risk of dehydration.

Journey break at control post

- The highly relevant welfare consequences for horses during the control post stage are gastro-enteric disorders, handling stress, injuries, respiratory disorders, resting problems and sensory overstimulation.
- Among the major hazards for animal welfare during the control post stage are inappropriate handling, exposure to novel stimuli, limited access to bedding, low space allowance, time off-feed or introduction of unfamiliar feed.
- When control posts are used, animals must be unloaded and reloaded. These procedures involve hazards potentially leading to welfare consequences such as handling stress, heat stress, injuries and sensory overstimulation (Section 3.4).
- In addition, control posts involve biosecurity risks as animals can be exposed to infectious diseases through direct or indirect contact with other animals and opportunistic pathogens.
- The type and severity of welfare consequences associated with a stay in a control post depend on the management (e.g. cleaning and disinfection procedures), the housing conditions (type of stable, ventilation, bedding, etc.), the equipment (e.g. ramps to (un)load, drinkers) and training of the staff.
- Across the categories of horses typically transported on journeys involving journey breaks, the scientific focus on control posts has been limited. This means that whether control posts in their current state fulfil their intended function is not known, and there is a (presently unquantified) risk that even though control posts conform to the current regulation, their use may be associated with animal welfare consequences.
- If a stay in a control post or similar should be beneficial for the welfare of horses during transport, any journey break needs to be long enough for each animal to eat, drink and rest. Assuming the recommendations made on microclimatic conditions (Section 3.5.3.1), space allowance (Section 3.5.3.2) and maximum journey times (Section 3.5.3.3) and given the limited amount of data on recovery periods, a period of 12–24 h would seem to be enough to allow for feeding, drinking and recovery from fatigue. Setting the required period will, however, depend on the journey experienced. It will also depend on the state of the animals. The duration of a rest at a control post, allowing welfare consequences from the transit stage to be mitigated, constitutes a gap in knowledge.

4.6. Conclusions on transport of horses by Roll-on-roll-off ferries

- There are limited scientific studies focused on the welfare of horses during RO-RO journeys. Hence, this assessment is based on expert opinion and general knowledge about RO-RO ferries and animal transport.

- Typically, the welfare consequences, hazards, preventive and corrective/mitigating measures for transport by road (Sections 3.3, 3.4 and 3.5) also apply here. In addition, transport by RO-RO ferries presents further concerns.
- The main welfare concerns related to transport of horses on RO-RO ferries are: (1) a combination of waiting time in the port before and after the voyage plus the duration of the sea journey leading to the total time spent inside vehicles exceeding the recommended journey time; (2) weather disruption leading to delay or cancellation of journeys, as well as to motion stress; (3) reduced ventilation due to lack of natural ventilation (wind) inside the vessel; and (4) difficulties in attending to animals and unloading them in case of emergencies.

4.7. Conclusions on transport of horses by air

- Horses travel frequently by air and Europe exports many horses to other continents.
- The welfare consequences, hazards, preventive and corrective/mitigating measures explained in the transport by road (Sections 3.3–3.6) also apply here. However, there are additional concerns for horse welfare during air transport expressed by the EFSA Experts: narrow space (often with restriction of neck movement), lengthy waiting times and variation in microclimatic conditions. Other factors that may lead to welfare consequences are motion stress and auditory stimuli.
- The available evidence to evaluate the welfare of horses when transported by air is scarce. This constitutes a gap in knowledge.

4.8. Conclusions on transport of donkeys

- Despite some differences in behaviour and physiology between horses and donkeys, no scientific evidence was found to support different conditions for transporting donkeys.
- No scientific information on the transport of crosses between donkeys and horses (mules and hinnies) are available. Hence, any conclusions made with reference to horses can also be made with reference to the crosses between horses and donkeys.
- Due to the subtle reaction of donkeys to pain, diseases or painful conditions may result unnoticed during the fitness for transport assessment, becoming a risk for the welfare of the animals during transport.
- Donkeys with predisposing factors and concurrent disease have a higher risk of hyperlipaemia when a stressful event occurs (including transport).
- Pair bonding is often seen in donkeys and is important when handling donkeys during transport. Consequently, donkeys could benefit from being transported in small groups of two to four familiar animals.

4.9. Conclusions on specific scenarios

A) Long journey to slaughterhouses

- The welfare consequences, hazards, preventive and corrective/mitigating measures explained in the transport by road (Sections 3.3–3.6) also apply here.
- There are additional concerns for horse welfare during long journeys to slaughterhouses, specifically related to the exposure to the hazards due to the long journey time.
- Horses destined for slaughter within the EU might be in a poor state of health before transport, and can sometimes be transported under poor conditions.
- The proportion of horses deemed unfit for transport at the point of origin has been reported to be an issue, because these animals tend to be of lower value and less is invested in ensuring that conditions of transport are satisfactory.

