

ADOPTED: 1 June 2022

doi: 10.2903/j.efsa.2022.7404

Welfare of small ruminants during transport

EFSA Panel on Animal Health and Welfare (AHAW),
Søren Saxmose Nielsen, Julio Alvarez, Dominique Joseph Bicout, Paolo Calistri,
Elisabetta Canali, Julian Ashley Drewe, Bruno Garin-Bastuji, Jose Luis Gonzales Rojas,
Christian Gortázar Schmidt, Virginie Michel, Miguel Ángel Miranda Chueca, Barbara Padalino,
Paolo Pasquali, Helen Clare Roberts, Hans Spoolder, Karl Stahl, Antonio Velarde,
Arvo Viltrop, Christoph Winckler, Bernadette Earley, Sandra Edwards, Luigi Faucitano,
Sonia Marti, Genaro C Miranda de La Lama, Leonardo Nanni Costa, Peter T Thomsen,
Sean Ashe, Lina Mur, Yves Van der Stede and Mette Herskin

Abstract

In the framework of its Farm to Fork Strategy, the Commission is undertaking a comprehensive evaluation of animal welfare legislation. The present Opinion deals with the protection of small ruminants (sheep and goats) during transport. The main focus is on welfare of sheep during transport by road but other means of transport and concerns for welfare of goats during transport are also covered. Current practices related to transport of sheep during the different stages (preparation, loading and unloading, transit and journey breaks) are described. Overall, 11 welfare consequences were identified as being highly relevant for the welfare of sheep during transport based on severity, duration and frequency of occurrence: group stress, handling stress, heat stress, injuries, motion stress, predation stress, prolonged hunger, prolonged thirst, restriction of movement, resting problems and sensory overstimulation. These welfare consequences and their animal-based measures are described. A wide variety of hazards, mainly relating to inappropriate or aggressive handling of animals, structural deficiencies of vehicles and facilities, unfavourable microclimatic and environmental conditions and poor husbandry practices, leading to these welfare consequences were identified. The Opinion contains general and specific conclusions in relation to the different stages of transport. Recommendations to prevent hazards and to 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 spatial thresholds (minimum space allowance). The development of welfare consequences over time were assessed in relation to maximum journey time. The Opinion covers specific animal transport scenarios identified by the European Commission relating to the export of sheep by livestock vessels, export of sheep by road, roll-on-roll-off vessels and 'special health status animals', and lists welfare concerns associated with these.

© 2022 Wiley-VCH Verlag GmbH & Co. KgaA on behalf of the European Food Safety Authority.

Keywords: small ruminants, sheep, goats, transport, animal welfare assessment, Farm to Fork Strategy, welfare consequences, animal-based measures, hazards, quantitative thresholds

Requestor: European Commission

Question number: EFSA-Q-2021-00433

Correspondence: ahaw@efsa.europa.eu

Panel members: Søren Saxmose Nielsen, Julio Alvarez, Dominique Joseph Bicout, Paolo Calistri, Elisabetta Canali, Julian Ashley Drewe, Bruno Garin-Bastuji, Jose Luis Gonzales Rojas, Christian Gortázar Schmidt, Mette Herskin, Miguel Ángel Miranda Chueca, Virginie Michel, Barbara Padalino, Paolo Pasquali, Helen Clare Roberts, Hans Spoolder, Karl Stahl, Antonio Velarde, Arvo Viltrop and Christoph Winckler.

Waiver: In accordance with Article 21 of the Decision of the Executive Director on Competing Interest Management a waiver was granted to Sandra Edwards, Genaro C Miranda de La Lama and Luigi Faucitano, experts of the Working Group WG/P/AHAW/2020/05 - AHAW Welfare Farm to Fork. Pursuant to Article 21(6) of the aforementioned Decision, the concerned experts were allowed to take part in the drafting and in the discussion of the scientific output but were not allowed to take up the role of, or act as, chairman, vice-chairman or rapporteur of the Working Group. Any competing interests are recorded in the respective minutes of the meetings of the Working Group WG/P/AHAW/2020/05 - AHAW Welfare Farm to Fork.

Declarations of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output: hearing experts Nancy De Briyne, Michael Cockram, Yolande Seddon and Clive Phillips. EFSA would like to thank Mariana Geffroy, Mimi Kalcheva and Maria Veggeland from EFSA, for all the support provided in this Opinion. EFSA wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

Suggested citation: EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), Nielsen SS, Alvarez J, Bicout DJ, Calistri P, Canali E, Drewe JA, Garin-Bastuji B, Gonzales Rojas JL, Gortázar Schmidt C, Michel V, Miranda Chueca MA, Padalino B, Pasquali P, Roberts HC, Spoolder H, Stahl K, Velarde A, Viltrop A, Winckler C, Earley B, Edwards S, Faucitano L, Marti S, Miranda de La Lama GC, Nanni Costa L, Thomsen PT, Ashe S, Mur L, Van der Stede Y and Herskin M, 2022. Scientific Opinion on the welfare of small ruminants during transport. EFSA Journal 2022;20(9):7404, 101 pp. <https://doi.org/10.2903/j.efsa.2022.7404>

ISSN: 1831-4732

© 2022 Wiley-VCH Verlag GmbH & Co. KGaA on behalf of the European Food Safety Authority.

This is an open access article under the terms of the [Creative Commons Attribution-NoDerivs](https://creativecommons.org/licenses/by/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.

Reproduction of the images listed below is prohibited and permission must be sought directly from the copyright holder:

Figure 8: © Elsevier; Figure 9: © British Society of Animal Science; Figures 10, 12 and 13: © British Veterinary Association; Figure 11: © Elsevier



The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.



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 seven specific scenarios. This Opinion deals with the protection of small ruminants (sheep and goats) during transport.

The scientific assessment was carried out by breaking down the transport of small ruminants 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) to assess the welfare consequences, and hazards leading to the welfare consequences. In addition, recommendations to prevent hazards and to correct or mitigate welfare consequences were developed. Recommendations were also developed in relation to quantitative thresholds for microclimatic conditions within the means of transport. In addition, the development of welfare consequences over time were assessed in relation to maximum journey duration.

While the Opinion focuses primarily on the road transport of sheep, there are specific sections dealing with export by road, transport by roll-on-roll-off ferries, livestock vessels, as well as air and rail transport. Welfare concerns (defined as an area or a topic to which special attention should be given in order to potentially avoid welfare consequences), related to the transport of goats are covered in a separate section of the Opinion.

According to the European Commission's TRACES system, around 3 million sheep were transported annually between member states between 2019 and 2021 across all means of transport, with approximately 95% of those taking place by road. For goats, the number of animals transported annually in that period averaged 66,000, with 96% of those transported by road.

In total, 11 welfare consequences were selected as being highly relevant for the welfare of sheep during transport based on severity, duration and frequency of occurrence. These were (i) group stress, (ii) handling stress, (iii) heat stress, (iv) injuries, (v) motion stress, (vi) predation stress, (vii) prolonged hunger, (viii) prolonged thirst, (ix) restriction of movement, (x) resting problems and (xi) sensory overstimulation. The occurrence of each type of welfare consequence varied depending on the stage and means of transport. Small ruminants may experience one or more high-level 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. They included 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/untrained handlers leading to inappropriate handling, inappropriate use of dogs for controlling sheep, structural deficiencies of vehicles and facilities, poor driving skills and transport conditions, unfavourable microclimatic and environmental conditions and poor 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. 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 very 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 leading to sheep being 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 sheep are: handling stress, heat stress, injuries, predation stress and sensory overstimulation. Across the highly relevant welfare consequences, the major hazards are inappropriate handling, unsuitable facilities, projections, separation from their social group, high temperatures, delays, noise, sights and odours. The main preventive measures are establishment and maintenance of proper facilities, avoiding loading during hot hours and education and training of handlers.

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

If the negative impact on animal welfare from the microclimatic conditions during journeys is to be fully prevented, sheep should be transported in their thermal comfort zone, the upper threshold of which is estimated to be 25°C. The risk of heat stress, and the severity of heat stress, is likely very high when the thermal conditions reach the upper critical temperature estimated to be 28°C for fleeced sheep, and 32°C for shorn sheep.

In relation to the horizontal space allowance for sheep during road transport, the available evidence suggests that a k-value of at least 0.037 in the allometric equation relating space to live weight, is required. With regard to vertical space, low height constitutes a hazard for several welfare consequences such as heat stress and restriction of movement, and no studies have established a proper deck height for sheep during transport. As long as no research has established evidence-based thresholds for vertical space, it is recommended that the space above the highest point of the animals is at least 15 cm in vehicles with mechanical ventilation and 30 cm in naturally ventilated vehicles.

The amount of time the animals are exposed to hazards during transport 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, 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: motion stress and sensory overstimulation start as soon as a vehicle starts moving, and continues while the vehicle is moving, potentially leading to fatigue and negative affective states such as fear and distress; 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; Resting problems are expected to increase with increasing duration, as the lack of resting becomes more problematic for the animals and may lead to fatigue; Even when a transport vehicle is fitted with water drinkers, prolonged thirst may lead to dehydration and associated negative affective states, and physiological changes that are likely to be associated with thirst have been identified after 12 hours of transport; and due to practical difficulties in feeding animals on a transport physiological changes indicative of hunger can be present after 12 hours of transport.

Per definition, breaks in journeys (either while a vehicle is stationary or when animals are unloaded in a control post, for example), function to remove the animals from the hazards they are exposed to during transit and allow them to recover from the associated welfare consequences. No studies have documented successful feeding of sheep during the transit stage, and it is not considered practically possible. Thus, if sheep are to recover from the welfare consequences experienced during transit they need to be unloaded from the vehicle.

At control posts, along with the mitigation of welfare consequences, there is also potential for exposure to hazards leading to 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 sheep 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 or not, is not known.

Most of the hazards, welfare consequences, preventive, corrective and mitigating measures, will be the same for sheep and goats, because they are generic to some extent for the road transport of small ruminants. However, there are differences between the two species of animals in terms of their biology that should be understood to improve operators' safety and animal welfare during transport. Goats are more curious, bold and agile than most breeds of sheep. During loading and unloading, goats are more reactive than sheep, because they are more aggressive and they exhibit more exploratory behaviours, although this varies according to the husbandry systems and life experience of the goats.

Conditions rendering goats unfit for transport are very similar to the ones listed for sheep, but no list has been developed for goats.

The procedures of unweaning and prolonged transport immediately after unweaning are stressful and exhaust the body reserves of unweaned lambs. Therefore, from an animal welfare point of view, weaning lambs sometime before transport would be advantageous.

The specific scenarios relevant to small ruminants that EFSA was asked by the Commission to consider were the export of sheep by road, the export of sheep in livestock vessels, the transport of sheep in roll-on-roll-off ferries and the transport of 'special health status animals', i.e. the transport of sheep where unloading them before the final destination might jeopardise their health status.

In the years 2019–2021, the yearly export of sheep to third countries by road has varied from 113,000 to 199,000 animals. A number of concerns specifically related to the export of sheep by road have been identified, including long delays at border crossings when leaving the EU, and the absence of certified resting points outside of the EU. In general, journeys of sheep to third countries can involve journeys over several days and there can be serious difficulties with thermoregulation due to high ambient temperatures. There are also specific health risks related to sheep being exported.

Every year, the EU exports approximately 3 million sheep and goats by sea, mainly to the Middle East and Africa. The largest livestock vessels can carry up to 75,000 sheep. Specific concerns relating to the exporting of sheep include long waiting times at ports, starvation due to inappetence during the journey, heat stress, the presence of noxious gases on the vessel, lack of availability of space on the vessel, motion stress and improper handling on arrival at the destination.

The concerns addressed for the welfare of sheep transported on roll-on-roll-off ferries are the risk of prolonged journey time, weather disruptions, inadequate ventilation, difficulties in attending to animals in case of emergencies and motion stress. Research is needed to evaluate the welfare of sheep during transport in roll-on-roll-off ferries to form the basis for future recommendations.

'Special health status animals' are sheep that are transported through an area of lower health status than at their farm of origin. In such cases, unloading of the animals may pose a biosecurity risk. The failure to unload sheep under these circumstances may present a large risk to their welfare. If, due to biosecurity concerns, sheep are not unloaded to provide required rest, feed and water, facilities must be available on the vehicle to provide the necessary resting, feeding and drinking, and suitable microclimatic conditions. However, no scientific studies have been found that can demonstrate the effective feeding and watering of sheep while staying in a vehicle.

Table of contents

Abstract.....	1
Summary.....	3
1. Introduction.....	8
1.1. Background and Terms of Reference as provided by the requestor.....	8
1.1.1. Background.....	8
1.1.2. Terms of Reference.....	8
1.1.2.1. Assessment of common transport practices.....	8
1.1.2.2. Assessment of seven specific transport practices.....	9
1.2. Interpretation of the Terms of Reference.....	9
2. Data and methodologies.....	12
2.1. Data.....	12
2.1.1. Data from literature.....	12
2.1.2. Data from Public Consultation.....	12
2.2. Methodologies.....	12
2.2.1. Experts' opinion.....	15
2.2.2. Literature searches.....	15
3. Assessment.....	16
3.1. The transport of sheep within the EU.....	16
3.2. Welfare consequences associated with transport of sheep.....	16
3.2.1. Negative affective states.....	18
3.2.2. Definition and interpretation of ABMs for highly relevant welfare consequences during animal transport.....	19
3.3. Preparation of sheep for transport.....	25
3.3.1. Current practices.....	25
3.3.2. Highly relevant welfare consequences.....	25
3.3.3. Fitness for transport.....	27
3.3.3.1. Introduction.....	27
3.3.3.2. Assessment of fitness for transport in sheep.....	28
3.3.3.3. Transport of animals with reduced fitness.....	32
3.4. Loading/unloading.....	33
3.4.1. Current practice.....	33
3.4.2. Highly relevant welfare consequences.....	33
3.5. Transit stage.....	36
3.5.1. Current practice.....	36
3.5.2. Highly relevant welfare consequences.....	36
3.5.3. Quantitative examination of thresholds to protect the welfare of sheep during the transit stage: microclimatic conditions, space allowance and journey time.....	42
3.5.3.1. Threshold of microclimatic conditions.....	42
3.5.3.2. Threshold of space requirements during journeys.....	47
3.5.3.3. Thresholds for journey time.....	54
3.6. Journey breaks.....	63
3.6.1. Provision of water and/or feed on the vehicle while stationary.....	64
3.6.2. Control posts.....	64
3.6.2.1. Current practice.....	65
3.6.2.2. Highly relevant welfare consequences.....	65
3.7. The transport of goats.....	69
3.8. The transport of unweaned lambs.....	71
3.9. Specific scenario: The export of sheep by road.....	72
3.10. Specific scenario: The export of sheep in livestock vessels.....	73
3.11. Specific scenario: Transport of sheep on roll-on-roll-off ferries.....	75
3.12. Specific scenario: The transport of sheep by air.....	75
3.13. Specific scenario: The transport of sheep by rail.....	76
3.14. Specific scenario: 'Special health status animals'.....	76
3.15. Uncertainty analysis.....	77
4. Conclusions.....	77
4.1. General conclusions on transport of sheep.....	79
4.2. Conclusions on the preparation of sheep before transport.....	80
4.3. Conclusions for loading/unloading of sheep during road transport.....	80
4.4. Conclusions for the transit stage during road transport of sheep.....	81
4.5. Conclusions for journey breaks and control posts.....	83
4.6. Conclusions on welfare concerns during transport of goats.....	84

4.7.	Conclusions on welfare concerns during transport of unweaned lambs	84
4.8.	Conclusions on specific scenarios	84
5.	Recommendations	85
5.1.	General recommendations on the transportation of sheep	85
5.2.	Recommendations for preparation of sheep before transport	85
5.3.	Recommendations for loading/unloading of sheep during road transport	85
5.4.	Recommendations for the transit stage of sheep during road transport	86
5.5.	Recommendations for journey breaks and control posts	87
5.6.	Recommendations for transport of goats	87
5.7.	Recommendations for specific scenarios	88
	References	88
	Abbreviations	99
	Appendix A – Template used during the selection of the highly relevant welfare consequences	101

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 jeopardize their health status.

For all scenarios, 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 small ruminants 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.

This Opinion focuses on sheep, as they are transported in much higher numbers inside and outside EU than goats. The available information on goat transport is summarised in a separate section. For both species, 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 (WCs) of transport to some extent differ depending on, for example, the previous husbandry conditions.

The Opinion deals 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 small ruminants until the initiation of loading of the animals onto a vehicle or other means of transport. In effect, in this Opinion, the preparation of sheep for transport essentially involves the gathering of the animals to holding facilities and the keeping of them there prior to transport. Loading starts when the first animal is moved from the holding pen into the means of transport and ends when the last animal is loaded and 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 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 (CPs). Legislation regarding drivers of animal transport vehicles affect animal transport, especially on journeys with only one driver, as drivers have to have rest breaks where 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: A summary of the EU drivers' hours rules and sector specific working time rules (Department for transport UK, 2014)

Drivers' hours rules Regulation (EC) 561/2006	Working time rules Directive 20,002/15/EC
<p>Driving</p> <ul style="list-style-type: none"> • 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 	<p>Working time (including driving)</p> <ul style="list-style-type: none"> • Working time must not exceed average of 48 h a week (no opt out)¹ • Max working time of 60 h in one week (provided average not exceeded) • Max working time of 10 h if night work performed.²
<p>Breaks</p> <ul style="list-style-type: none"> • 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) 	<p>Breaks³</p> <ul style="list-style-type: none"> • 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.
<p>Rest</p> <ul style="list-style-type: none"> • 11 h regular daily rest⁵; 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. 	<p>Rest</p> <ul style="list-style-type: none"> • 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 totalling 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 (e.g. markets, auctions) but refers to them where appropriate. Destination is not covered but only specified when important considerations are found. In the case of animals arriving for slaughter,

additional information can be found in the EFSA Opinion on Welfare of Sheep and Goats at Slaughter (EFSA AHAW Panel, 2021).

Within the small ruminant species, different animal categories exist, such as lamb and sheep. This scientific assessment focuses on non-juvenile animals, but, when relevant and when information is available, other categories (such as unweaned lambs) are mentioned. When specific studies are referred to, the average body weights of the involved animals are mentioned (when available) as well as the choice of category involved in the study (e.g. shorn sheep). For most of the sections of the Scientific Opinion, conclusions and recommendations concerning non-juvenile sheep of an unspecified weight, age, fleece or horn status are drawn.

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

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

Second, for the specific industry practices (specific scenarios) listed in the mandate that relate to sheep, 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 WCs), and, where possible, suggests recommendations.

In relation to the specific transport practice 'export by livestock vessels', the assessment covers the journey up to and including the unloading of the animals in question at the port of destination in a third country. A third country is a country that is not a member of the European Union (or one of the four EFTA countries). In relation to the second specific transport practice, 'the export of livestock to third countries by road', the assessment covers the journey up to and including the unloading of the animals in question at the premises of destination in said third country.

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 then be linked to one or more ABMs that are indicative of it.

During preparation for transport, animals might present various health conditions (including WCs such as injuries) 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 of the Scientific Opinion, focusing on fitness for transport, is developed as part of the assessment of the preparation stage.

For the purposes of this scientific assessment, failure to implement or non-compliance with the current rules as specified in the transport regulation are 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.

Across animal species and animal categories, the assessment is not split according to the current legislation, for example, 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 EFSA's interpretation of the transport mandate, a Public Consultation was launched in the period 15 April–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, for example 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 WCs 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').

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 sub-questions. These were interrelated, meaning that the outcome of each sub-question was necessary in order to proceed to the next sub-question. The approach to develop the sub-questions was based on evidence from the scientific literature and expert opinion. The translation of the assessment questions into sub-questions is mapped in Table 2.

Table 2: Specific assessment questions and sub-questions of the mandate

Assessment Questions		Sub-questions	
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
		<p>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.</p> <p>Approach: Expert opinion via group discussion.</p> <p>Relationship with assessment question: This sub-question is necessary for the overall assessment question requiring the description of the practices.</p>	<p>Aim: All the animal transport practices per animal category identified and selected from sub-question 1 are described narratively.</p> <p>Approach: Literature review.</p> <p>Relationship with assessment question: this corresponds to the assessment question and is necessary for the next assessment question.</p>
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
		<p>Aim: to identify the welfare consequences and provide a definition for them. EFSA generates a list of welfare consequences common for all mandates.</p> <p>Approach: Expert opinion via group discussion (see focus and full resulting list in Section 3.2).</p> <p>Relationship with assessment question: the list of all possible welfare consequences is necessary for the next assessment question asking to identify the highly relevant ones per each system.</p>	<p>Aim: To select the highly relevant welfare consequences for each of the previously defined animal transport scenarios per species or animal category.</p> <p>Approach: Expert opinion via EKE (see Section 2.2.1).</p> <p>Relationship with assessment question: this corresponds to the assessment question, is related to sub-question 1 in which relevant welfare consequences are identified only for current transport scenarios.</p>
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
		<p>Aim: The ABMs for the assessment of the welfare consequences previously identified as relevant are selected (only for feasible ABMs).</p> <p>Approach: Expert opinion via group discussion.</p> <p>Relationship with assessment question: this corresponds to the assessment question and is related to sub- question 4 in which ABMs are identified only for the highly relevant welfare consequences.</p>	<p>Aim: The ABMs for the assessment of the welfare consequences previously identified as the highly relevant are described.</p> <p>Approach: Literature review.</p> <p>Relationship with assessment question: related to sub-question 5.</p>

Assessment Questions	Sub-questions	
iv. Identify the hazards leading to these welfare consequences	7. Identify the hazards leading to the highly 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 sub- question 4 in which hazards are identified only for the highly relevant welfare consequences.	8. Describe the hazards leading to the most relevant welfare consequences Aim: The hazards are described. Approach: Literature review. Relationship with assessment question: related to sub- question 6.
v. Provide recommendations to prevent, mitigate or correct the hazards	9. Identify the preventive and corrective measures for the highly 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 sub- question 4 in which preventive and corrective measures are identified only for the most relevant welfare consequences.	10. Describe the preventive and corrective measures for the highly relevant welfare consequences Aim: Preventive and corrective measures are described. Approach: Literature review. Relationship with assessment question: related to sub- question 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 sub-questions 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 (Sub-question 4), the mandate requested the identification of the highly relevant WCs for each of the defined animal transport practices.

The starting point was the list of 33 specific WCs identified under Sub-question 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 Sub-question 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. The appendix 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 was elicited in three phases:

- 1) First phase: the experts individually went through the list of WCs and identified those that fell in the 'non-applicable' or 'slightly relevant' categories. Their individual judgements were then collated, and those WCs unanimously identified as belonging to these two categories were removed and not considered for further assessment. Those WCs for which there was no consensus as to whether they were 'non-applicable' or 'slightly relevant' remained for further assessment and required 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 were asked to individually rank all of the remaining WCs in the list that were not already identified as highly relevant (and thus kept) or non-applicable or less relevant (and thus removed) from the most to the least relevant. Their individual rankings were 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 opinions only report, for each of the defined animal transport stages, those WCs that were selected to be highly relevant from this exercise (since the mandates ask 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 (space allowance, microclimatic conditions, development over time) relevant to the assessment.

2.2.2. Literature searches

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

First, broad literature searches were carried out 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 sheep, the search results (general + specific) yielded a total of 807 (669 + 138) records that were exported to an EndNote library together with the relevant metadata (e.g. title, authors, abstract). Titles and abstracts were firstly 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 272 + 91 relevant records for the search concerning publication dates from 2011 to 2021. Experts screened these papers and selected 203 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 sheep within the EU

Transport of animals between Member States (MS) and exports from the EU are 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 inside MS (i.e. to slaughterhouses or between farms) are not recorded in this database (Rojek, 2021).

According to TRACES, around 3.5 million sheep were transported between MS per year in 2019–2021, across all means of transport. Road transport constituted around 90%.

3.2. Welfare consequences associated with transport of sheep

During the last decades, several scientific reviews (e.g. Cockram, 2007; Nielsen et al., 2011), textbooks (e.g. Grandin, 2019) and international organisations (e.g. WOA, 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 WCs. 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 and handling of animals at multiple facilities by different groups of stockpersons), 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 sheep, the following WCs were selected as highly relevant: group stress, heat stress, handling stress, injuries, motion stress, predation stress, prolonged hunger, prolonged thirst, resting problems, restriction of movement and sensory overstimulation (Table 3). It is clear from this table that most 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: Welfare consequences 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
Group stress	The animal experiences stress and/or negative affective states such as pain, fear and/or frustration and stress resulting from a high incidence of aggressive and other types of negative social interactions, often due to hierarchy formation and competition for resources or mates.				X

WCs and definitions		Transport stages			
		Preparation	Loading/ unloading	Transit	Journey break
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. loading/unloading).	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
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
Predation stress	The animal experiences stress and/or negative affective states such as fear and/or pain resulting from being attacked or perceiving a high predation risk.	X	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	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	
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 movements	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	

WCs and definitions		Transport stages			
		Preparation	Loading/ unloading	Transit	Journey break
Sensory overstimulation	The animal experiences stress and/or negative affective states such as fear, discomfort due to visual, auditory or olfactory under/overstimulation by the physical environment.		X	X	X

WC: welfare consequence.

3.2.1. Negative affective states

The description of each WC reported in Table 3 refers to one or more negative states, many of which have affective components (e.g. pain, fear, fatigue). These are the major negative affective states that derive from the occurrence of the WC, 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).
Stress¹ and Distress	STRESS ¹ : Stressors are events, internal or external to the body involving real or potential threats to the maintenance of homeostasis. When stressors are present, the body will show stress responses (biological defence to re-establish homeostasis – for example 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).
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 threaten 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 is not in itself a negative affective state, but 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 the assessment of welfare during transport for livestock species (Messori et al., 2015a, 2017), and – so far – no validated protocol for the assessment of welfare before, during and after transport has been described. Consequently, no benchmark values documenting optimal animal welfare status during or after transport exist and could be used in this Scientific Opinion. In addition, benchmark values documenting good animal welfare do not exist for any of the ABMs listed in 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, without using any specialised equipment or laboratory test, at a low cost, and with no (or only minimal) interference with normal operation procedures (Llonch et al., 2015; Messori et al., 2015a). 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 animal 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 is very difficult, if not impossible to see animals when they are in a livestock truck, for example. 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 do not overcome all of these challenges, as data generated by such sensors need to be analysed in some way leading to an interpretation of the scenario in question. Currently, technological tools within this area are under development in sheep (e.g. Zhang et al., 2020), but not yet applicable in practice. This constitutes a gap in knowledge. Until sensors and associated interpretative or alarm systems are available at a practical level, the stage of transport appears to be the biggest influence on the feasibility of an ABM during animal transport. 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).

During preparation, loading, unloading and journey breaks off the vehicle, the animals can be properly inspected and ABMs in principle 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, e.g. latency to eat 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 for transport inspectors even when the animals can be inspected (Llonch et al., 2015). Examples of these are physiological indicators requiring invasive procedures. Tables 5–14 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 group stress

Table 5: ABMs for the assessment of group stress in sheep during journey breaks

ABM	Definition and interpretation of the ABM
Butt	<p>Definition: A strike with the head into the side or rump of the other sheep (Erhard et al., 2004).</p> <p>Interpretation: An act of aggression.</p>

ABM	Definition and interpretation of the ABM
Threats	<p>Definition: <i>Threat (head):</i> Butting movement towards the opponent without contact (Fisher and Matthews, 2001). <i>Threat (back):</i> The aggressor takes a few steps back and shows a 'low stretch' (the sheep extends the neck forward and horizontal to the ground. <i>Threat (forward):</i> The aggressor approaches the other sheep turning the head downwards pointing her horns towards the opponent (Erhard et al., 2004).</p> <p>Interpretation: Threat (with the head, back or forward) and butt are often displayed as a sequence of behaviours in an effort to exert dominance over other sheep.</p>
Push and butt-push	<p>Definition: <i>Push:</i> Pressing shoulder against shoulder. <i>Butt-push:</i> pushing with the forehead or horns against the opponent's body. In the butt-push, there is less emphasis on the impact of the horns (butt), but on the pushing movement (Erhard et al., 2004).</p> <p>Interpretation: An attempt to move other sheep.</p>

ABM: animal-based measure.

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

Table 6: ABMs for the assessment of handling stress in sheep during preparation, loading/unloading and journey breaks

ABM	Definition and interpretation of the ABM
Falling	<p>Definition: Animal showing a loss of balance during loading/unloading causing other part(s) of the body (beside legs) to touch the floor (Consortium of the Animal Transport Guides Project, 2018).</p> <p>Interpretation: Sheep may fall on the ramp or in the barn/truck as a result of hasty or violent handling, behaviour of other animals, slippery ground, slope or obstacles.</p>
Heart rate	<p>Definition: The number of heartbeats per unit of time, usually per minute.</p> <p>Interpretation: Acute stress response leads to a release of catecholamines into the serum and consequently to an increase in heart rate (Damián et al., 2021). However, heart rate is also subject to regulation by the autonomic nervous system through sympathetic nerves that increase and parasympathetic nerves that decrease heart rate. Heart rate is affected by factors other than acute stress. The exercise associated with movement in response to handling and loading will also increase heart rate.</p>
Slipping	<p>Definition: Animal showing a loss of balance with a leg sliding unintentionally over a small distance (Consortium of the Animal Transport Guides Project, 2018).</p> <p>Interpretation: Sheep can slip on the ramp or in the barn/truck as a result of hasty or violent handling, behaviour of other animals, slippery ground, slope or obstacles.</p>

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

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

ABM	Definition and interpretation of the ABM
Rectal temperature	<p>Definition: Rectal temperature is measured as an indicator of core temperature. Rectal temperature of sheep varies between 38.3°C and 39.9°C under thermoneutral conditions (Goodwin, 1998; Kearton et al., 2020).</p> <p>Interpretation: Body temperature rises during heat stress when the physiological and behavioural mechanisms for the dissipation of heat can no longer maintain equilibrium because of heat gained from excessive environment heat combined with metabolic heat production. Sheep lose approximately 20% of total body heat via respiratory moisture in a neutral environmental temperature (12°C). The moisture loss increases and accounts for approximately 60% of the total heat loss at high ambient temperature (35°C) (Thompson, 1985). An increase in the ambient air temperature from 18°C to 35°C is accompanied by significant increases in rectal temperature in sheep (Marai et al., 2007).</p>

ABM	Definition and interpretation of the ABM
Panting	<p>Definition: The first phase of panting is characterised by rapid, shallow breaths associated with an increase in respiratory rate resulting in an increase in respiratory volume (Hales and Webster, 1967). Open-mouth panting is related to the second phase of gasping which is characterised by slower and deeper breathing, associated with open-mouth gasping and a greater increase in respiratory volume than that observed in the first phase of gasping (Hales and Webster, 1967).</p> <p>Interpretation: Rapid panting is a response to hot environmental conditions or acute physical exercise (Lees et al., 2019).</p>
Respiratory rate	<p>Definition: Frequency of breathing, usually measured as the number of breaths that can be counted according to the flank movements per minute (Da Silva et al., 2002).</p> <p>Interpretation: Respiration rate increases with body temperature in order to maintain homeostasis.</p>
Salivation	<p>Definition: The secretion of biofluid by the three major salivary glands located in the mouth - paroid, submandibular and sublingual - together with the secretions from the minor submucosal glands of the oral cavity (Proctor, 2000).</p> <p>Interpretation: Intense salivation is one of the clinical signs of heat stress in sheep (Dos Santos et al., 2019). When animals experience heat stress they exhibit profuse salivation or drooling (Caulfield et al., 2014).</p>
Sweating	<p>Definition: Secretion of fluid or moisture from the sweat glands to the surface of the skin.</p> <p>Interpretation: At high temperatures, evaporative cooling is the main mechanism for heat dissipation in most mammals (Blackshaw and Blackshaw, 1994) and is the only form of heat loss once the ambient temperature exceeds the skin temperature (Cunningham, 2002). When the effective temperature increases above the comfort zone, the animals 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 or stress.</p>

ABM: animal-based measure.

iv) ABMs for the assessment of the welfare consequence injuries

Table 8: ABMs for the assessment of injuries in sheep during loading/unloading and journey breaks

ABM	Definition and interpretation of the ABM
Lameness	<p>Definition: Locomotory changes involving impaired movement or deviation from normal gait or posture (Van Nuffel et al., 2015).</p> <p>Interpretation: Most lameness is a consequence of pain but it can also occur from mechanical defects resulting in physical incapacity. Behavioural changes associated with lameness indicate attempts by the animal to protect the affected limb from pain and further injury (Whay, 2002).</p>
Non-ambulatory sheep	<p>Description: Unable to stand or move without assistance and/or unable to bear weight on two legs (Consortium of the Animal Transport Guides Project, 2018).</p> <p>Interpretation: Can occur as a result of injury, lameness, disease or exhaustion. It makes a sheep unfit for transport and to prevent unnecessary suffering the sheep requires additional care or euthanasia.</p>
Skin lesions and wounds	<p>Definition: Macroscopically visible loss of skin integrity due to lesions and wounds (Phythian et al., 2019).</p> <p>Interpretation: High prevalence of skin lesions can be caused by violent handling, highly reactive animals, collisions with facilities, falls and entrapment, especially on the back, thorax and limbs (Llonch et al., 2015).</p>

ABM: animal-based measure.

v) ABMs for the assessment of the welfare consequences motion stress and sensory overstimulation

Table 9: ABMs for the assessment motion stress in sheep during transit, and sensory overstimulation during loading/unloading, transit and journey breaks

ABM	Definition and interpretation of the ABM
Heart rate	<p>Definition: The number of heartbeats per unit of time, usually per minute.</p> <p>Interpretation: The acute stress response leads to a release of catecholamines into the serum and consequently to an increase in heart rate (Damián et al., 2021). However, heart rate is also subject to regulation by the autonomic nervous system through sympathetic nerves that increase and parasympathetic nerves that decrease heart rate. Heart rate is affected by factors other than acute stress. The exercise associated with movement in response to handling and loading will also increase heart rate.</p>
Plasma cortisol	<p>Definition: Measure of cortisol concentrations in plasma. Cortisol is one of the major glucocorticoids secreted from the adrenal cortex, it is heavily involved in the response to emotional and physical stress as well as in homeostatic mechanisms (Katsu and Iguchi, 2016). When a threat is perceived by an animal, large quantities of cortisol are released in blood, due to the activation of the hypothalamic–pituitary–adrenal (HPA) axis (Andanson et al., 2020).</p> <p>Interpretation: Plasma cortisol concentration is a good indirect measure of the stress experienced by an animal when exposed to adverse conditions during the initial part of a journey (Miranda-de la Lama et al., 2009). With increased journey duration feedback mechanisms can reduce its concentration and the plasma cortisol concentration may no longer reflect the continued perception of sheep that transportation remains an aversive stimulus. Continued hypothalamic stimulation results in the continual secretion of corticotropin-releasing hormone even though plasma cortisol concentration may have fallen (Smith et al., 2003).</p>

ABM: animal-based measure.

vi) ABMs for the assessment of the welfare consequence predation stress

Table 10: ABMs for the assessment of predation stress in sheep during preparation and loading/unloading

ABM	Definition and interpretation of the ABM
Avoidance behaviour, especially expressed as sudden movements	<p>Definition: Moving away from another animal or away from a source of aversive stimulus (Napolitano et al., 2011).</p> <p>Interpretation: During loading/unloading, sheep may be exposed to sheepdogs that provoke an avoidance response as an antipredator behavioural response (Beausoleil et al., 2005).</p>

ABM: animal-based measure.

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

Table 11: ABMs for the assessment of prolonged hunger in sheep during transit and journey breaks

ABM	Definition and interpretation of the ABM
Beta-hydroxybutyrate (BHBA) in blood	<p>Definition: The concentration of ketone bodies in the blood is an indicator of their use as an alternative energy source, especially in the brain (Katz and Bergman, 1969).</p> <p>Interpretation: The production of ketone bodies during fasting through transport can be considered an adaptive mechanism to minimise glucose consumption, thus avoiding excessive muscle and lipid catabolism (Knowles et al., 1995).</p>
Glycogen depletion	<p>Definition: Glycogen, the form in which the body stores sugar, is mainly concentrated in muscles and liver. Glycogen depletion occurs after fasting periods and intense exercise when the body mobilises carbohydrate reserves (mainly glycogen from the liver), until this becomes exhausted after about 12–24 h (Gardner et al., 2014). When glycogen storage in the liver is depleted, stored adipose tissue triglycerides are released into the circulation as fatty acids and glycerol.</p> <p>Interpretation: Fasting for 24 h can reduce liver glycogen concentration to minimal values (Warriss et al., 1989). In addition, stress or vigorous exercise before slaughtering can also cause glycogen depletion in muscle tissues having a direct impact on muscle pH, meat colour and consequently, meat quality (Gardner et al., 2014).</p>

ABM	Definition and interpretation of the ABM
Latency to feed consumption immediately after unloading	<p>Definition: A quantification of the time interval from unloading and until the animal is observed eating for the first time.</p> <p>Interpretation: A short latency to eat is a reflection of a high motivation to eat. Can be affected by other factors such as fear.</p>
Non-esterified fatty acids in blood	<p>Definition: Measurement of non-esterified fatty acids (NEFA) or free fatty acids, which are fundamental units in the structure of lipids in membranes and lipoproteins, in blood. NEFA are an important source of energy for the heart and for skeletal muscle.</p> <p>Interpretation: A higher concentration of non-esterified fatty acids (NEFA)/free fatty acids in sheep is an indicator of lipolysis due to the need for its use as an alternative energy source (Knowles et al., 1995). Transport could increase plasma NEFA concentration because feed deprivation during transport causes depletion of hepatic glycogen, so that NEFA become the main source of energy through mobilisation of body fat (Zhong et al., 2011). However, it can also be confounded by effects of exercise and stress (Warriss et al., 1989).</p>

ABM: animal-based measure.

viii) *ABMs for the assessment of the welfare consequence prolonged thirst*

Table 12: ABMs for the assessment of prolonged thirst in sheep during transit

ABM	Definition and interpretation of the ABM
Hard faecal pellets	<p>Definition: Faeces with a dry, firm and hard consistency.</p> <p>Interpretation: Dehydration as a product of water deprivation results in hard, dry faeces in sheep because the body tries to conserve water in the blood by intestinal absorption of water from the faeces (Asplund and Pfandes, 1972).</p>
Plasma osmolality	<p>Definition: Increased concentration of the particles dissolved in the plasma.</p> <p>Interpretation: Lack of access to water coupled with physical exercise and heat during travel causes hypertonic dehydration, which is expressed in an elevation of plasma osmolality (Parrott et al., 1987). Increased plasma osmolality indicates hypertonic dehydration which occurs when water loss is proportionally greater than electrolyte loss, with water passing from the cell to the extracellular space as a compensatory mechanism (Walz and Taylor, 2012).</p>
Packed cell volume	<p>Definition: The ratio of the volume occupied by packed red blood cells to the volume of the whole blood as measured by a hematocrit.</p> <p>Interpretation: Changes are confounded by potential splenic contraction due to stress, dehydration and loss of red blood cells (Turner and Hodgetts, 1959).</p>
Plasma total protein concentration	<p>Definition: Dehydration leads to a decrease in blood plasma volume, which increases the concentration of plastic proteins such as albumin and globulins.</p> <p>Interpretation: A journey in hot conditions, high densities and without water intake will cause animals to lose fluids and consequently have a marked elevation of this indicator.</p>
Intake of water after unloading	<p>Definition: Intake of water during the initial hours after unloading..</p> <p>Interpretation: Shows the transport-induced thirst threshold as a function of drinking time (Pascual-Alonso et al., 2017).</p>
Latency to drink	<p>Definition: Time until sheep starts drinking after unloading.</p> <p>Interpretation: Shows the transport-induced thirst threshold as a function of drinking time (Pascual-Alonso et al., 2017). The shorter the latency, the higher the thirst.</p>