5. Recommendations

The following section lists the recommendations of this Scientific Opinion. Within each transport stage, recommendations are often layered and should be read as a whole.

5.1. General recommendations on transport of horses by road

- Protocols to assess the welfare of horses during transport should be developed and validated, preferably involving aspects of pre- and post-transport housing conditions.
- In order to have useful ABMs in animal transport, research should be carried out to develop these, including the identification and validation of technological solutions in this setting, aiming to assess outcomes more than input.

5.2. Recommendations for preparation horses before transport

- Protocols to assess animal welfare during the preparation phase, including scenarios characterised by repeated reloading of animals should be developed and validated.
- In order to avoid welfare consequences such as prolonged hunger and thirst in later transport stages, feed and water should be accessible during preparation and feed and water should be provided in a way where animals can have easy access to them.
- If possible, horses should be trained for loading and transport.
- People handling horses should be educated in horse behaviour and training.

A) Fitness for transport

- In order to avoid welfare consequences, such as pain and discomfort, animals should be fit for transport. Guidelines based on ABMs for conditions leading to animals being unfit, including thresholds, should be established and validated. Among suggested candidate ABMs are lameness score, pyrexia, dyspnoea, ataxia, disorientation/abnormal behaviour, abnormal navel, wounds, aspect, demeanour, swollen joints, abscesses, hernias, late pregnancy, bone fractures, body condition score, eyesight deficiency.
- In order to avoid doubt and misclassification of animals in relation to fitness for transport, the concept should be properly defined, owners and professional groups (including farmers, stockpersons, drivers, hauliers, inspectors and veterinarians) should be well educated and questions on responsibility between the groups should be clarified.
- Risks associated with transport of animals with a number of conditions potentially involving negative affective states during transport, such as pregnancy, should be examined.
  - — A contingency plan needs to be made on how to care for horses that become unfit for transport during the journey.

5.3. Recommendations for loading/unloading of horses during road transport

- — In order to minimise handling stress and other welfare consequences during loading and unloading, handlers should be properly educated and trained in the use of non-coercive methods and tools.
- — In order to minimise handling stress and injuries, horses should be trained for loading and unloading.
- — Loading and unloading facilities should be fit for purpose in order to avoid welfare consequences such as injuries.
- — Delays in loading increase exposure to hazards such as high effective temperatures, may lead to welfare consequences such as heat stress, and should be avoided.

5.4. Recommendations for the transit stage during road transport of horses

A) Microclimatic conditions

- Among the environmental factors affecting the heat load placed on horses during transport, it is recommended to take dry bulb temperature and at least humidity into account when horse welfare during transport is evaluated. The relationship between these can be expressed by different indexes. Further research should be carried out to assess costs and benefits of different choices of index.

- If a negative impact on animal welfare from the microclimatic conditions during journeys is to be fully prevented, horses should be transported in their thermal comfort zone, the upper threshold of which is estimated to be 20°C.
- In order to reduce the risk of welfare consequences due to exposure to high effective temperatures, the temperature inside vehicles transporting horses should not exceed the upper critical temperature estimated to be 25°C.
- To reduce the risk of heat stress, horses should always be well-hydrated by ensuring water is freely available before and after the journey.
- Means of transport should be equipped with sensors recording microclimatic conditions as close as possible to the position of the animals in the vehicle, and (at least in large vehicles) at several locations to include hot as well as cold spots, and representative points in between, thereby allowing monitoring of the microclimate (preferably a combination of temperature and humidity) of the load, and the adjusting of the ventilation if the conditions exceed the comfort zone levels. Technical issues (e.g. accuracy, maintenance, placement, reliability and calibration) relating to this improved approach should be clarified.
- Future research should be carried within:
  - Development of systems to maintain the microclimatic conditions in stationary as well as moving vehicles by e.g. air conditioning.

B) Space requirements

Individual – horizontal space

- The width of the individual stall should be at least 40 cm more in total than the width of the animal at its widest point (see Figure 13 for a graphical explanation).
- When transported individually, a horse should have at least 40 cm of free space in addition to the body length of the horse measured from the tail to the nose while with the neck is parallel to the ground, plus 50 cm if feed in a hay net is provided in transit (Figure 14).
- Horses must be able to lower their head below the wither height to clear their respiratory tract, and this is impossible if they are cross-tied or tied short (< 60 cm rope).

Group – horizontal space

- Unhandled horses should be transported in a small group composed of compatible animals free to move around with a density of < 200 kg/m^2.

All – vertical space

- Research should establish evidence-based thresholds for vertical space, taking the microclimatic conditions and space for natural movements into account. The minimum internal height of a compartment holding horses is recommended to be the [height of the withers of the tallest animal in a compartment (cm) + 75 cm].
- Future research should be carried within:
  - The minimum space allowance for horses transported in an individual stall by road, including tying conditions.
  - Space requirements for horses transported with reduced fitness for transport (e.g. transported to a veterinary clinic).
  - The establishment of spatial requirements for mares transported with their foal.

C) Journey times

- The number and the severity of hazards that animals are exposed to during transport influence the resultant welfare consequences, but on the basis of evidence on continuous welfare consequences involving stress and negative affective states, for the benefit of animal welfare, the journey duration should be kept to a minimum.
- To limit the impact of transport on animal welfare, in an effort to reduce the exposure to hazards and related welfare consequences, continuous, progressively developing and sporadic, it is recommended to consider that:
  - Animals experience motion stress and sensory overstimulation throughout the entirety of the journey potentially leading to fatigue, fear and distress;

  - Pain and/or discomfort from health conditions or injuries will worsen over time during transport and may lead to suffering;
  - Resting problems severity is expected to increase with increasing duration and may lead to fatigue;
  - Clinical respiratory disorders can be present after journeys of 10–14 h;
  - Gastro-enteric disorders such as gastric ulceration can be seen after 12 h in unfed horses.
  - Behavioural indicators of thirst first reported after 3 h and physiological biomarkers of dehydration after as little as 1–3 h.
  - Physiological biomarkers indicative of prolonged hunger have been reported after 12 h of transport.

- During transport, horses should be provided with feed and water *ad libitum* or at least at regular intervals (of no more than 4 h) for a period of 30 min while the vehicle is stationary.
- Future research is recommended in the following areas:

  - Investigation of relationships between journey time, journey conditions and ABMs considered to reflect affective states of horses for all animal categories, including knowledge about the progressively developing welfare consequences and their changes over time. Such research could inform the appropriate limits on journey duration if the recommended conditions of temperature and space are not fulfilled.

5.5. Recommendations on journey breaks and control posts

Based on general knowledge about horses and the current practice, the following is recommended to protect the welfare of horses during this transport stage. New scientific knowledge may lead to adjustments of these.

- Journey breaks can be in a stationary vehicle or after unloading from the vehicle.
- The microclimatic conditions during a journey break should allow for horses to be kept in their thermal comfort zone.
- Feed (e.g. hay) should be provided for *ad libitum* intake and horses should have free access to drinkers and fresh water at all times.
- Control posts should have proper loading and unloading facilities, and staff with experience in handling horses.
- Horses that are singly stalled during transport should be housed in single stables. Horses that are being transported in a group, should be housed with that group and only that group.
- Animals showing signs of weakness or disease should be inspected and treated accordingly. Animals unfit for further transport should not follow the consignment at reloading, but be slaughtered, treated or euthanised according to the prognosis of their condition. Contingency plans should be in place for injured and sick animals.
- Due to the risk that a stay in a control post (including unloading and reloading) leads to welfare consequences, it is recommended that the number of times that horses stay in a control post should be as low as possible.
- Assuming the recommendation made on microclimatic conditions (Section 3.5.3.1) and space allowance (Section 3.5.3.2) and maximum journey times (Section 3.5.3.3), a period of 12–24 h should be given in a control post to allow for feeding, drinking and recovery from fatigue after a long journey.
- Future research in the following areas is recommended:

  - Examine whether control posts fulfil their function, and how they should be designed and managed to protect horse welfare.
  - Scientifically define an appropriate duration of stays at control posts.

5.6. Recommendations on transport of horses by Roll-on-roll-off

- The recommendations for road transport (see Sections 3.3, 3.4, 3.5 and 3.6) are applicable in this context therefore it is recommended to apply them.
- Sufficient ventilation on the deck where the animals are located should be ensured.
- Due to the exposure to the hazards generic to road transport plus the additional concerns listed, voyage duration should not be considered resting time.

- Transporters must ensure that contingency plans in case of emergencies are in place, e.g. ferry disruptions.
- Animals should not be shipped when the effects of weather conditions anticipated for the voyage and at the point of destination are likely to cause them injury or suffering. Take into account factors such as the forecast wind direction and strength, state of the sea and whether or not the vessel is stabilised.
- The driver or animal attendant must be able to have access to the animals at regular intervals during the voyage in order to check and care for them.