ABM: animal-based measure.

ix) ABMs for the assessment of the welfare consequence resting problems

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

ABM	Definition and interpretation of the ABM
Disturbed lying movements	<p>Definition: The sheep perform more intention movements to lie down (i.e. repeatedly sniffing the surface, often accompanied by swinging the head from side to side close to the surface or bending one front leg without lying down) as well as lying attempts – that is, placing one or both carpal joints on the surface, followed by standing up again.</p> <p>Interpretation: Tired animals show a high motivation to lie down to lessen the effects of the journey (Pascual-Alonso et al., 2017).</p>
Frequent posture changes	<p>Definition: Observation of sheep actively and frequently changing posture during the journey, in contrast with animals standing still during the journey (Ruiz-De-La-Torre et al., 2001).</p> <p>Interpretation: Sheep tend to change posture as a sign of discomfort or as a mechanism to maintain balance during the journey.</p>
Lying behaviour after a journey	<p>Definition: Percentage of animals lying down after journey.</p> <p>Interpretation: Increased lying behaviour after a journey might occur due to inadequate space for animals to lie down and rest during a journey.</p>
Prolonged standing	<p>Definition: Measurement of the number of sheep maintaining an upright position for all or most of the journey, rather than lying down (Cockram et al., 2012).</p> <p>Interpretation: Sheep tend to spend more time standing during the journey because of the impossibility of lying down due to lack of space and/or excessive vibration-motion of the truck (Ruiz-De-La-Torre et al., 2001).</p>

ABM: animal-based measure.

x) ABMs for the assessment of the welfare consequence restriction of movement

Table 14: ABMs for the assessment of restriction of movement in sheep during transit

ABM	Definition and interpretation of the ABM
Falling	<p>Definition: Animal that exhibits a total loss of balance during travel and the animal's body has full contact with the floor (Jones et al., 2010; Consortium of the Animal Transport Guides Project, 2018).</p> <p>Interpretation: Insufficient space allowance and/or poor ride characteristics of the roads in transit make it difficult for animals to balance, i.e. loss of balance, slip, knee drop, leading to falls.</p>
Latency to stand up after a falling event	<p>Definition: Time required by the animals to regain the standing up posture after falling down.</p> <p>Interpretation: An increased latency to stand up after falling could occur due to inadequate space to adjust posture.</p>
Loss of balance	<p>Definition: Group events where all or most of the sheep in the compartment lose stability and need to move their feet to remain upright.</p> <p>Interpretation: Under similar circumstances, loss of balance events occur more frequently at reduced space allowances.</p>
Overlying	<p>Definition: Animals lying down (or attempting to) on other conspecifics.</p> <p>Interpretation: When space is restricted, overlying can occur as some animals are observed to lie down on another animal when both attempt to lie down at the same time.</p>
Slipping	<p>Definition: Animal showing a loss of balance with a leg sliding unintendedly over a small distance (Consortium of the Animal Transport Guides Project, 2018).</p> <p>Interpretation: Insufficient space allowance makes difficult for the sheep to balance and could lead to an increase in the number of slips.</p>
Trampling	<p>Definition: The action of stamping, pawing, walking or standing on by other sheep while lying down or after they have fallen.</p> <p>Interpretation: Overcrowding can cause trampling of animals that are lying down by animals standing on them (Jones et al., 2010).</p>

ABM: animal-based measure.

3.3. Preparation of sheep for transport

Across sheep categories as well as journey types and journey durations, the preparation of sheep for transport may differ substantially. However, in terms of animal welfare, it is an important phase, as careful preparation of sheep for transport can substantially improve the welfare impact of the journey.

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 sheep until the initiation of loading of the animals onto a truck or other means of transport. In effect, in this Opinion, the preparation of sheep for transport essentially involves the gathering of the animals to holding facilities and the keeping of them there prior to transport itself. Fitness for transport is included in the preparation phase. The loading of animals into the transport vehicle is covered in Section 3.4. For matters related to logistics, paperwork and planning, such as for example route planning, readers are recommended to check recent recommendations from the EU Transport Guides (Consortium of the Animal Transport Guides Project, 2018). Recommendations for facilities at the various types of premises in question and for the means of transport can also be found in the Transport Guides. These issues will not be covered in this Scientific Opinion.

3.3.1. Current practices

Sheep are kept on farms that vary from the extensive, mountainous terrain where they range on open, unfenced pasture, to intensive feedlots where sheep are kept permanently in small pens (Campo et al., 2016). Gathering for transport is an infrequent practice, in the former case usually once a year, and sheep are therefore highly susceptible to stress as a result of their lack of familiarity with the process (Wickham et al., 2015). In hill farms, ewes and lambs on the higher areas are gathered annually to separate lambs for more intensive fattening, near the homestead or on another property. This traditional system of seasonal movement was formerly accomplished by droving the sheep on foot, but now transport vehicles are often used.

The preparation of sheep for transport essentially involves the gathering of sheep to holding facilities and the keeping of the sheep there prior to transport itself. The means of gathering and the type of premises will depend on the type of journey being prepared for. The transport of sheep from one farm to another will involve the rounding up of the sheep 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 the sheep are being transported from one EU MS to another, 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. If the sheep are to be exported to third countries, they will very likely have to spend time in quarantine premises in order to meet the requirements of the relevant health certificate. This can involve large numbers of sheep, sometimes even several thousand. These sheep have to be transported by road to the quarantine premises before the quarantine process can begin.

Moving sheep between farms may involve them passing through a market. Such sales involve sheep being transported to a market and then from the market to another farm in a different vehicle and sometimes with different sheep from different farms. Sheep transiting through live markets are at particular risk of poor welfare due to bad handling (and the associated slips, falls, throwing and wool pulling), hurried loading and, less commonly, isolation (Gregory et al., 2009). However, markets will not be specifically dealt with in this Opinion.

Sheep travelling on vessels have several transport phases, initially on trucks, then onto the vessel and finally on trucks again.

3.3.2. Highly relevant welfare consequences

The highly relevant WCs selected during the preparation stage are handling stress and predation stress. The selected ABMs for the assessment of these WCs are shown above in Section 3.2.2. Below, hazards (in bold), preventive (PRE), corrective and mitigating measures are described.

i) Handling stress

There is increasing evidence that the attitude and behaviour of the stockperson towards the animals in their care can have a significant impact on fear, welfare and productivity (Losada-Espinosa et al., 2020). On the other hand, sympathetic management by farmers can reduce the impact of handling, particularly repeated handling, even in the case of sheep that are generally fearful of

humans and sheepdogs. Therefore, the main hazards leading to handling stress during the preparation phase are:

Inexperienced, untrained or aggressive handlers: Handlers with these characteristics tend to cause fear, unpredictability and accidents in the group.

- PRE: To prevent this hazard, handlers should be properly educated and trained to rationalise and differentiate between their good and bad practices when gathering, loading and/or unloading animals from the truck. Handler training should preferably be done at the loading/unloading site or at a similar location. Key aspects that should be taught to handlers are the escape zone, flight distance and aspects of group herding, as well as leading animals with flags, avoiding moving the animals too fast and avoiding the use of sticks and electric prods. The risks and accidents that can occur due to excessive behavioural reactivity of the isolated animal should be taught, as well as the repercussions such as bumps, bruises, fractures and trampling on the animal itself and the handlers (Losada-Espinosa et al., 2020). Practical teaching should include pedagogical dynamics for handlers to develop or strengthen the degree of empathy towards the animals. More detailed information on the training of handlers can be found in the transport guidelines (Consortium of the Animal Transport Guides Project, 2018).

Inappropriate handling: Inadequate handling during unloading and loading may occur when sheep are reluctant to move and there is an increased pressure to get them on/off the truck as a group for example due to limited time availability. Therefore, pushing, pulling the wool or holding the animals by the skin are due to difficulties in getting the sheep moving in the right direction or to follow their conspecifics (Gregory et al., 2009). At other times, a handler may hold one or more animals by the fleece or leg to load (or unload) them into the truck to get the other animals to follow. All such practices induce stress and fear in the animals.

- PRE: To prevent this hazard, handlers should be properly educated and trained (see above) in handling sheep. It may be advantageous to set a minimum group size of four to five individuals as this has been suggested to ensure that animals remain calm (Fisher and Matthews, 2001).

Poor handling facilities: Poorly designed or poorly maintained facilities are a stress factor for handlers and animals. In these conditions, handlers are less efficient, tire more quickly and may suffer from occupational accidents, as well as becoming rougher in their handling of the animals.

- PRE: To prevent this hazard, handling facilities should be fit for purpose (Cockram and Velarde, 2022).

Lack of previous handling experience of the animals: Animals more accustomed to positive contact with humans and with being handled are likely to be less fearful of being loaded and transported.

- PRE: Sheep should be habituated to be handled before the transport.

Corrective/mitigating measure for handling stress

Corrective measures include removal of the specific person employing inappropriate handling or provision of assistance to him/her on the spot.

ii) Predation stress

Herding dogs specialise in gathering and controlling the movement, direction, and speed of sheep and in sorting and partitioning particular individuals within a larger animal group in cooperative response to the guidance of their handler (Ridgway, 2021). However, when using dogs in loading/unloading, it should be borne in mind that sheep may perceive dogs as potential predators and have an innate aversion to them, especially when they feel cornered or are momentarily separated from the group and even if they are brought into enclosed spaces. The main hazards leading to predation stress are:

Sheep not habituated to dogs: In animals not accustomed to being handled with herding dogs the risk and severity of predation stress will be higher.

- PRE: To prevent this hazard, sheep should be habituated to dogs.

Inappropriate use of dogs in enclosed or confined spaces: Handling sheepdogs in enclosed spaces with inclines and aisles such as loading and unloading areas can cause animals to become frightened, move abruptly or crowd and avoid contact with the dog and consequently collide with each other, potentially injuring themselves.

- PRE: To prevent this hazard, handlers should pay extra attention to the work of the dog in enclosed or confined spaces. The dog should never be allowed in the truck with the animals, although it may accompany the driver in the cab.

Untrained or over excitable dogs: The use of untrained or overly excitable dogs will cause animals to be frightened, excited, injured or fatigued, which will affect their fitness for the journey or their recovery during post-transport rest (Dwyer, 2009).

- PRE: To prevent this hazard, handlers should pay extra attention to only use properly trained dogs, e.g. dogs that have been socialised with the sheep from puppyhood and preventing the dog from being uncontrolled during loading/unloading, as well as removing the dog when its assistance is no longer required, as its presence is a distraction to the sheep and a source of unpredictability.

Corrective/mitigating measure of predation stress

Corrective measures include a secure resting area for the sheep with shade and access to water where there is no visual or olfactory contact with the dog(s).

3.3.3. Fitness for transport

3.3.3.1. Introduction

Depending on the length and quality of the journey, transport can represent significant hazards, even for healthy and fit sheep. These difficulties are increased for weak, diseased or injured sheep. Those sheep are most likely already experiencing WCs, or negative affective states, such as pain, before being transported. Thus, weakened animals, in this condition, will be less able to deal with the hazards and additional challenges associated with transport, such as loading and unloading, interacting with other animals, maintaining stability, avoiding fatigue and responding to feed and water restriction (as reviewed by Cockram, 2019b), and to extreme thermal environments in the vehicle itself.

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

Sheep sent for slaughter with pre-existing conditions are more likely to die in transit, become non-ambulatory, or be condemned as unfit for human consumption upon arrival at the slaughterhouse than those that are fully fit (Cockram, 2019b). In one study of lambs transported to slaughter in the UK, 0.008% were recorded as dead-on-arrival (DOA). There was a relationship between lambs with pre-existing conditions (identified post-mortem as condemnations due to abscess, arthritis and pleuritis) and the an increased risk of mortality during or shortly after transport (Knowles et al., 1994).

Thus, if animals are not properly assessed, and unfit animals are allowed to enter the logistic chain, it is a hazard for their welfare, predisposing them to different WCs during the journey (Table 3), and potentially leading to negative affective states such as discomfort, pain and suffering. Typical characteristics leading to animals being unfit for transport are related to health impairment. Some characteristics rendering animals unfit for transport, however, do not directly relate to health, but to certain age groups or certain stages of the production cycle.

The criteria and circumstances leading to a decision of 'unfit for transport' probably vary considerably across the different categories of sheep, thereby posing extra challenges for the professionals involved in decision-making: farmers, livestock drivers, veterinarians and competent authorities, as well as posing challenges to the welfare of sheep that may be transported despite being unfit for the intended journey.

Based on the above, it is clear that assessment of fitness for transport is not simple. Several studies, albeit in cattle and pigs, have reported doubt in the decision-making of involved professionals (Herskin et al., 2017, 2020; Thodberg et al., 2020; Dahl-Pedersen, 2022), and a comparison between and within three different professional groups (drivers, veterinarians and farmers) regarding fitness for transport of dairy cows, showed at best moderate agreement (Dahl-Pedersen et al., 2018). Such

knowledge is not available for sheep, but due to their tendency to not show signs of discomfort, it is likely that assessment of sheep fitness for transport is at least as challenging as assessment of fitness for transport in the other species. Hence, in order to prevent the significant welfare hazard of allowing unfit animals to enter means of transport, it is highly important that the inspection is carried out correctly. If the concept of fitness for transport is not well-defined, if guidelines for fitness of transport are not comprehensive and broadly available, if professionals are not properly educated, and if questions about responsibility are present, the risk of animals entering means of transport as unfit will be higher. This is even more important in the case of long journeys, such as journeys by sea vessels.

From a scientific point of view, the concept of fitness for transport has received limited attention, and at present, thresholds for ABMs as indicators of animals being unfit for transport have most often not been established or validated. If sheep are to be fully protected from the consequences of being transported while in reality unfit for transport, knowledge about the risk associated with transport of animals with a number of conditions potentially leading to negative affective states (e.g. wounds), as well as the establishment of ABMs useful to identify these and their thresholds (suitable for use across professional groups), are needed.

3.3.3.2. Assessment of fitness for transport in sheep

When fitness for transport is assessed, signs of illness, such as sickness behaviour, altered gait, increased respiration and discharges, should be evaluated (Lovatt, 2010). Even when assessed as fit for transport and transported to slaughter, signs of pathology, that may or may not have been clinically apparent before loading, may be detected at slaughter and might or might not have affected their ability to respond to the challenges of transport (as reviewed by Cockram and Velarde, 2022). Examples of adverse consequences of pathology that would impair the biological fitness of sheep for transport are reduced respiratory function, reduced exercise tolerance (Scott and Gessert, 1998), impaired ability to walk and maintain posture, and reduced capacity to thermoregulate and respond to periods of feed and water restriction (Cockram, 2019b).

- *Conditions leading to sheep being unfit for transport*

If, before transport, a sheep has a painful condition, transport will almost certainly aggravate the pain. Movement of, or pressure on, an area of inflammation, such as an arthritic joint, will cause additional pain. Therefore, body movement during loading, unloading, in response to vehicular movements or other animals, and during postural changes, are likely to cause movement of sensitive tissue and cause additional pain. Sheep should not be transported to slaughter with a fracture as this causes additional suffering. Bone fractures are painful; pressure or movement and mechanical distortion of fractures cause additional pain (Cockram, 2019b).

Lameness is common in sheep (Winter, 2008; Kaler and Green, 2009) and can be detected using locomotion scoring (Kaler and Green, 2008). When a sheep appears lame or is reluctant to walk, pain is most likely involved. Prolonged standing and challenges to stability when the vehicle or other animals shift position are likely to cause the condition of a lame animal to deteriorate during a journey. A lame animal that lies down during transport is at risk of being injured or trampled by other sheep that remain standing. To be fit for transport, a sheep must be able to stand, bear weight on all legs and be able to adjust footing to maintain balance during the journey. The sheep must also be able to walk up and down ramps when loaded and unloaded. Although some lambs with footrot are transported to slaughter (König et al., 2011) they have greater sensitivity to mechanical stimulation of their feet than non-lame sheep (Ley et al., 1989). As they have to make frequent foot changes to maintain stability during transport they likely experience pain each time that they move their feet.

Some conditions make a sheep unfit for transport, because they reduce their ability to perform an important physiological function, e.g. pneumonia can reduce exercise tolerance and capacity to deal with heat. Emaciated or weak sheep may have reduced ability to obtain feed and water, are more susceptible to the combined effects of fasting and cold exposure and are less able to respond to other animals and events affecting their stability (Cockram, 2019b). Shorn sheep are more susceptible to wind-chill than fleeced sheep (Alexander, 1974) and are therefore more vulnerable to transport in cold conditions.

Sheep with a clinical disease may feel ill (e.g. inappetence, thirst and fever), be more susceptible to cold and heat and may also be at increased risk of experiencing other negative affective states, such as fear (because of disorientation or reduced ability to respond to perceived danger) and distress (Cockram and Hughes, 2018).

Most cull sheep should be considered vulnerable as they are at a greater risk of deteriorating during the journey and potentially become unfit. Common health issues that could affect the fitness of cull ewes for transport, especially if they experience long journeys, are emaciation, lameness, mastitis and 'broken mouth'/dental issues (Herrtage et al., 1974; Ridler and West, 2010; McLaren et al., 2020). Ewes in poor body condition are likely to be less able to cope with prolonged periods of feed restriction and are more sensitive to stress, as shown by greater plasma NEFA concentrations and a lower plasma glucose concentration than those in better body condition (Caldeira et al., 2007). Some non-ambulatory cull ewes sent to slaughter show signs of hypocalcaemia and hypoglycaemia (Shorthose and Shaw, 1977).

A ewe with an engorged udder is not fit for transport. During lactation, the udder fills with milk and if the pressure is not relieved by milking or suckling by the offspring, the udder becomes painfully engorged. In this condition, a ewe may appear reluctant to lie down, have inflamed mammary glands (swollen, painful, warm or red) and could appear lame. The risk of udder engorgement can be reduced by making arrangements for the lactating ewe to be milked before the start of a journey and at regular intervals during the journey, drying-off several weeks before the journey and keeping the journey as short as possible.

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 fetus(es);
- 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 being transported if the pregnant female is not slaughtered;
- 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. According to the biology of sheep, giving birth to one or more precocial young means that the investment – in terms of energy resources and weight – of pregnant sheep differ from species, such as rabbits, where the young are born in an altricial state (as reviewed by Nowak et al. (2000)). Sheep give birth to one or typically more precocial offspring, the weight of which at birth can be more than 10% of the mother's own body weight (Dwyer, 2017). This depends on the breed, as modern sheep breeds have been selected for increased litter size and increased productivity traits in the offspring such as heavier birth weight.

Pregnancy in itself does not make a sheep unfit for any transport (Roussel et al., 2006), but in late pregnancy there are physical and physiological changes that increase the challenges experienced by a ewe during transport. The relation between pregnancy stage and fitness for transport in ewes has, however, only been studied marginally. Ewes transported in late pregnancy are at increased risk of metabolic conditions such as pregnancy toxemia due to the effects of fasting and motion stress (Saba et al., 1966). Pregnancy is associated with changes in the cardiovascular system that could reduce the exercise capacity of the ewe (Lotgering et al., 1983), a finding that was documented in sheep that were 117–138 days pregnant (term 147 days), and increase the risk of heat stress (Romo-Barron et al., 2019). The increase in the volume of the allantoic and amniotic fluid compartments with gestation (Wintour et al., 1986) is likely to affect the locomotion of the ewe. In addition, it is also suggested that the stress of transport could cause abortion (Vidić et al., 2007).

From Table 15, it is clear that there is consensus not only among different guidelines from, e.g. industry (e.g. MLA, 2019), but also governmental (CHAR, 2022) and intergovernmental sources (WOAH, 2011) to recommend that pregnant sheep are not transported beyond 90% of pregnancy. However, no evidence to support these guidelines has been found, as, for example, illustrated from Figure 1, showing the fetal growth curve of lambs.

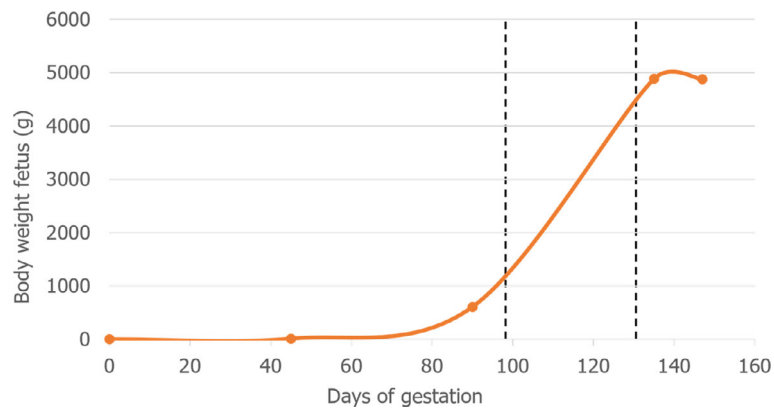


Figure 1: Fetal growth curve of lambs (data from Pillai et al., 2017)

The gestation length of sheep is 147 days, and fetal growth increases significantly from approximately half term, and continues to increase steeply until few days before the lamb(s) is/are born. Figure 1 displays data from Pillei et al., 2017 that studied 27 multiparous Western White-faces ewes, 3 years or older, giving birth to 47 offspring (15% singleton, 49% twin and 17% triplet). The dotted lines are inserted to indicate the initiation of the last trimester (approximately day 100) and the last 90% of pregnancy (approximately day 130).

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. In her review chapter, Dwyer (2017) concluded that the evidence of the potential consequences of prenatal stress in sheep currently is rather patchy and inconclusive. It can, however, not be excluded that transport stress experienced while *in utero* can affect lambs.

The 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. In addition, 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 other 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.

Juvenile animals have a reduced capacity to withstand the challenges of transport. They may be at increased risk of hypothermia and hypoglycaemia and may be weak and lacking coordination. Young lambs may also be at greater risk of an infectious disease due to their underdeveloped immunity. These factors make them more susceptible to extreme conditions such as cold temperatures and long journeys without access to food, water and rest.

Conditions affecting fitness for transport are listed in several regulations (Government of Canada, 2022; Council Regulation (EC) No 1/2005¹ and there are several guidance documents and decision trees available to assist in the assessment of the fitness of sheep (Australian Animal Welfare Standards and Guidelines, 2012; Consortium of the Animal Transport Guides Project, 2018; Alberta Farm Animal Care Association and Alberta Lamb Producers, 2019; Meat and Livestock Australia (MLA), 2011; WOA, 2011). World Organisation for Animal Health (2011) have also produced guidance on the assessment of the fitness of sheep before live export by sea.

Table 15 provides an extensive list of examples of conditions that would make a sheep unfit for transport. However, the 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 sheep unfit in relation to the intended journey.

Table 15: List of conditions that can make a sheep unfit for transport

General condition	Specific condition	References	
Sickness/illness	Not specified further	WOAH, CHAR	
	Pathological processes	CR2005	
	Cardiovascular or respiratory disorders/laboured breathing	AFACA, CATGP, CHAR	
	Apparent lack of coordination/disorientation	AFACA, CATGP	
	Generalised nervous system disorder	CHAR	
	Shock or dying	CHAR	
	Fever	CHAR	
	Infected navel	CHAR	
	Gangrenous udder	CHAR	
	Mastitis	AFACA, MLA	
	Bloated to the extent that it exhibits signs of discomfort or weakness	AFACA, CHAR	
	Gastrointestinal disruption	AFACA	
	Contagious ecthyma (orf)	AFACA, MLA	
	Orchitis	AFACA, MLA	
	Swollen penis	MLA	
Urinary calculi causing abdominal distention	AFACA		
Pathophysiological state	Weakness	AFACA, CATGP, CR2005, MLA, WOAH	
	Emaciation	Not specified further	AAWSG, CATGP, CHAR, MLA
		BCS < 2 out of 5	AFACA
	Fatigue/exhaustion	AFACA, CATGP, CHAR, WOAH	
	Dehydration	AAWSG, AFACA, CHAR, MLA	
	Distress	AAWSG	
	Hypothermia	Not specified further	CHAR
		Cold stress or frostbite	AFACA
	Hyperthermia	Not specified further	CHAR
		Heat stress	AFACA, MLA
	Engorged udder	MLA	
	Eye lesion	Blind in both eyes	AAWSG, MLA, WOAH
Blind		CATGP	
Severe squamous cell carcinoma		CHAR, MLA	
Injury	Not specified further	AAWSG, AFACA, CHAR, CR2005, WOAH	
	Severe open wound or a severe laceration	AFACA, CHAR, CR2005	
	Disabled/infirmity	CHAR, WOAH	
	Unhealed wounds after recent surgery	CATGP, WOAH	
	Severe haemorrhage	CATGP	
	Abscess	AFACA	
	Has sustained an injury and is hobbled to aid in treatment	CHAR	
	Ingrown horn	MLA	
	Flystrike	AFACA, MLA	
Prolapse	Prolapsed uterus or a severe rectal or severe vaginal prolapse	AFACA, CHAR, CR2005	

General condition	Specific condition	References	
Hernia	Hernia that (i) impedes movement, including when a hind limb of the animal touches the hernia as the animal is walking, (ii) causes signs of pain or suffering, (iii) touches the ground when the animal is standing in its natural position or (iv) has an open wound, ulceration or obvious infection	CHAR, MLA	
In pain	Cannot be moved/transported without causing additional suffering	AAWSG, CHAR, WOAH	
	Experience severe pain when moving	CATGP	
	Fracture	AFACA	
	Fracture that impedes mobility or causes pain or suffering	CHAR	
Lameness	Unable to bear weight on each leg	AAWSG, AFACA, CATGP, CHAR, MLA, WOAH	
	Lame in one or more limbs to extent that it exhibits signs of pain or suffering and halted movements or a reluctance to walk	CHAR	
	Swollen joints	AFACA	
Non-ambulatory		AFACA, CATGP, CHAR, CR2005, MLA, WOAH	
Reproductive state	Pregnancy	Final 10% of gestation period	CATGP, CHAR, CR2005, MLA, WOAH
		Likely to lamb	AFACA
		Within 2 weeks of lambing	AAWSG
	Recent lambing	Given birth within the previous 48 h	AFACA, CATGP, CHAR, WOAH
		Given birth within previous week	CR2005
Newborn	Unhealed navel	CHAR, CR2005, WOAH	
	< 1 week old	AFACA, CATGP, CR2005	

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

- AAWSG: Australian Animal Welfare Standards and Guidelines (2012).
- AFACA: Alberta Farm Animal Care Association and Alberta Lamb Producers (2019).
- CATGP: Consortium of the Animal Transport Guides Project (2018).
- CHAR: Government of Canada (2022).
- CR2005: Council Regulation (EC) No 1/2005.¹
- MLA: Meat & Livestock Australia and LiveCorp (2011).
- WOAH: World Organisation for Animal Health (2011).

3.3.3.3. Transport of animals with reduced fitness

Examples of additional measures that could be used to reduce the risk of suffering by animals that are vulnerable or may show clinical signs of lowered fitness during transport include: increased contingency planning, reducing journey duration, adjusting ventilation, increasing bedding, avoiding extreme weather conditions, avoiding loading via steep ramps, loading last and unloading first, providing space to lie down, increasing monitoring frequency, providing feed, water and rest more frequently and use of analgesics or other applicable medication.

However, the effectiveness of mitigation measures to avoid the additional suffering likely to be associated with the transport of animals of lowered fitness is questionable, and research is needed in this area. There are also differing opinions on the types of conditions that would make a vulnerable sheep fit for transport, even when additional mitigation measures are used. Further research is needed to establish guidelines for this.

3.4. Loading/unloading

3.4.1. Current practice

The loading and unloading operations are a strategic element of transport and are based on a series of practices of handling and driving the animals on or off the vehicle through a series of fences, aisles, chutes, docks, ramps and/or hydraulic lifts (Pulido et al., 2019). The quality of these operations is highly dependent on factors in the production environment such as: available infrastructure, microclimatic conditions, handlers' skills, training and attitudes, and logistical planning (Pulido et al., 2019). In addition, factors inherent to the animals also influence the loading/unloading procedures, such as the commercial category, previous experiences, herding behaviour of the breed and fitness for the journey (Miranda-de la Lama et al., 2010). Specifically, loading may be preceded by potentially stressful practices such as social separation, social mixing, herding, handling and physical restraint that have an affective and behavioural impact on animals (Burnard et al., 2015). The response of animals to unloading depends on the cumulative effect of travel time, road conditions, density, microclimatic conditions, weather conditions as well as the degree of fatigue of the animals (Messori et al., 2015b).

The infrastructure for loading/unloading tends to be very heterogeneous in terms of design and manufacture, being highly dependent on the degree of innovation of the logistic chain, although in general it can be classified into three categories: (1) fixed ramps or docks, (2) mobile/portable ramps or docks and (3) automated lifts. The first is usually part of the equipment present in sheep breeding and production centres and abattoirs, while the second and third can be part of the infrastructure of the premises or be part of the equipment of specialised trucks.

3.4.2. Highly relevant welfare consequences

The highly relevant WCs selected during the loading and unloading stage are handling stress, heat stress, injuries, predation stress and sensory overstimulation. The selected ABMs for the assessment of these WCs are shown above in Section 3.2.2. Below, hazards, preventive and, corrective/mitigating measures are described.

i) Handling stress

Most of the hazards contributing to the risk of handling stress during the loading and unloading of the animals are almost identical to the ones present during the preparation stage (ie. inexperienced handlers, inadequate handling, use of physical force/instruments and poor handling facilities) (Section 3.3.2). In addition, the hazards specific for handling stress during the loading and unloading and their preventive measures are:

Slippery floors and loading area: Floors and ramps constructed of non-adhesive or dirty materials (slurry, sludge and litter) cause fear and loss of balance in the animals. This results in animals engaging in avoidance behaviour, refusing to walk or falling.

- PRE: To prevent this hazard, the loading area should have non-slippery floors and be properly cleaned before the loading/unloading.

Separation of male and female animals that are used to be together: When sexually mature males and females are used to be together, the separation of them in association with loading onto a means of transport, and the keeping of them separated from each other during journeys, may be associated with increased risk of handling stress. The Consortium of the Animal Transport Guides Project (2018) also recommended handling of the animals in groups.

- PRE: Compatible groups should be loaded/unloaded together to avoid adverse animal WCs. The following guidelines should be applied when assembling groups of animals; animals reared together or with a strong social bond should be maintained as a group even though they are of different sex and size; aggressive animals should be segregated.

Corrective/mitigating measures of handling stress

As suggested by the Consortium of the Animal Transport Guides Project (2018), if an animal stops and refuses to move on, the following procedure should be applied: behave calmly, let the animal calm down, and check the animal is not sick, wounded, or unfit for transport. If the animal is sick, remove

from the race and decide on the course of action. Corrective measures include also removal of the specific person performing inappropriate handling or provision of assistance to him/her on the spot.

ii) Heat stress

Sheep are more able to tolerate severe heat stress than many other animal species. There are more than 1,000 sheep breeds (and 600 goat breeds) throughout the world and the capacity and physiological and behavioural mechanisms to adapt to the thermal challenge differs between the breeds (Joy et al., 2020). The susceptibility to heat stress (Schoenian, 2019) may vary due to several factors, in particular (i) presence of fleece, horn and age of the animal and (ii) skin colour. The European sheep breeds are usually the least heat adaptive because they tend to have shorter bodies and legs, short, thick ears, tight skin and dense fleeces (EFSA AHAW Panel, 2011). The characteristics of the outer surface of a sheep's body are of great importance in the relationship between the animal and its ambient temperature. Carpet-type wool, as compared with denser wool types, seems to confer protection from solar radiation while at the same time allowing effective cutaneous evaporative cooling (Cain et al., 2006).

The main hazards that can lead to heat stress during this stage of transport are listed below, together with preventive measures, and corrective or mitigating measures for the WC. More detailed information on the influence of high temperature on sheep can be found in Section 3.5.3.1 dealing with heat stress during the transit stage.

High effective temperature: The loading/unloading of sheep when the microclimatic conditions, including solar radiation, involve temperatures above the thermal comfort zone (TCZ) increases the risk of heat stress.

- PRE: To reduce the risk of heat stress in hot climates or during summer, loading times should be strict and should be carried out in the early morning. Shading should be provided to protect the animals from solar radiation and ambient temperature. Shading nets have been shown to be effective in reducing exposure to direct solar radiation, producing a microclimate capable of minimising heat stress in sheep (Piccione et al., 2006). Animals should be given access to drinking water before loading. In the case of shorn sheep, routine wetting practices may be adopted prior to loading. Water application can be used in two methods: direct application to the animal and application to the surface of the pen (Lees et al., 2017). Handlers should be aware that handling animals in hot conditions causes animals to fatigue quickly. Therefore, herding should be calm, excessive noise and stimulation should be avoided, the order of loading should be planned and unnecessary procedures prior to loading should be avoided.

Long fleece in high temperature: Animals with a long fleece loaded, transported and unloaded at high temperatures are more vulnerable to experience marked heat stress expressed as increased ruminal and body core temperature and the two phases of panting, than short-fleeced animals (Beatty et al., 2008).

- PRE: To prevent this hazard, previous general recommendations should be applied.

Short fleece in solar radiation: The fleece provides insulating protection to the animal by reducing radiative heat gain in warm environments. This means that, in high ambient temperatures, shorn animals are more vulnerable to solar radiation and may experience heat stress (Lees et al., 2017).

- PRE: To prevent this hazard, shade should be provided during and loading and unloading of the animals.

Young lambs: Thermoregulatory mechanisms are acquired progressively with age, so young lambs are more vulnerable to experience heat stress than more mature individuals.

- PRE: To prevent this hazard, these animals should be handled calmly, applying the same recommendations as described previously.

Delays to loading and unloading: Delays in loading cause the animals to be more exposed to solar radiation and high temperatures, which constitutes a risk to the homeostasis of the animals. Similarly, delays in unloading the animals, especially if the truck waits without mechanical ventilation, constitute a high risk for heat stress in the animals.

- PRE: To prevent this hazard, the journey should be well planned in advance with a specific timetable, loading order and standardised emergency practices and protocols, avoiding any delays.

Corrective/mitigating measures of heat stress

If some animals are suspected to be heat stressed during the loading, the process should stop immediately and the affected animals should be moved to a shaded place and provided with water. Wetting procedures can be adopted, especially to shorn sheep, to reduce the effects of heat. Animals should be inspected before re-starting the loading to ensure they are fit for transport.

iii) Injuries

The main hazards that can lead to injuries during the loading and unloading of the sheep are related to the facilities and the handling of the animals.

Unsuitable facilities: If the loading and unloading areas are poorly designed or poorly maintained, it increases the risk of accidents such as slips and falls and consequent skin injuries, bruises and wounds.

- PRE: Facilities should be adequate and well maintained through periodic inspections and before use. In this regard, appropriate incentives should be provided to encourage good handling facilities such as: solid-sided runs that allow several sheep to move together and not be distracted during loading or unloading, non-slip floors and ramps, or having an external loading/unloading ramp or a loading dock that allows sheep to get on or off the vehicle on a flat surface, and finally covering the loading/unloading area with straw.

Projections: Social isolation and separation, rough handling and unpredictable handlers are the main causes of sheep to accidentally or deliberately project themselves against facilities or handlers to escape aversive stimuli. Frequently, these attempts cause injuries of varying degrees of severity that will affect fitness during loading/unloading and even for fitness for transport.

- PRE: Facilities should in principle be designed without projections and other injurious structures. In addition, facilities should be checked periodically to detect floors, ramps, fences or docksides in poor condition, as well as edges against which the animals can project themselves. Railings on ramps and raceways should be of appropriate height so that animals cannot jump over them, with the gaps sufficiently narrow at the bottom to prevent sheep or their limbs being caught, slipping through or becoming injured (Consortium of the Animal Transport Guides Project, 2018). The repair of damage should be based on eliminating any physical element that could cause injury, for which surfaces, or structural elements detected as dangerous can be cushioned or covered.

Inappropriate handling: Inadequate handling during unloading and loading, as described above in the preparation phase, increases the risk of sheep getting injured. Such actions may be related to the level of education of the handler.

- PRE: To prevent this hazard, handlers should be properly educated and trained following the principles previously mentioned in preparation section.

Corrective/mitigating measures of injuries

If any animal is injured during the loading/unloading, they should be moved apart and examined to determine the severity of the injury and treated accordingly. If palliative measures are needed, a first aid kit containing antiseptic spray for livestock use should always be available. It is also advisable to have emergency veterinary assistance for suturing animals that require it or euthanasia in severe cases.

iv) Predation stress

The hazards that can lead to predation stress during the loading and unloading of the animals are the same ones previously presented on the preparation phase related to the presence of untrained dogs or untrained personnel on working with dogs, especially in enclosed or confined spaces (see Section 3.3.2).

v) Sensory overstimulation

The hazards leading to sensory overstimulation are all the changes and/or major exposure of the diverse stimuli the animals are exposed to during the loading/unloading. Among relevant examples are:

Sudden change in, and/or major exposure to diverse stimuli: Even under ideal conditions, loading/unloading poses a challenge for sheep which can be exacerbated by stimuli such as:

- tactile experiences (types of floors, platforms and/or ramps of different appearance, size, degrees of inclination and materials),
- visual stimuli (changes in brightness, limitations to the visual field and visual contact with objects or animals that produce aversion),
- auditory stimuli (vocalisations, unfamiliar noises),
- olfactory stimuli (pheromones, dust and gases).

All of the above increases the perception of risk in sheep causing fear, nervousness and anxiety as a result of sensory overstimulation (Burnard et al., 2015; Pulido et al., 2019).

- PRE: It is advisable that in the design and/or maintenance of chutes, passageways and loading/unloading docks, care should be taken to avoid large contrasts of light that cause the animals to refuse to pass through. Also, the facilities must have the necessary conditions so that the animals can intuitively and easily reach the dock or loading/unloading surface. In addition, facilities should also be inspected regularly to locate hazards that can cause sensory overstimulation such as sources of stimuli like noise, light, odours, dust and heat.

Corrective/mitigating measures of sensory overstimulation

Corrective measures should focus on providing enough time and appropriate environment allowing the sheep time to recover.