5.7. Recommendations on transport of horses by air

- Until evidence-based thresholds are established for these means of transport, alignment with the recommendations for microclimatic conditions for road transport is recommended.
- Waiting times should be reduced and animals should be given comfortable conditions during the waiting time, ensuring access to feed and water and temperatures within thermal comfort zone.
- During the journey horses should be regularly checked and watered, with a hay net constantly available.
- Horses should be accustomed to travel at least by road before travelling on a plane.
- Future research in the following area is recommended:
    - Development and validation of protocols to assess horse welfare during the air transport.

5.8. Recommendations on transport of donkeys

- In the absence of data confirming any difference, alignment with the recommendations for transport of horses is recommended.

Fitness for transport assessment of donkeys should consider their subtle reaction to pain and focus on identifying underlying conditions that could lead to hyperlipaemia.

5.9. Recommendations on specific scenario: Long journey to slaughterhouses

- Alignment with the recommendations for road transport of horses (Sections 3.3–3.6) is recommended.
- Unfamiliar horses should not be mixed in the transport vehicle.
- Small groups are recommended when horses are transported loose.

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Abbreviations

ABM	animal-based measure
ACTH	adrenocorticotropic hormone
AST	Aspartate Aminotransferase
bpm	Breaths per minute
CgA	chromogranin A
CK	Creatine kinase
EKE	Expert Knowledge Elicitation
FEI	Federation Equestre Internationale
HR	Heart rate
HRV	Heart rate variability
LCT	Lower critical temperature
PRE	preventive measure
RH	relative humidity
RO-RO	Roll-on roll-off (ferries)
RR	Respiratory rate
RT	Rectal temperature
SAA	serum amyloid A
TCZ	Thermal Comfort zone
THI	temperature-humidity index
TNC	Thermoneutral zone
TRACES	TRAde Control and Expert System
TRPB	Transport-related problem behaviour
UCT	Upper critical temperature
WBCT	Wet-bulb Globe Temperature
WC	Welfare consequence

Appendix A – Template used during the selection of the highly relevant welfare consequences

Name	Example			
Animal species	Example			
Animal type	Example			
Husbandery system	Example			
Code	***1***	Clearly relevant		
	32	Clearly not relevant		
	33	Not applicable		
	2 to 31	***Please rank the remaining WCs***	***VVV***	

ID	***Abbr***	Welfare Consequence	***Ranking***	Binding
B03	***SCS***	Group stress	0	0
H03	***HEA***	Heat stress	1	0
H04	***CLD***	Cold stress	1	0
B02	***RSP***	Resting problems	1	0
B06	***GFS***	Motion Stress	1	0
H01	***HNG***	Prolonged hunger	1	0
B01	***MOV***	Restriction of movement	1	0
B04	***VAS***	Sensory Overstimulation	1	0
B05	***HNL***	Handling stress		0
B09	***CMF***	Inability to perform comfort behaviour		0
B12	***EXP***	Inability to perform exploratory or foraging behaviour		0
H02	***H2O***	Prolonged thirst	1	0
H05	***LOC***	Locomotory disorders (including lameness)		0
H06	***SKL***	Skin lesions and wounds		0
H10	***RES***	Respiratory disorders		0
H12	***GED***	Gastro-enteric disorders		0
H15	***MTB***	Metabolic disorders		0
H16	***MUS***	Muscle disorders		0
H17	***UMB***	Umbilical disorders		0
B08	***SEP***	Separation stress	32	0
B16	***PLY***	Inability to perform play behaviour	32	0
H07	***MAN***	Pain resulting from management procedures	32	0
H08	***BNL***	Bone lesions (incl. fractures and dislocations)	32	0
H09	***SKD***	Skin disorders (other than pododermatitis or skin lesions)	32	0
H11	***EYE***	Eye disorders	32	0
B07	***ISO***	Isolation stress	33	0
B10	***WSX***	Inability to perform sexual behaviour	33	0
B11	***USX***	Inability to avoid unwanted sexual behaviour	33	0
B13	***MAT***	Inability to express maternal behaviour	33	0
B14	***SUC***	Inability to perform sucking behaviour	33	0
B15	***CHW***	Inability to chew and ruminate	33	0
H13	***RPD***	Reproductive disorders	33	0
H14	***MAS***	Mastitis	33	0

Please sort this table by the "Ranking" and complete the "Binding" (No. of cells with same rank)