3.5. Transit stage

3.5.1. Current practice

There is a diverse range of vehicles used to transport sheep, from small trailers to small ordinary trucks and specialised triple-decker trailers. Occasionally sheep may be transported in the same truck with other species, such as goats. There are three typical models of operation in the sheep industry. The first consists of the farmer and/or the livestock company having a trailer or truck or fleet to meet their mobility needs. The second is that the operations are carried out by specialised companies; and the third is based on a hybrid strategy, where the livestock company has its own vehicles and leases services when its installed capacity is exceeded, or to cover non-routine journeys that would alter the planned scheme (Miranda-de la Lama et al., 2009, 2010).

Throughout the journey, even under favourable conditions, sheep are exposed to a number of potential stressors that can compromise their health and welfare, such as the microclimatic conditions inside the means of transport, weather conditions, social mixing, handling, feed and water withdrawal, vehicle motion, noise and environmental contaminants, potentially leading to fatigue, stress and injuries (Miranda-de la Lama et al., 2014). Conditions during the journey should ideally be adapted according to breed, age, physiological state and body condition, which will reduce stress and its impact on animal health and welfare.

Adult animals usually have more behavioural and physiological resources to cope with stress during transport than juveniles. Health, body condition and previous experiences may be decisive factors in how individuals cope with transport. Sex may also affect coping ability, as females are known to be more susceptible to stress during transport than males, as well as exhibiting marked inappetence after transport (Aoyama et al., 2003).

3.5.2. Highly relevant welfare consequences

The WCs selected as highly relevant for sheep during the transit stage are heat stress, prolonged thirst, prolonged hunger, motion stress, restriction of movement, resting problems and sensory overstimulation. The ABMs used to assess each WC have been defined above (Section 3.2.2). The hazards leading to the WCs, are identified below as well as preventive measures and mitigating or correcting measures.

i) Heat stress

The main hazards, preventive and corrective measures for heat stress are listed below. More detail on the effective temperature and relative humidity (RH) is provided in the final part of this section (Section 3.5.3.1).

High effective temperature: High effective temperature (i.e. temperature combined with high humidity), inside the truck is the main hazard for animals developing heat stress during the journey.

- PRE: Temperature inside the truck should be kept in the TCZ as described in detail in Section 3.5.3.1.

High environmental temperature: The air entering a transport vehicle will be at the temperature and humidity of the air outside the truck.

- PRE: The only preventive measure for this hazard is to avoid transporting the sheep during the hottest period of the day.

Solar radiation: The incident solar radiation on the roof and walls of the vehicle causes the interior of the vehicle to heat up.

- PRE: The main preventive measure for this hazard is to avoid transporting the sheep during the hottest period of the day. Avoid stopping transport vehicle (unless air-conditioned), only transport the sheep in cool part of day (night if necessary). In the longer term, the roofs and walls of trucks could be insulated and/or constructed of radiation-reflective material.

Low ventilation rate: Ventilation replaces the air in the vehicle with air from outside.

- PRE: To reduce the risk of high effective temperatures inside the truck, appropriate ventilation should be ensured. This will also help to avoid concentration of gases and fumes reducing the risk of sensory overstimulation as well.

Stocking density: Lowering space allowance increases the number of sheep that can be loaded onto a vehicle per surface area and if this occurs, the total amount of metabolic heat and moisture that is produced in the vehicle will increase.

- PRE: To prevent this hazard, animals should be transported in their TCZ, as explained below in Section 3.5.3.1, where the upper threshold of the comfort zone and the upper threshold of the thermoneutral zone (TNZ) are identified as the basis of a quantitative recommendation on microclimatic conditions during transport of sheep.

Corrective/mitigating measures of heat stress

When signs of heat stress are identified, it is recommended to complete the journey as soon as possible and to avoid stopping the vehicle unless cooling of the vehicle (e.g. by air-conditioning) is possible. When heat stress is observed, provide convective air cooling with mechanical fans and increase ventilation by increasing number and surface area of side shutters. In case of emergencies, sheep can be wetted with a hose. Water spillage should be avoided on the floor from drinking devices to limit the humidity inside the truck. Upon arrival at the destination, sheep should be unloaded into a shaded pen with drinking water, plenty of space and several hours of rest time.

ii) Prolonged hunger

The WC prolonged hunger is regarded as highly relevant during the transit stage. The prevalence is expected to be high, as no studies have documented the successful feeding of sheep during journeys. Depending on factors such as time off-feed before the journey starts, sheep may not be hungry during the initial phase of the journey, but hunger will develop over time. 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 more and more 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: Hours without feed is the most important hazard for hunger to develop.

- PRE: In order to reduce this hazard, the time off-feed should be short. It is, though, critical that sheep have an opportunity to drink after eating, otherwise they are at risk of developing dehydration. Therefore, even though the time off-feed will be longer, it is a good practice, after sheep are fed, to remove feed, and provide water for a couple of hours before loading.
- PRE: Journey times recommended in Section 3.5.3.3 should be respected.

Space allowance: For longer journeys, if sheep need to eat onboard the vehicle in order to avoid hunger, extra space is most likely required to provide access to troughs, allowing all sheep in a compartment to eat simultaneously.

- PRE: See Section 3.5.3.2 for examination on the space needed for sheep to be fed and watered on the vehicle.

Corrective/mitigating measures of prolonged hunger

The most effective measure to correct hunger is to offer feed by stopping the truck or unloading the animals to allow sheep to feed. The animals must be familiar with the type of feed provided; this is especially important in animals coming from extensive systems (little experience with pellet feeding) and lambs that are usually accustomed to what their mothers eat.

iii) Prolonged thirst

The WC prolonged thirst is regarded as a highly relevant during the transit stage. The prevalence may be high if water is not provided to the animals or if they are not able to obtain enough water (due to, e.g. lack of familiarity with drinking devices, neophobia or fear of other animals). Depending on factors such as time off water before journey start and/or microclimatic conditions, sheep 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 and more problematic for the animals. Prolonged thirst may lead to dehydration, discomfort and suffering. Additional information on this WC is included in Section 3.5.3.3. The main hazards, preventive and corrective/mitigating measures are:

Time off water: The absence of water during the journey is a risk for sheep, leading to electrolyte imbalance in plasma measurements. These effects can be exacerbated if the journeys are done in hot environments, if they involve high stocking densities, or if they are associated with exacerbated motion stress.

- PRE: To prevent this hazard, animals should be given access to water until the moment of loading and on arrival at the destination. On journeys with breaks, the first resource to offer after unloading is access to water.

Inadequate/insufficient drinking devices and/or inexperience in drinking from them: Poorly designed drinking devices, too few drinking devices, or devices designed for other species or age categories constitute a risk of compromised water intake during the transit stage. In addition, if the animals are not familiar with the operation of the device, they will likely not drink adequately (Messori et al., 2015b). Water supply systems may become inadequate during very hot weather when demand is high (Jubb and Perkins, 2015). Low intake of water during journeys will be evident on arrival with animals highly motivated to drink, and they may show signs of dehydration despite having water during the transit stage.

- PRE: To prevent this hazard, sheep should not be transported in vehicles with inadequate/insufficient drinking devices. In addition, in trucks equipped with drinking nipples, periodic checks should be carried out to ensure that the pivots are functional and have the necessary flow for the animals to drink.

Reduced intake of water: Technical failures may lead to inaccessible, inefficient completely failing water systems. Only a few studies have examined the intake of water during transport. Dalmau et al. (2014) used ruminal temperature sensors during 24-h journeys in trucks provided with nipple drinkers. Based on the lack of abrupt changes in ruminal temperature (known to be associated with intake of water), the authors concluded that the animals seemingly did not drink during the journey. Signs of thirst such as licking rain from bars were observed, though. Thus, it is possible that sheep, even when water is available on trucks during the transit stage, and they are familiar with the equipment, for some reason are reluctant to drink while in transit.

- PRE: If it is correct that sheep drink less water than required during transit, this hazard cannot be fully prevented during the transit stage, not even by giving access to drinkers on trucks. The only preventive measure, then, is to provide free access to water before loading, and to

limit journey duration, so that the WC prolonged thirst will not develop. If journey breaks are involved, sheep should be watered there.

High effective temperature in truck: High temperatures inside the truck (as defined in Section 3.5.3.1) will aggravate the WC of prolonged thirst. Therefore, transport should be carried out with microclimatic conditions inside the vehicle within the TCZ.

- PRE: See Section 3.5.3.1 for examination of heat stress and how it can be prevented.

Space allowance: If sheep need to drink onboard the vehicle in order to avoid the WC prolonged thirst, extra space is most likely required to provide access to drinkers.

- PRE: Previously, a space allowance corresponding with a k-value of 0.037 (see Section 3.5.3.2) was recommended for sheep transported for long periods and requiring to be fed and watered on the vehicle (SCAHAW, 2002). However, no scientific validation of this requirement has been found.

Unweaned lambs: Dehydration particularly affects lambs, especially those still on milk at the time of transport. The severity of dehydration depends on the degree to which the lamb has developed ruminal fermentation, the amount and composition of food residues in the rumen, and the experience the lamb has had in drinking water from a drinking device (Dwyer, 2008).

- PRE: It is possible that artificially suckled lambs or lambs that have seen their mothers handle these devices may be better able to drink.

Corrective/mitigating measures of prolonged thirst

In the case that prolonged thirst signs are observed in the animals during the transit stage, the truck should be stopped, and all sheep given the opportunity to drink water, providing enough time for this.

iv) Resting problems

Resting problems is regarded as a highly relevant WC during the transit stage. The prevalence is at least moderate, as resting problems may affect a large proportion of animals in a moving vehicle, depending on factors such as the driving quality and the space allowance. Even with ample space, and the possibility to lie down, it is not documented that all sheep in a compartment will or can rest during journeys. Therefore, the duration of resting problems depends on the journey duration, and severity is expected to increase with increasing duration, as the lack of resting becomes more and more problematic for the animals. Resting problems may eventually lead to fatigue. Below, the main hazards are identified and preventive, corrective and mitigating measures are suggested.

Truck motion: The moving truck is not only stressful for sheep in itself, but can also lead to resting problems as transported sheep lie down less and in less relaxed postures than sheep kept in stationary vehicles (Cockram et al., 1996, 2004). See the section on Motion stress below for a review of the consequences of truck motion.

- PRE: Truck motion is inherent to road transport. However, good suspension vehicles and driving through main roads could help reduce the consequences of this hazard.

Inadequate floor: Poorly maintained or poorly designed corrugated patterns do not allow animals to comfortably maintain balance during the transit stage, predisposing them to slipping, falling and constant changes of position.

- PRE: To prevent this hazard, truck floors should be made out of non-slippery materials and be regularly checked to ensure the proper maintenance.

Insufficient vertical space: Low decks are problematic because animals will be forced to adopt abnormal positions during the transit stage, and this can be exacerbated if the floor also does not provide the necessary conditions for a comfortable resting position. Additionally, vertical structures such as broken or uneven vehicle and compartment walls will prevent animals from using them to support their weight to mitigate the effects of vibration and movement.

- PRE: The roof of the deck should allow animals to stand up in normal posture and to move around in the truck to get access to flooring to lie down. Additional information on the minimum vertical space is provided in Section 3.5.3.2, where a quantitative assessment was done for the vertical space required by sheep during the transit stage.

Inadequate bedding: Without adequate bedding (Teixeira et al., 2014) (type and/or quality and/or quantity), animals will be less motivated to rest lying down during journeys and will be exposed to slips, falls and weakness. Even on journeys where proper bedding is provided from the beginning, bedding may become a limiting factor for journey duration if it gets too soiled or trampled and is no longer useful as lying substrate.

- PRE: Adequate bedding material should be provided and it should be dry with a high ability to absorb fluids. Sufficient amounts of bedding allow for more comfort and facilitate the resting of animals. Lambs ≤ 20 kg need special attention: they need to be given adequate bedding or equivalent material to secure comfort appropriate to the number of animals transported, the duration of the journey and forecasted weather conditions (Consortium of the Animal Transport Guides Project, 2018).

Insufficient horizontal space (space allowance): At some stage of the journey, sheep may need to lie down either when the vehicle is in motion or stationary (Menchetti et al., 2021).

- PRE: See Section 3.5.3.2 for a detailed examination of space needed by sheep to rest and the preventive space required.

Corrective/mitigating measures of resting problems

In situations where severe resting problems have been identified, sheep can be offloaded and allowed to rest.

v) Restriction of movement

The main hazard leading to the restriction of movement is inadequate space for the animals, either in the vertical or horizontal dimensions.

Insufficient horizontal space (space allowance): During transport, sheep need space to adopt postural changes to brace themselves while standing, and to adjust their footing to maintain stability in response to changes in acceleration (including braking, stopping and changes of direction). Additional information on this topic is covered in detail in Section 3.5.3.2 where detailed research was compiled to provide quantitative recommendations of space allowance during transport.

- PRE: To prevent insufficient space allowances, planning of the journey should be done considering the minimum space provided per weight of animals transported as referred in Section 3.5.3.2.

Insufficient vertical space: In addition to the horizontal or floor space required in a truck compartment, the vertical space should be considered.

- PRE: The deck height should always allow the animals to stand up in normal posture, to perform natural movements, and to be able to move around in the truck and access resources. See Section 3.5.3.2 on recommendations on vertical space.

Corrective/mitigating measures of restriction of movement

In extreme cases, when severe restriction of movement is detected, the journey should be stopped.

vi) Sensory overstimulation and motion stress

The two WCs, motion stress and sensory overstimulation share mediating biological mechanisms, ABMs and to some extent also hazards. They are, thus, treated in combination during the transit stage of transport.

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 has a direction (generally in three planes) (Figure 2), a magnitude (how far) and a velocity (how quickly – what rate). There is significant research demonstrating that certain frequencies of vibration often encountered on commercial transport vehicles are aversive to animals (Parrott et al., 1994).

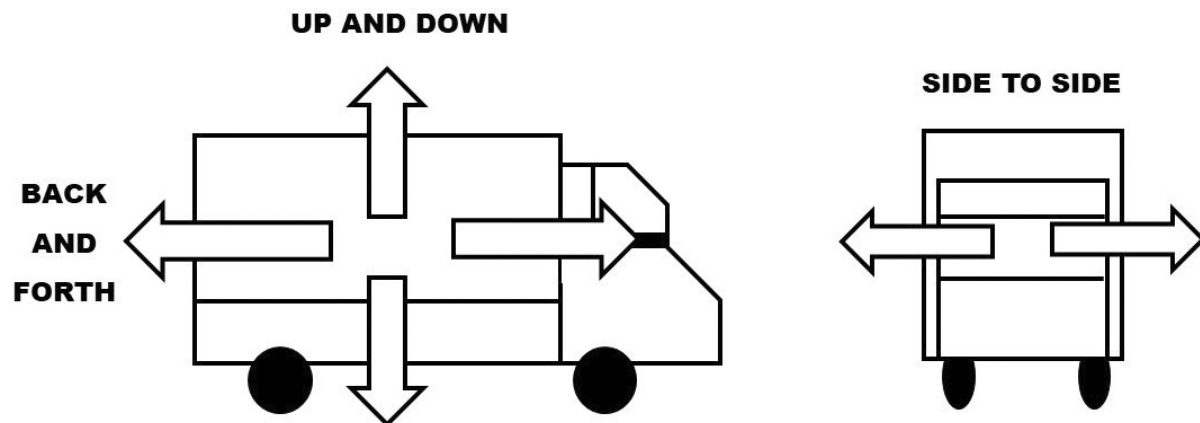


Figure 2: Schematic drawing showing the three planes of vibratory movements animals are exposed to during transport by road. Adapted from the guidelines on transport of livestock from Humane Slaughter Association, 2022

Sensory overstimulation and motion stress are each regarded as a highly relevant WC in the transit stage. The prevalence is high, as motion stress is likely to affect all animals in a moving vehicle. The duration depends on journey duration and onset of truck motion. Severity depends on the driving conditions and vehicle design, and will most likely increase over time as animals become more fatigued. An extreme case of motion stress, with a low prevalence but a very high severity, is truck accidents. These are, thus, not covered specifically in this Scientific Opinion but may have severe consequences in terms of animal welfare. See Section 3.5.3.3 for additional information on sensory overstimulation and motion stress and how the WCs evolve during journeys.

The main hazards leading to these WCs, their preventive and corrective measures are listed below:

Rolling and pitching of truck: While the vehicle is moving, all sheep 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 truck to lose stability and the animals can lose their balance. It is likely that sheep find movement stressful as sheep placed in an oscillating pen to simulate transport showed a plasma cortisol and adrenaline response (Parrott et al., 1994).

- PRE: Among the preventive measures for this hazard are planning the journeys on motorways, with vehicles equipped with good suspension and driven by experienced and skilled drivers. In addition, truck vibration can be reduced through a suspension system which, in the case of freight trucks, can be either leaf spring or air suspension. Both suspension systems improve vehicle contact with the road surface and indirectly reduce vehicle vibration (Dalla Costa et al., 2017). Low inflation of the tires and air suspension systems have been recommended by some authors vs leaf spring systems (Aradom, 2013).

Poor driving: The driver's ability to control the truck affects the quality of driving. Acceleration, braking, cornering and driving techniques affect the ability of animals to maintain a stable posture, increasing excitability, reactivity and injury (Cockram et al., 2004). Long working hours, poor route design, changes in sleep cycles cause fatigue to the drivers leading to road accidents during livestock transport (Miranda-de la Lama et al., 2011). Other factors include the age of the driver, due to the combination of experience and good health (González et al., 2012).

- PRE: The route should be planned avoiding city traffic, industrialised areas and roads with many roundabouts, corners, etc. Drivers should utilise smooth, defensive driving techniques, without sudden turns or stops, to minimise uncontrolled movements of the animals. In order to be able to do this, drivers should be properly educated.

Slippery floors: Wet truck floors can be a risk element for animals due to leaking water from water troughs or nipples, excess slurry, or very wet animals with long wool, no or little bedding material and in extreme cases wear and tear of non-slip floors. See Section 3.5.3.2 on the relationship between space allowance and the risk of slipping.

- PRE: The presence of bedding material can also make the floor non-slip and this factor is essential to prevent slipping and falling (Consortium of the Animal Transport Guides Project, 2018) as well as to reduce resting problems. On longer journeys, maintaining bedding cleanliness and dryness can be challenging, the lack of which likely can have negative effects on animal welfare. However, no studies have been found on the relationship between bedding cleanliness and journey duration.

High stocking density: As described in detail in the quantitative section of space allowance, sheep need space in order to be able to balance and adjust their posture to the acceleration and other events of the transit stage. Therefore, higher stocking densities impede animals' ability to properly balance, increasing the risk of motion stress.

- PRE: Animals should be transported with sufficient space allowance as defined in Section 3.5.3.2.

Excessive stimuli (i.e. noise, odours, sight of rapidly passing surroundings): Exposure to sensory stressors of a simultaneous and changing nature at varying intensities and frequency may lead to stress and negative affective states such as fear. The plasma cortisol response of sheep to transport can be reduced by restriction of visual stimuli of the moving environment (da Cunha Leme et al., 2012).

- PRE: As much as possible, the maintenance of the vehicle (including cleanliness) should be controlled to eliminate unnecessary light ingress (without affecting ventilation), sources of noise and to avoid dust accumulation.

Corrective/mitigating measures of sensory overstimulation and the motion stress

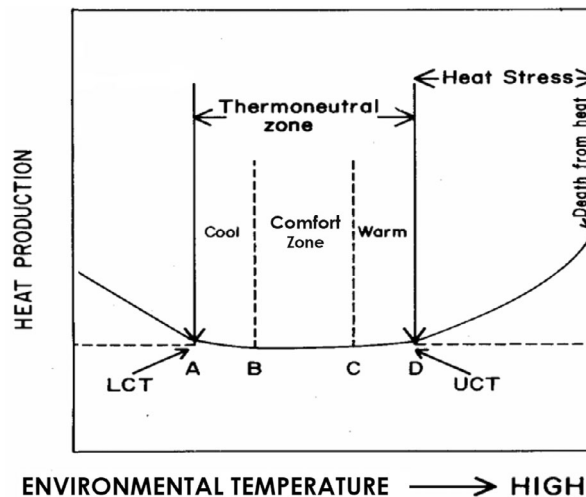
If the transit stage is particularly problematic in terms of sensory overstimulation, consideration should be given to stopping and unloading the animals to rest, or to trying to bring the journey to an end as soon as possible.

3.5.3. Quantitative examination of thresholds to protect the welfare of sheep during the transit stage: microclimatic conditions, space allowance and journey time

3.5.3.1. Threshold of microclimatic conditions

A) 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 sheep transport is based on the thermoregulatory concepts and model as described by EFSA (2004) (Figure 3), and originally formulated by Mount (1974). The figure covers a range of environmental temperatures from cold to very hot. Since the selection of highly relevant WCs was not done per animal category (cold stress likely more relevant for lambs close to weaning than for older categories of sheep), but per animal species, cold stress was not chosen as a highly relevant WC for sheep. Thus, this assessment of thresholds for microclimatic conditions focusses on temperatures higher than B as indicated on Figure 3.



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

The following three concepts from the figure need to be introduced:

Thermoneutral zone: 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) and the upper critical temperature (UCT) (marked as A and D on Figure 3). 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 humidity, radiation, heat loss to the floor, air velocity around the animal, but also motor activity (e.g. maintaining balance during transport) (Bracke et al., 2020).

Thermal comfort zone: According to Silanikove (2000), subdivision of 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 3) 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 panting in sheep, and may start to display thermoregulatory behaviour. The TCZ is sometimes called the safe zone as it is referred in EFSA AHAW Panel, 2022b.

Upper critical Temperature: 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 increases and metabolic rate increases (Silanikove, 2000). As described by Norris and Kunz (2012), heat is transferred by four mechanisms: radiation (from a hot object to a cooler object via electromagnetic waves), conduction (between two solid objects in contact with one another), convection (through the movement of a gas or liquid) and evaporation (conversion of water from the liquid to gas phase). 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 3 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, sheep 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 sheep under basic thermoregulatory studies, transport often includes deprivation of feed and water, exposure to vibration and motion forces, 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 3, 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 and 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 WC heat stress, addressing a situation where an animal experiences stress and/or negative affective states such as discomfort and/or distress.

B) Heat stress and animal welfare during transport of sheep

As reviewed by Rashamol et al. (2019), not only the ambient temperature, but also other environmental conditions influence the heat load placed on animals. Examples of these are: the RH, the solar 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 truck, the truck type, the type of ventilation shutters and many more. These will all influence the microclimatic conditions experienced by sheep and should, in theory, all be taken into account when microclimatic conditions of sheep 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.

In the context of animal transport, ventilation functions to replace the metabolic heat and moisture produced by the animals inside the truck with air of a certain temperature and humidity from outside the truck. 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 truck, but only if ventilation is very efficient.

However, in a passively ventilated vehicle, air flow over the surface of the vehicle results in a pressure gradient in which there is lower pressure towards the front sides of the vehicle than at the rear sides and tail. There may be higher pressure on the front (forward face or headboard). The net effect is that in a passively ventilated configuration air movement will tend to involve entry of air towards the rear, forward movement of air towards the front of the vehicle and exit of air at the front sides of the structure (Figure 4).

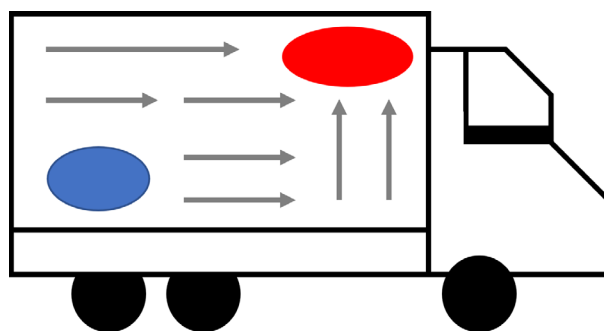


Figure 4: Predominant patterns of air flow (white arrows) in a moving passively ventilated vehicle. Red areas indicate where air heated by the animals accumulates and blue areas show colder spots

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, e.g. cross winds. The buoyancy and free convection regimes will tend to create a thermal gradient within the load with the upper locations being warmer than those below. In more open configurations, cross winds may provide some beneficial effects. 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.

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 the type of transport vehicle used in the EU, the pressure field around the vehicle drives the passive ventilation, i.e. air will tend to enter towards the rear of the vehicle and exit towards the front side of the load. The presence of the walls on the vehicle limits air entry and exit along the sides of the vehicle and the route for air flow will be determined by the location of any openings in the structure. This results in uneven distribution of thermal conditions within the load with hot-spots (red) and cold-spots (blue) indicated in Figure 4.

Using the equation provided by Mitchell and Kettlewell (2008), the ventilation rate required to limit a rise in internal air temperature to 5°C = total metabolic heat production/[specific heat capacity of air (1,226 J/m³ per °C) × rise in air temperature (5°C)]. With 84 sheep the required ventilation rate would be 3,528/(1,226 × 5) = 0.58 m³/s. With 67 sheep, the required ventilation rate would be 2,814/(1,226 × 5) = 0.46 m³/s.

Thus, there is a ventilation rate (by mechanical or passive ventilation), for a certain range of environmental temperatures that, in theory, reduces the effective temperature within the 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 generated by the animals, solar radiation and heat loss from the vehicle. This ventilation rate is unknown for sheep transport, as far as we know, as relevant studies have not been found.

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 sheep.

When trucks are stationary, e.g. for loading and unloading, the risk for heat stress increases. Naturally ventilated vehicles must, therefore, either have sufficiently large, or otherwise properly designed ventilation spaces to reduce heat build-up when stationary, or mechanical ventilation should also be provided, and be turned on (see also EFSA, 2004).

However, very little information exists on how effectively different transport ventilation designs are able to keep sheep within the required temperature ranges when using mechanical vs passive ventilation. Further research is needed in this area. Vehicle designs and ventilation capacities must enable the animals to be transported safely within the required temperature ranges, independent of the local climatic conditions through which the vehicle will travel.

The water vapour content of the air is important because it impacts the rate of evaporative heat loss through the skin and respiratory tract (Bohmanova et al., 2007). 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 increased 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.

During transport, the humidity inside a vehicle is produced by both external humidity and the moisture generated by the animals loaded. Therefore, the relationship between air temperature and humidity is crucial from the point of view of animal welfare during transport (Miranda-de la Lama et al., 2014).

Generally, air water vapour content is assessed by 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. When the ambient temperature is above the TCZ, a high level of humidity in the air will reduce evaporative heat loss and therefore make the animal feel warmer. 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 that take into account humidity effects.

C) Thermal heat indices in sheep during transport

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, the 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 this use of THI 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. Recent studies have, however, called for further development of indices used to assess heat stress in livestock due to limitations in for example THI, such as (1) lack of integration of all environmental parameters, and (2) either not reflecting current high producing animals or not specifying production level. Factors like these may limit the usefulness of the indices to accurately predict or assess the thermal status of sheep and other livestock (Wang et al., 2018; Herbut et al., 2018).

To avoid the limitations presented by the different available THIs or other comparable indexes, psychrometric principles (related to the humidity and temperature of air) have been used to develop other thermal comfort indexes, such as the specific enthalpy of air (de Castro Júnior and Silva, 2021). Enthalpy is the heat energy of the air surrounding the animal, and dictates the degree of heat loss to the microclimate. Physically, the specific enthalpy of air (h) is defined as the total amount of energy existent in a unit of dry air mass (kJ/kg of dry air) and can be calculated using simple tools such as thermometer and hygrometer, and mathematical models, as recently reviewed by de Castro Júnior and Silva (2021). In the future, time derivatives of temperature or enthalpy could be used as non-invasive indirect welfare indicators during animal transport and they appear to be more sensitive than values of temperature or RH. However, at the present stage, it is considered that too little data are available to be able to give recommendations based on enthalpy. Thus, this Scientific Opinion focuses at the temperature immediately surrounding the sheep and measures of humidity. The combined ambient temperature and RH can be reported by different indices such as wet-bulb temperature (Twb), apparent equivalent temperature (AET), previously used by Mitchell (2006) to assess heat stress during transport of broilers, or Enthalpy Comfort Index (ECI) employed in tropical regions as a qualitative indicator of thermal environment of livestock (Rodrigues et al., 2011). Irrespective of the index chosen to monitor temperature and humidity, vehicles should be equipped with sensors recording temperature and humidity as close as possible to the position of the animals therein, and at several locations to include the top 'hot spot' at the front, the 'cold spot' at the rear and bottom of the load and representative points in between (Figure 4). The livestock driver should then monitor the microclimate of the load and adjust the ventilation if the conditions exceed the TCZ levels. Technical issues (e.g. accuracy, maintenance, placement, reliability and calibration) relating to this improved approach will need to be addressed. However, none of these indices have been validated for sheep during transport.

D) Identification of environmental conditions to protect sheep from heat stress during transport

The characteristics of the outer surface of a sheep's body are of great importance in the relationship between the animal and its ambient temperature. Carpet-type wool, as compared with denser wool types, seems to confer protection from solar radiation while at the same time allowing effective cutaneous evaporative cooling (Cain et al., 2006). Coat depth and wind speed are important moderators of these responses (Turnpenny et al., 2000).

Differences in heat stress tolerance and behaviour have been observed in unshorn and shorn sheep. According to the results from Dikmen et al. (2011), during the summer, there was a tendency of lower rectal temperature for shorn lambs because a greater amount of heat could be transferred via conduction, convection, radiation and also evaporative heat loss could also be used more effectively. On the other hand, unshorn lambs experience lack of heat transfer from skin to environment because of less thermal conductivity caused by fleece thickness. A thick layer of fleece gives an advantage to unshorn lambs to tolerate better hot and dry environments with high solar radiation compared to the shorn ones.

Faurie et al. (2004) observed that lactating ewes were more susceptible to elevated core body temperature than non-lactating ewes, which is to be expected from their higher metabolism rate.

Identification of the upper threshold of the TCZ (point C): The first response of sheep to an increase in effective temperature is vasodilation, with higher temperatures producing heat loss through sweating and panting. The relationship between ambient temperature and leg/ear skin temperature, which is indicative of vasodilation, was measured by Blaxter et al. (1959a). The threshold for increased evaporation through vasodilation was determined to be 25°C, with this process completed by 27°C for shorn sheep (Blaxter et al., 1959b). Turnpenny et al. (2000) also independently modelled the relationship between effective temperature and leg/ear skin temperature with very similar results. The onset of vasodilation, as indicated by a sharp increase in skin temperature at an ambient temperature of 25°C, can be considered the most effective measure of the upper threshold of the TCZ (point C).

Identification of the Upper Critical Temperature (point D): Once the UCT, the upper limit of the TNZ, is reached, the rate of evaporative heat loss increases exponentially, meaning that signs of heat stress, such as panting and, to a lesser degree in sheep, sweating increase even further in an effort to stop the rise in core body temperature above normal. During transport of sheep, the WC heat stress, may start when the animals are no longer in their TCZ, and the risk of heat stress, and the severity of heat stress, is likely high when the thermal conditions reach the UCT.

Based on a recent review of literature, The EFSA Scientific Opinion on Welfare of small ruminants at slaughter (EFSA AHAW Panel, 2021) suggested that the TZN has an upper limit of 28°C for fully fleeced sheep and 32°C for shorn sheep.

E) Summary of microclimatic conditions

Sheep are more able to tolerate severe heat stress and periods of water and feed scarcity than other livestock species although differences exist between breeds.

During transport, sheep 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. In addition, transport often includes 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, sheep should be transported in their TCZ. This means that, during transport of sheep, the WC heat stress may start when they are no longer in their TCZ, and the risk and severity of heat stress is likely high when the thermal conditions reach the UCT.

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

Based on the available information, an estimate of 25°C can be proposed for the upper threshold of the TCZ, and estimates of 28°C and 32°C can be proposed for the UCT for shorn and fleeced sheep, respectively. For lambs, less information is available. For variations of dry temperature and relative humidity, the higher the levels of relative humidity, the lower the upper thresholds of TCZ and UCT will be, when measured as a dry temperature only.

Although sensors recording dry temperature have commonly been used in the transport of livestock so far, it would be a significant refinement to use improved sensors that take into account humidity effects.

3.5.3.2. Threshold of space requirements during journeys

A) Introduction and methodology

The stocking or loading density refers to the live weight of sheep within a specified area of floor space (or occasionally the number of sheep of a specified live weight range per unit area).

The space allowance can be quantified as the floor area per sheep. In this Scientific Opinion, space allowances are given as m² per animal, as well as the estimated k-value for the allometric equation for space allowance (Petherick and Phillips, 2009).

The spatial dimensions of compartments holding sheep during transport are important for their welfare – in the horizontal as well as the vertical plane – and lack of space may lead to several WCs such as restriction of movement, resting problems and heat stress.

Due to the limited research available, the multiple factors that can influence how sheep respond to space during transport, the variability in types of sheep and in journey conditions, it is considered preferable to provide minimum rather than target or recommended space allowances for different types of sheep. The evidence for WCs when overcrowding occurs is stronger than that available for determining optimal conditions. The minimum space requirement will vary with the breed of sheep, 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 sheep during transport, the following approach will be used:

During transport, sheep require a minimum space allowance that will accommodate (a) their physical size and allow them to, (b) adjust their posture in response to acceleration and other events, (c) rest in a normal standing or lying posture, (d) thermoregulate, and (e) eat and drink, if feed and water are provided in the means of transport. Recommendations for a minimum space allowance will be set by the first limiting factor that reduces the ability of the sheep to undertake one of the above biologic functions, i.e. whichever of the above requirements needs the most space.

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

In this work, the WCs, hazards and AMBs listed above are used to draw conclusions. Studies involving space allowances comparable to, or larger than, the Council Regulation (EC) No 1/2005¹ were included. Throughout, it is specified whether data were collected as part of surveys or intervention studies.

B) Horizontal space

i) Space to accommodate physical size

The space occupied by sheep in a standing posture depends on the size of the sheep, and allometric equations are available that relate live weight to floor space. Allometric equations ($A = kW^{2/3}$, where k is a constant and W represents live weight (in kilograms)) are used to estimate the space that a stationary animal occupies as a consequence of its mass (Petherick and Phillips, 2009). The power factor is derived from theoretical relationships between length, volume, weight and surface area (Warriss, 1998). Using an exponent of 2/3 makes the assumption that livestock species have a similar shape. Therefore, allometric equations provide estimates of space requirements rather than definitive calculations of areas. A k -value of at least 0.02 has been proposed to provide sufficient space for a standing posture (Petherick and Phillips, 2009). Variations in the k -values used in allometric equations produce a range of recommended space allowances for each live weight range (Warriss, 1998; Visser, 2014). Planimetric measurements have been made to estimate the surface area of pigs, rabbits and birds occupied by the animals when in different positions (i.e. when standing and lying). However, little such research was identified for sheep.

Specific factors can affect how much space some animals require. For example, sheep with horns may require more space as do heavily pregnant ewes vs non-pregnant ewes. Behavioural characteristics that affect social interactions between animals can also affect how they utilise the space available and respond to changes in space allowance. For example, there can be social interactions between horned sheep, but no research on relationships between social interactions and space allowance during transport was identified.

Jones et al. (2002) (based on planimetric examination) estimated that groups of lambs (weighing an average of 46 kg) physically occupied between 0.22 and 0.24 m²/animal when standing, and that groups of unshorn ewes (weighing an average of 65 kg) physically occupied between 0.26 and 0.28 m²/animal when standing. These space requirements represent the absolute minimum space required to allow sheep of these sizes to stand side by side. By estimating the percentage of free/unoccupied space in trucks transporting sheep for 6 h, Jones et al. (2010) found that – for different weight classes and shorn/fleeced animals – provision of space according to k -values of 0.018–0.026 led to less than 10% unoccupied space, thereby confirming the findings reported by Jones et al. (2002) on the space requirements of sheep to fit into the means of transport.

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

During transport, sheep need increased space, compared to stationary conditions, to adopt postural changes to brace themselves while standing, and to adjust their footing in order to maintain stability in response to acceleration (including braking, stopping and changes in direction). When sheep are transported with little available free space, their opportunity for movement is restricted, and they

provide some lateral support for each other. However, a substantial body of other research suggests that sheep are at reduced risk of injuries and stress when they are provided with additional space during a journey. For example, after a 24-h journey, the plasma CK activity in fleeced lambs (37 kg) was reported to be greater in those transported at a space allowance of 0.22 m²/animal than in those transported at 0.25–0.34 m²/animal (Knowles et al., 1998). Similarly, Teke et al. (2014) compared lambs (29 kg) transported for 2.25 h at 0.20 m²/animal and then lairaged for 1 h vs animals transported at 0.27 m²/animal, and found increased plasma cortisol concentration and plasma CK activity in the former group. These results suggest that for lambs of an average weight of 29 kg, a space allowance of >0.20 m²/animal can reduce the risk of stress and muscle injuries. There are, however, also studies using physiological indicators such as the serum concentration of cortisol or lactate dehydrogenase activity, between sheep kept at different space allowances, and not showing any effects (Akin et al., 2018) and also studies based on plasma CK activity that did not report any effects (Cozar et al., 2016).

In a series of studies, Jones et al. (2010) observed the stability of different types of sheep during a 6-h journey and identified several significant effects of space allowance. The variation in space allowance was achieved by changing the group size of sheep in a given compartment size, and therefore space allowance was potentially confounded with group size. Rates (events/h) of falling, trampling, slipping and the time required by individual sheep to stand up after falling were measured in each group of animals. The rate of loss of balance was measured as a group event. For comparison purposes, the space allowances stated by the authors were expressed as k-value estimated from the allometric equation mentioned above. For the calculations of the k-value, the average weight of the animals in each compartment was used, although there was some variability of weights (and consequently small differences in k-values) within each compartment of sheep. No data were available for certain categories of animals for some events, as it was not reported in the original paper due to a lack of statistical significance between space allowances for that specific animal-based measure.

Falling: The falling rates (sudden drop to the floor from standing) for the shorn categories (lambs and ewes) are presented in Table 16. Overall, the results suggest a comparable development with respect to the effect of space allowance on falling rates, across shorn lambs and ewes, with a decrease of falls with higher space allowances.

For shorn ewes, the rate of falling dropped 10 times from 3.5 falls per hour and group with a k-value of 0.018 to 0.3 falls per hour at a k-value of 0.026. Based on this data, a k-value of ≥ 0.026 is required to minimise the risk of sheep falling.

Table 16: Fall rates (events per hour per compartment) recorded in shorn sheep (ewes and lambs) at different space allowances expressed as k-value during a six-hour journey (Jones et al., 2010). Space allowance per animal (area, m²), $A = kW^{2/3}$, where k is a constant and W is the weight of the sheep in kilograms. Empty cells indicate that data are not available

k-Value	Shorn ewes	Shorn lambs
0.018	3.5	
0.021	1.1	2.3
0.026	0.3	0.3
0.029		0.2
0.037	0	0
0.09	0	0

Latency to stand up after a falling event: The data on the time taken to stand subsequent to having fallen are presented in Table 17. Again, the greater the space allowance, the quicker the sheep were able to regain their feet. Sheep took an average of between 3 and 7 min to stand, having fallen, although exceptions occurred as one animal took more than an hour to stand. At a k-value of 0.026 or greater, ewes and shorn lambs were able to regain their feet immediately. However, in this study, fleeced lambs required more space, as at a k-value of 0.034 they still took some time to stand up after falling. Thus, based on the data presented, a k-value of ≥ 0.034 is required to reduce the risk of difficulty regaining a standing posture after falling.

Table 17: Average time (seconds) of sheep (ewes and lambs of unspecified age) to stand up after being forced to the floor at different space allowances expressed as k-values during a 6-h journey (Jones et al., 2010). Empty cells indicate that data are not available

k-value	Shorn ewes	Fleeced ewes	Shorn lambs	Fleeced lambs
0.018	174			
0.021	60		435	
0.024		368		
0.026	0	0	0	34.3
0.029			0	
0.033				172
0.034		0		
0.037	0		0	
0.038				0
0.045				0
0.048				
0.086		0		0
0.09	0	0	0	

Trampling: Trampling is when sheep are stamped, pawed, walked or stood on by other sheep while lying down or after they have fallen. Both shorn and fleeced ewes responded similarly in their relationship between space allowance and the rates of trampling during transport (see Table 18). The greater the space allowance, the lower the rate of trampling for all sheep.

There was a marked decrease from 3.6 trampling rate at a k-value of 0.018–0.1 at a k-value of 0.034–0.037 (data not available for all categories at all k-values). In shorn lambs, the rates of trampling were considerably higher than the other groups (10 times more approximately), potentially due to the much higher proportion of animals lying down at the same time in this group in comparison with the others (see Section 3.5.3.2Biii). Nevertheless, the data for the trampling rate followed the same trend as the other groups, with a significant reduction of trampling rate until space allowance of 0.037, and a marginal reduction of this rate in higher space allowances. Thus, based on the data presented, a k-value of ≥ 0.037 is required to significantly reduce the risk of trampling.

Table 18: Trampling rates (events per hour per compartment) recorded in shorn sheep (ewes and lambs) and fleeced ewes at different space allowances expressed as k-value during a 6-h journey (Jones et al., 2010). Empty cells indicate that data are not available

k-Value	Shorn ewes	Fleeced ewes	Shorn lambs
0.018	3.6		
0.021	2		37
0.024		1.5	
0.026	0.6	0.08	12
0.029			12
0.034		0.1	
0.037	0.1		6
0.048		0	
0.086			
0.09	0	0.04	0.7

It is not directly comparable to transport conditions, but effects of space allowance on trampling and aggressive behaviour have also been found when sheep were not transported. Menchetti et al. (2021) reported that lambs of 25 kg, that were not transported, had a greater frequency of trampling and aggressive behaviour when kept at a k-value of 0.026 than at a k-value of 0.033.

Loss of balance: Loss of balance incidents were defined as group events where all or most of the sheep in the compartment lose stability and need to move their feet to remain upright. The effect of increased space allowance on the loss of balance rates is essentially the same for shorn lambs, fleeced

lambs, shorn ewes and fleeced ewes (Figure 5). The rate drops from 20 incidents per hour at a k-value of 0.018 in shorn ewes to an average of 2.4 losses of balance per hour at a k-value of 0.09, but these incidents were never fully eliminated.

Although loss of balance events cannot be fully eliminated, higher space allowances reduce the risk of sheep losing balance during road transport and only a marginal reduction of the loss of balance events were observed after k-values higher than 0.037.

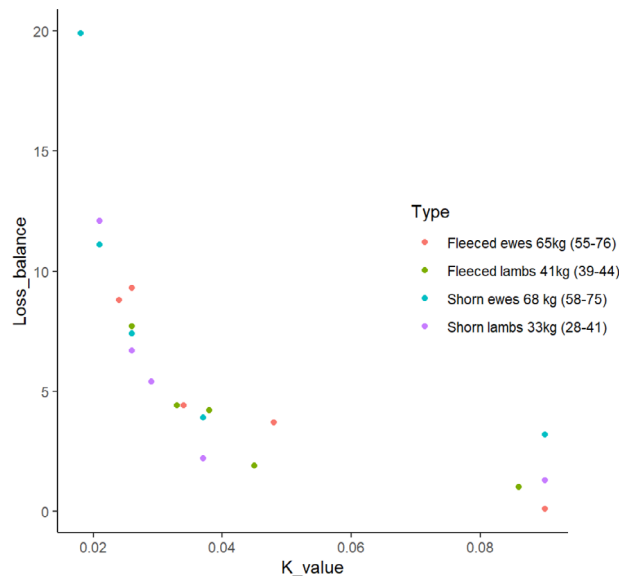


Figure 5: Loss of balance events per hour per compartment recorded by sheep category (indicated by different colours) at different space allowances expressed as k-value during a 6-h journey (data from Jones et al., 2010)

Slip rate: A slip is a loss of grip between the floor and one or more feet and was measured as the number of slips per hour in each group. The slipping rate for all three categories of sheep involved in the study was essentially the same with respect to changes in space allowance, with a decrease in the rate of slipping for all groups of sheep from a maximum rate of slipping seen at a k-value of 0.021 to a minimum seen at a k-value of 0.09 (see Figure 6). Slips could not be eliminated completely by increasing space allowance. However, from a welfare point of view, slips are considered less problematic than losses of balance or falls.

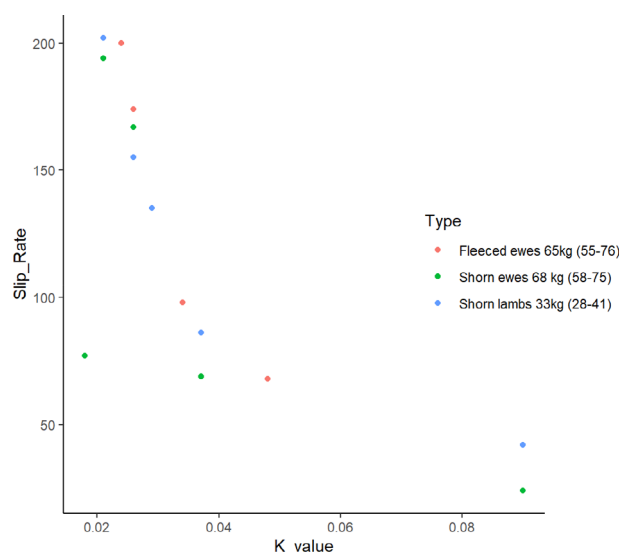


Figure 6: Slip rates recorded by sheep category at different space allowances expressed as k-value during a 6-h journey (data from Jones et al., 2010)

iii) Space required to rest in lying posture

For long journeys, sheep may need to lie down either when the vehicle is in motion or stationary. Studies done under housing conditions show that, when sufficient space is provided, ewes spend a large proportion of the day lying down, and the proportion of time lying increases with increasing space allowance (Bøe et al., 2006). Initially, Petherick and Baxter in 1981 and Petherick in 1983 estimated the space required for other species (pigs) to lie down on the sternum using a k-value of 0.019. These equations were revised considering data from different experiments in different animal species and a k-value of at least 0.027 has been proposed to estimate the minimum space that would allow all the sheep within a pen or vehicle to lie down simultaneously during transportation (Petherick and Phillips, 2009). This value corresponds with a space allowance of 0.28 m²/animal for shorn sheep of 33 kg and 0.45 m²/animal for shorn ewes of 68 kg. However, this is a theoretical value and only takes into account the area of the floor occupied by the animals, but not the extra space needed for any animal to change position from standing to lying or vice versa. In addition, k-values do not take the transport or not transport conditions into consideration.

Whether sheep need to lie down and rest during transport is dependent on factors such as age, health and physical condition, and journey duration and conditions. It is probably more important for sheep to lie down on a long journey than on a short one. The effect of space allowance during transport on lying behaviour has been studied by several authors as discussed below.

Cockram et al. (1996) observed that few sheep lay down during the first 3 h of a journey, with some of them starting to lie down after about 0.75 h. However, between 3 and 12 h of the journey, more lying was observed when the sheep had sufficient space to lie down. A later study observed a steady increase in lying behaviour during the first 6 h of a journey (Cockram et al., 1997). Transport conditions, e.g. the road conditions and the frequency of acceleration events associated with driving, and journey duration, influence the lying behaviour of the sheep during transport (Cockram et al., 2004). If the stocking density, driving quality, road conditions and suspension characteristics of the means of transport provide comfortable conditions, sheep will attempt to lie down during the journey. Thus, in order to avoid potential negative WCs in terms of thwarted lying motivation leading to resting problems, and later fatigue, sheep should be allowed space to lie during transport.

Lambs (29 kg) transported for 3 h did not show a significant difference in lying behaviour at 0.2 m²/animal (k-value 0.021) compared with 0.4 m²/animal (k-value 0.042) (Akin et al., 2018). After 3 h of a 12-h journey, fleeced sheep (30–40 kg) transported at 0.22 m²/animal lay down less during the journey than those at space allowances of 0.27, 0.31 and 0.41 m²/animal. During the first 12 h after the 12-h journey, the sheep that had been transported at 0.22 m²/animal lay down more than those that had been transported at 0.41 m²/animal (Cockram et al., 1996).

During a study simulating sea transport, sheep of 25 kg spent significantly more time lying at higher space allowance (0.35 m²/animal) than those exposed to the same treatment at 0.30 and 0.26 m²/animal (Navarro et al., 2018). Although the conditions of the journey are different than during road transport (different motion stressors), these results support the relationship between space allowances and lying time.

During a 6-h journey, Jones et al. (2010) recorded the lying behaviour, expressed as the maximum percentage of sheep in the compartment lying at any one time (Table 19). This was done for different categories of weaned lambs and sheep and fleeced/shorn.

Table 19: Maximum percentage of sheep in the pen lying at any one time per animal category and space allowance expressed as k-value (Jones et al., 2010). Empty cells indicate that data are not available

k-Value	Shorn ewes	Shorn lambs
0.018	3.7	
0.021	4.5	46.0
0.026	12.2	68.2
0.029		78.5
0.037	13.7	95.2
0.09	31.2	100

In this study, marked differences were observed in this behaviour between shorn lambs and shorn ewes, as fewer shorn ewes were observed to lie down, even when space was not restricted, at similar

space allowances. However, this difference may not reflect lack of a motivation to, or need to lie down, as there may be other factors, e.g. related to the social relations between adult animals vs juveniles, that may explain the lower observation of lying. The lying behaviour of fleeced lambs and ewes was not reported in the study, because the differences were not statistically significant. Notably, the study used different group sizes, and effects of group size and space allowance can, thus, not be fully separated.

The percentage of shorn ewes lying down increased from 3.7% at a k-value of 0.018 to 31% at a k-value of 0.09. The percentage of shorn lambs lying down increased from 46% at a k-value of 0.021 to 100% at a k-value of 0.09. The results, however, suggest that shorn lambs (33 kg) would benefit from a space allowance ≥ 0.39 m²/animal (k-value of greater than 0.037). Fewer shorn ewes were observed to lie down even when space was not restricted.

iv) Space required to thermoregulate

Reducing stocking density can reduce the risk of heat stress by reducing the number of sheep and the total production of metabolic heat and moisture by the sheep, and increasing the evaporative mechanisms via increased exposure of body surface area to ventilation. However, no research was identified to provide specific quantitative information on appropriate space allowances in relation to thermal conditions. Additional information on space allowance and microclimatic conditions (temperature, humidity and ventilation) during road journeys of sheep can be found in Section 3.5.3.1.

The minimal space allowance that would allow sheep to thermoregulate effectively will be influenced by the environmental conditions inside the means of transport, i.e. temperature and humidity and the effectiveness of the ventilation system (while the vehicle is in motion and is stationary). Lowering space allowance would increase the number of sheep that can be loaded onto a vehicle, and if this occurs, the amount of metabolic heat and moisture that they produce will increase. Unless this extra metabolic heat and moisture can be effectively removed by ventilation, low space allowances can be detrimental at warmer temperatures and raised humidity, and predispose to the WC heat stress. High stocking density can also affect the risk of heat stress by reducing air circulation between sheep, and also potentially cause obstruction to ventilation openings. In addition, if sheep are in lateral contact with each other due to high stocking density, their ability to dissipate heat is likely to be reduced.

Although research indicates that reducing stocking density can reduce the risk of heat stress (Fisher et al., 2002), no studies were identified to provide specific quantitative information on appropriate space allowances in relation to thermal conditions for sheep during transport. Council Regulation (EC) No 1/2005¹ recommends increasing space allowances by a percentage factor, e.g. 20%, when environmental conditions increase the risk of heat stress. However, estimations made by Randall and Patel (1994) of the influence of an increase in space allowance when sheep are transported in a naturally ventilated vehicle suggested that space allowance would need to be increased by 25–30% to reduce the internal temperature in the vehicle by more than 1°C. Estimating the actual influence of the reduction of stocking density on the microclimatic conditions inside trucks transporting animals is a complex exercise requiring 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 this process. Unfortunately, no experimental studies on the effect of changes in space allowance on heat stress in sheep during transport have been found.

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

If sheep need to drink and eat onboard the vehicle in order to avoid the WCs of prolonged hunger and prolonged thirst, extra space is most likely required to provide access to troughs and drinkers, allowing all sheep in a compartment to eat or drink.

Previously, a space allowance corresponding with a k-value of 0.037 was recommended for sheep transported for long periods and requiring to be fed and watered on the vehicle (SCAHAW, 2002). However, no scientific validation of this requirement has been found. Additional information on feeding and watering intervals can be found in Section 3.5.3.3.

C) Vertical space

In addition to the horizontal space in a truck compartment, the vertical space should be considered. 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, and should be prevented in order to avoid WCs such as heat stress and restriction of movement. Sheep should be able to at least adopt their natural standing posture, with the head raised, without getting in contact with the ceiling of the truck compartment. Jones et al. (2002) found that ewes spent approximately 80% of the time during journeys of 1 and

4 h with their heads above the withers. Thus, the withers cannot be considered as the highest point of the animals. Jones et al. (2002) estimated that the minimum deck height for a 65-kg cull ewe (data from a group weighing 42–92 kg) should be 95.5 cm, which included 22.3 cm above the withers of the tallest animal to allow for a head-up position. Based on recommendations from the Consortium of the Animal Transport Guides Project (2018), the space above the highest point of the animals should be at least 15 cm in vehicles with mechanical ventilation and 30 cm in vehicles with only natural ventilation. It has, however, not been possible to find studies validating these recommendations, and it is not clear whether they prevent all three hazards listed above. Additionally, the term 'highest point of the animals' has not been defined, and it remains unclear whether it refers to the highest point of the back of the animal or the head (and, in the latter case, in which position).

D) Summary of space requirements

The spatial dimensions of compartments holding sheep during transport, in the horizontal as well as the vertical plane, are of major importance for their welfare and lack of space may lead to several WCs such as restriction of movement, resting problems and heat stress – all potentially leading to distress.

Among the five biological functions of space, room to physically fit into the compartment in a standing posture was taken as a starting point of the assessment.

Ability to maintain stability on moving vehicles was the second biological function of space. The available scientific evidence suggests that the categories of sheep studied (different weight classes of weaned lambs and ewes, shorn or not) overall respond similarly to acceleration events, as larger space allowances allow the animals to brace themselves, adjust their posture and keep balance during the journey to reduce the risk of motion stress and restricted movements, which are among the highly relevant WCs selected for the transit stage. No studies on the space requirements for pregnant or horned sheep were identified.

Regardless of the sheep category, a synthesis of the available evidence suggests that a k-value of 0.037 is required for the animals to adjust posture in response to acceleration and to rest in a lying position.

In addition to the horizontal space in a truck compartment, the vertical space should be considered. 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, and should be prevented in order to avoid WCs such as heat stress and restriction of movement. Based on recommendations from the Consortium of the Animal Transport Guides Project (2018) the space above the highest point of the animals (not the withers, as head is often maintained at a higher level) should be at least 15 cm in vehicles with mechanical ventilation and 30 cm in vehicles without forced ventilation. It has, however, not been possible to find studies validating these recommendations.

3.5.3.3. Thresholds for journey time

As previously mentioned in this Scientific Opinion, and noted by Nielsen et al. (2011) and Cockram (2007), transport of animals is a complex stressor involving numerous aspects (related to the condition of the animals, their general biological characteristics, as well as the conditions under which the journeys take place including the duration), the majority of which may influence animal welfare to some extent.

Whether welfare issues arise during transport is not just dependent on the journey duration. It will depend upon multiple factors such as the type of animal (e.g. age and condition), their fitness for transport, the quality of the journey (including vehicle design, stocking density, ventilation, the standard of driving and quality of the road), the environmental conditions and the associated handling and management of the animals. When considering the implications of journey duration, it is important to consider the influence of each of the potential factors that can affect animal welfare.

Although quantitative limits are often 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 journey, 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 WCs, 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 animal 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: There have only been a limited number of studies that have investigated the effects of journey duration on the welfare of sheep, and the experiments available have often been done using conditions that conform to what is considered best practice. For example, most studies were conducted in journey conditions that were close to or within the TNZ of sheep. As the quality of these journey conditions is 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, 2019a,b).

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 DOA) 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 are dealt with in further detail below: prolonged hunger, prolonged thirst, motion stress, sensory overstimulation and resting problems. In addition, pain and/or discomfort associated with pre-existing or newly caused health conditions have been included. Below, the relationship between journey duration and these WCs is examined, ordered to align with the three different categories suggested by Cockram (2007): 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). In addition, it is taken as a prerequisite that the animals do not have unlimited access to food and water during the journey.

A) The welfare consequences motion stress and sensory overstimulation

Several studies involving transport of sheep have found increased plasma concentrations of cortisol (Broom et al., 1996; Cockram et al., 1997; Parrott et al., 1998), which is an indicator of stress. The studies typically examined plasma concentration of cortisol before and after journeys, thereby potentially involving many different WCs.

Another example of such a study, involving more WCs than motion stress, is Zhang et al. (2020). The authors measured cortisol and adrenocorticotrophic hormone (ACTH) levels in sheep during a 9-h journey using blood samples taken from the jugular vein at 1.5-h intervals during the journey. For comparison purposes, samples were taken from the same group of sheep at the same times of the day the day before the journey (Figure 7). The concentrations of cortisol and ACTH were significantly higher in the sheep throughout the journey when compared with the same sheep in non-transport conditions.

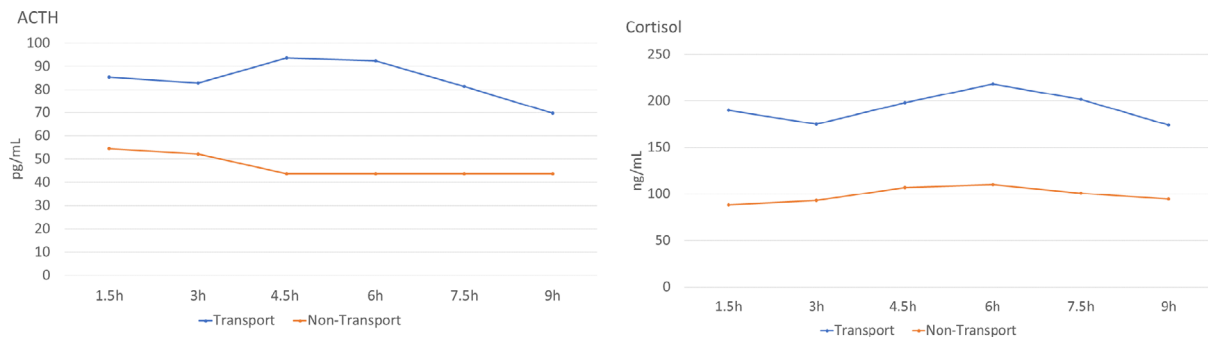


Figure 7: Plasma levels of ACTH (left) and cortisol (right) in sheep in normal husbandry conditions (orange lines) and during a 9-h journey (blue lines) (data from Zhang et al., 2020)

Zhang et al. (2020) used an approach based on wearable multi-sensor monitors to measure heart rate during a seven and a half hour journey transporting mutton sheep, collecting data during the journey. For heart rate, the results showed a rapid increase to approximately 130 bpm approximately 30 min after the start of the journey (Figure 8). During the initial period, it took approximately 1 h for the heart rate to decrease, after which a long-term dynamic change process was initiated, where the heart rate steadily increased to up to about 120 bpm at seven and a half h, likely due to the impact of vehicle motion and internal environmental factors. The normal heart rate of adult sheep is 65–80 bpm (Scott, 2007) although it may increase by more than 50% during handling before returning to near normal within 5–10 min.

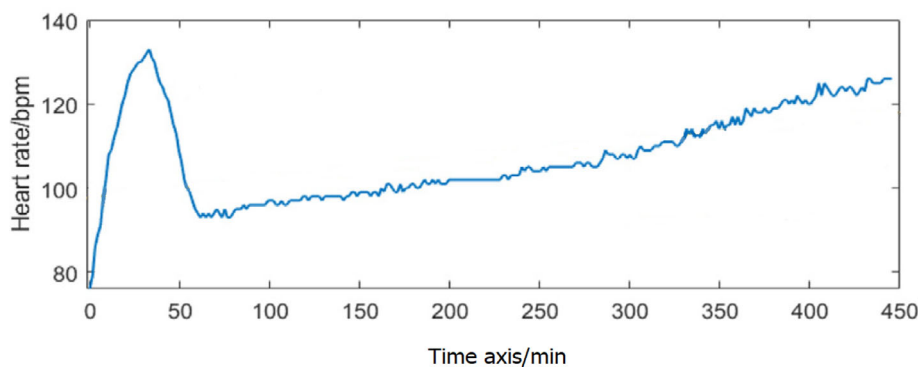


Figure 8: Heart rate (bpm) of sheep during a seven and a half hour journey (Zhang et al., 2020)

There are, however, studies that give information specifically on aspects of motion stress. Cockram et al., 1996 repeatedly measured heart rate and plasma concentration of cortisol in sheep during short stops in 12-h journeys (at 0.25, 3, 6, 9 and 12 h) and similarly during 12 h of confinement in a stationary vehicle. During transport, the heart rate and plasma concentration of cortisol were greater than during stationary confinement, indicating that aspects of the movement of the truck were acting as stressors. In addition, Ruiz-de-la-Torre et al. (2001) found that when driven on minor or rough roads, fewer sheep lie down than when the vehicle was driven on smooth roads or motorways. Based on qualitative behavioural assessment, Wickham et al. (2015) showed that sheep appeared more alert/anxious/nervous when driven erratically with repeated rapid accelerations and decelerations than when driven smoothly.

As correlational evidence, Parrott et al. (1998) measured plasma cortisol concentrations in sheep during a 31-h journey by road (see Figure 9). They found increased cortisol in response to loading and at the start of the first part of the journey, and then steadily declining to reach a nadir at hour 7 subsequently followed by a small increase. Cortisol concentrations remained low until the final 2 h of the journey, and then a marked increase was apparent. Most of this journey was on motorways, but the last 2 h was on side roads with bends at intervals where increased cornering and acceleration caused problems for the sheep, as suggested by increased cortisol concentrations during these hours.

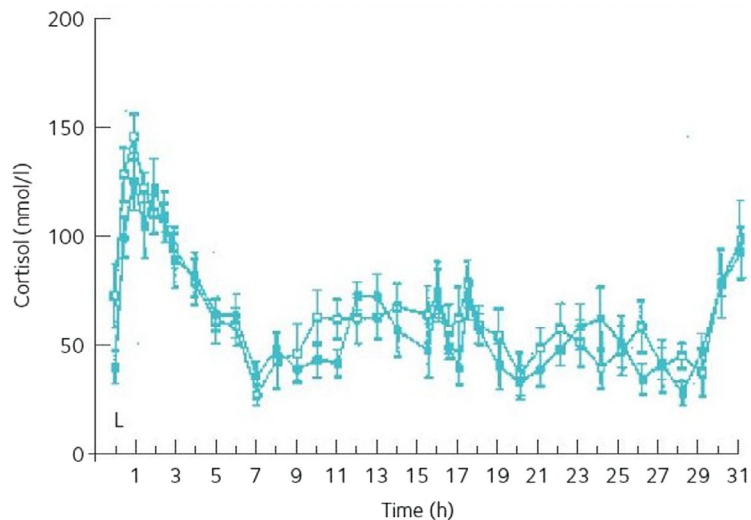


Figure 9: Cortisol in blood plasma during a commercial journey of 31 h in fleeced (■) and shorn lambs (□) (Parrott et al., 1998)

Cockram (2019a) argued on the basis of studies by Smith et al. (2003) that a decline in cortisol after some hours of transport need not be an expression of lowered aversion, but is likely due to negative feed-back as the control of the hypothalamic–pituitary–adrenal axis is multifactorial and multi-level. Thus, even though it is no longer reflected in the peripheral plasma cortisol concentration, sheep likely continue to perceive transport as an aversive stimulus (Cockram, 2019a).

In a study of transport simulation, Parrott et al. (1994) exposed a sheep to stand in an oscillating pen of a transport simulator together with a companion for 60 min. Blood samples were taken 15 and 5 min before and 10, 20, 30 and 60 min after the start of treatment. The physiological responses of the sheep were compared to treatments where sheep were in a control home environment, isolated or standing in water. Blood was analysed for plasma concentrations of cortisol, prolactin and adrenaline. All the stressors significantly increased plasma cortisol concentrations, whereas only transport simulation increased prolactin secretion. All the stressors stimulated adrenaline release within the first 10 min but the most marked effects were seen after transport simulation and isolation, both of which induced a significant increase throughout the treatment period. For the transport simulation treatment, also cortisol, prolactin and adrenaline were still raised 60 min after termination of the treatment.

In a study involving transport of sheep in a truck where half of the animals were allowed visual stimuli vs restricted access to visual stimuli of the moving environment, and transported for 1.5 h, da Cunha Leme et al. (2012) found increased plasma concentration of cortisol in the animals exposed to the visual stimuli, when blood sampled upon arrival at destination.

In addition, Cockram et al. (2004) studied behaviour of sheep during transport, and associated it with the behaviour of the truck driver as well as road conditions (such as driving speed and information about cornering). The study was done on mixed roads, and showed that differences in driving style affected the frequency of losses of balance by the sheep and also the degree of disturbance to the sheep and on their ability to rest during the journey. Losses of balance were common, but falls were rare. About 80% of the losses of balance were associated with driving events, such as acceleration, braking, stopping, cornering, gear changes and uneven road surfaces. It is likely that driving events were also responsible for many interruptions to both lying and rumination. Clear benefits of motorway driving compared with single carriageway driving were fewer losses of balance, more lying down, more rumination and fewer disturbances amongst the sheep.

In a study of physiological and haematological indicators of stress, Miranda-de la Lama et al. (2011) compared 3-h journeys of weaned lambs on paved vs unpaved roads. Lambs transported via the unpaved roads had significantly higher plasma levels of cortisol, lactate, glucose and CK levels (sampled before and after transport) than the lambs transported on the paved roads.

B) The welfare consequence resting problems

During either 12 h of transport or a 12-h stay in a stationary vehicle, sheep were observed to lie less than when kept in straw-bedded pens before the treatments (Cockram et al., 1996). Furthermore,

the transported sheep lay down less and ruminated less than non-transported sheep (Cockram et al., 1996). In addition, almost all of the time that sheep spend lying down during transport, they kept their head raised. Sheep in stationary vehicles tended to keep the neck relaxed and the head lowered (Cockram et al., 2004).

Exertion and potentially fatigue during transport may result from long periods of standing rather than lying down, muscular tension required to brace the body in response to intense vehicular motion and frequent limb movements as a result of a loss of balance (Cockram et al., 2012). In these conditions, mortality rates can also increase, especially among weaker individuals with higher metabolic needs and lower fat reserves (Vaintrub et al., 2020).

Longer journeys lead to behavioural changes of sheep, as compared to control animals. As a journey progresses, the proportion of time spent lying increases (Cockram et al., 1997, 2004) and towards the end of a 24-h journey lying is the most prominent behaviour. Whether an increase in lying behaviour represents an adaptation to the environmental conditions and 'rest' or a deterioration in the ability of the sheep to stand and 'exhaustion' is, however, not clear (Cockram and Mitchell, 1999).

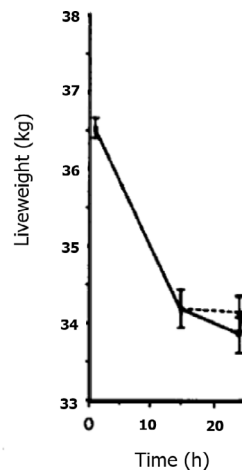
Irrespective of the space provided, resting problems may arise due to exposure to truck motion associated with driving events such as accelerations, cornering and uneven roads. Such truck motion may disturb the lying behaviour of sheep, potentially leading to fatigue. Due to a lack of knowledge, it has not been possible to conclude on the interval from journey initiation to initiation of resting problems.

C) The welfare consequence prolonged hunger

Being hungry is part of an animal's normal daily rhythm associated with meal intake (Roche et al., 2008; D'Eath et al., 2009). Although there are anatomical and physiological differences between the digestive systems of ruminants and monogastric animals, the mechanisms controlling feeding motivation are thought to be quite similar (Baile and Della-Fera, 1981; Roche et al., 2008). Hunger in ruminants is likely caused by a lack of gastrointestinal distention, by hormones that are influenced by circulating metabolites and metabolic signals from energy stores that reflect the energy status of the body relative to metabolic demand, and by time of day. These signals are integrated by the hypothalamus, thalamus and other areas of the brain to produce the feeling of being hungry (Tataranni et al., 1999; Roche et al., 2008), which can be identified by an increased motivation to consume feed (Jackson et al., 1999) and by animals showing increased activity to seek feed, e.g. foraging behaviour. Animals that have been fasted can show increased signs of arousal and anticipation before feed delivery (D'Eath et al., 2009). In hungry animals offered feed, the feeding rate is increased, and animals are likely to show increased competition to gain access to feed (Cockram, 2020a).

Despite the existence of motivational tools, including operant techniques (e.g. Franchi et al. (2019) in dairy cows) to quantify affective states such as hunger, these approaches have not been applied to transport conditions. In studies of the welfare implications of longer journeys carried out without access to feed, emphasis has been placed on physiological measurements that indicate a mobilisation of body energy reserves by monitoring peripheral blood concentrations of metabolites, as well as tissue concentrations of energy reserves, e.g. liver glycogen concentrations. Some studies have included post-transport measurements of feed intake, which are easier to interpret.

In sheep, the immediate effects of fasting are not as apparent as they are in monogastric animals. Although the weight of rumen contents and the production of volatile fatty acids decrease within 12 h of fasting, the continued fermentation of ingesta in the rumen provides dietary energy in the form of volatile fatty acids for several days after the last feed (as reviewed by Cockram, 2019b). Knowles et al. (1996) studied responses of lambs of mixed breed and sex (weight on average 36–37 kg) when exposed to journeys lasting at least 15 h combined with different resting periods, under commercial conditions. The study involved weighing of the animals before and after 15 h of transport (and later) for a large number of lambs. The authors report reduced body weight when the animals were weighed at 15 h, and suggest the weight loss was due to reduced gut fill (Figure 10). In addition, the authors mention that the lambs were highly motivated to eat when offered feed at this time.



Based on a combination of different treatments, the authors were able to sample the animals at $t = 0$ (from more than 500 lambs), and after 15 h of continuous transport and again after 24 h of transport (from 60 lambs each). The broken line refers to a treatment involving a break, and is not relevant here. The study continued focusing on resting periods, but these data are not shown here.

Figure 10: Covariate adjusted means of liveweight (kg) lost in lambs during commercial transport without access to feed or water (Knowles et al., 1996)

In response to reduced dietary energy, a fasted sheep starts to mobilise carbohydrate reserves in the form of glycogen from the liver, until this becomes exhausted after about 12–24 h, as reported by Warriss et al. (1989). The sheep in this study were slaughtered directly off pasture, after different periods of on-farm feed deprivation, but with continuous access to water. This is followed by mobilisation of body fat, as indicated by increased plasma concentrations of NEFA and increased transformation of fatty acids into ketone bodies, shown by an increased plasma concentration of β -hydroxybutyrate (Warriss et al., 1989; Karaca et al., 2016). In Warriss et al. (1989), it was observed that β -hydroxybutyrate increased progressively from 0.311 (SD; 0.070) to 0.717 (SD; 0.134) mmol/L between 0 and 72 h of fasting (Figure 11) and could be a possible index for food withdrawal. If fat reserves do not provide sufficient energy, protein in muscle is metabolised (Blaxter, 1962).

FASTING TIME IN SHEEP

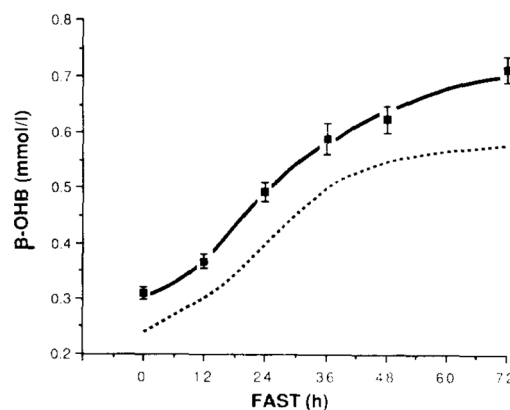
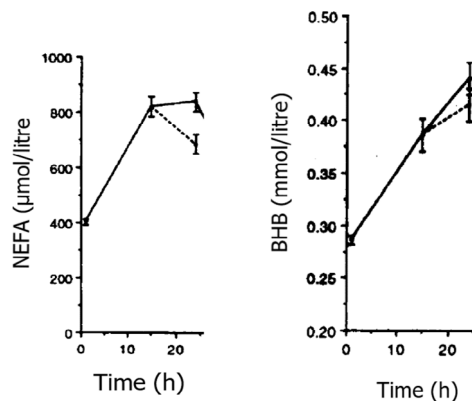


Figure 11: Relationship between fasting time (FAST) in hour and β -hydroxybutyrate concentration in plasma from blood collected by venopuncture (dotted line) and exsanguination (continuous line) (Warriss et al., 1989)

In the study by Knowles et al. (1996), the concentrations of β -hydroxybutyrate and NEFA increased significantly after the 15 h of transport without access to feed or water (Figure 12). In this study, no data are available before this point.



Based on a combination of different treatments, the authors were able to sample the animals at $t = 0$ (from more than 500 lambs), and after 15 h of continuous transport and again after 24 h of transport (from 60 lambs each). The broken lines refer to a treatment involving a break, and is not relevant here. The study continued focusing on resting periods, but these data are not shown here.

Figure 12: Covariate adjusted means of plasma concentration of NEFA and β -hydroxybutyrate in lambs during commercial transport without access to feed or water (Knowles et al., 1996)

After transport for 12 h without feed, the plasma concentration of NEFA of lambs was higher than in controls with access to feed and water. Fasting for 24 h can reduce liver glycogen concentration to minimal values (Warriss et al., 1989). These physiological responses of sheep to fasting indicate a mobilisation of body energy reserves in response to an energy deficiency (Warriss et al., 1989).

In a study involving older sheep (mean age 6.5 years), Pascual-Alonso et al. (2017) transported animals for 4 h without access to water and feed vs a control group staying on-farm with *ad libitum* access to feed; the sheep were weighed and blood sampled on the day before transport as well as upon arrival ($t = 0$), and again 4 and 24 h later. The transported sheep lost on average 0.98 kg, and had higher plasma concentrations of cortisol, glucose and NEFA.

There are, however, also studies that did not find any effects of journey duration on physiological indicators of hunger. In ewes transported for 12, 30 or 48 h without access to feed or water, Fisher et al. (2010) did not find statistically significant effects of journey duration on plasma β -hydroxybutyrate concentration. However, in the ewes transported for 48 h, metabolic effects associated with fasting and muscular exertion were greater than those seen after a 12-h journey. The changes identified were interpreted as indicating that the sheep transported for 48 h mobilised and utilised their adipose reserves and became ketogenic. However, in another paper reporting data from the same study, the ewes were reported to recover during a post-transport rest period, when they had access to feed and water (Li et al., 2011). Hungry animals, when offered feed, show increased feeding rate, and animals are likely to show increased competition to gain access to the feed (Cockram, 2020a). The first priority of sheep after journeys for up to 24 h is normally to eat, then drink, lie down and ruminate, rather than immediately drink or lie down. After a 12-h journey, sheep eat and lie down for longer, eat more hay and drink more water than before transport (Cockram et al., 1996). Indeed, motivation to eat is high after journeys of 12 or 24 h (Cockram et al., 1996, 1997). Knowles et al. (1996) also saw that lambs were highly motivated to eat when offered feed after a journey of 15 h. Also Pascual-Alonso et al. (2017) observed that the sheep transported for only four hours ate more, drank more and were more active than control animals (who had free access to feed during the period), when observed for 3 h post transport. Horton et al. (1996), though, found that after a 3-day journey, feed and water intakes in the day after transportation were lower than in controls.

When the behaviour of sheep during 12 h of transport or a stay of 12 h in a stationary vehicle was compared to the pretreatment behaviour, the occurrence of rumination was significantly reduced during the treatments (Cockram et al., 1996).

D) The welfare consequence prolonged thirst

If sheep do not drink during journeys, they will not be able to replace the water lost by passive diffusion through the skin and in respired air, sweat, urine and faeces. After loading, and with low space allowance, microclimatic conditions inside the truck, outside the TCZ, may create a microclimate

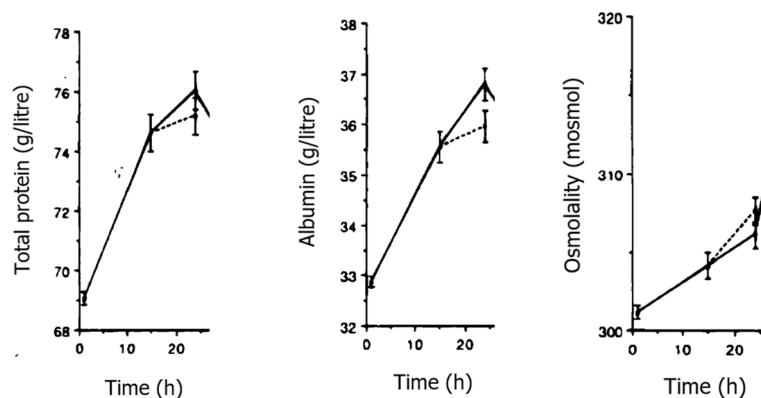
that increases evaporative water loss and favours dehydration. Thirst is a sensation that motivates animals to seek and drink water to maintain homeostasis (McKinley and Johnson, 2004). If thirst is severe and prolonged, it can be associated with dehydration and weakness. Thirst is initiated by an increase in the osmolality of body fluids and by a decrease in body fluid volume (de Araujo et al., 2003). Receptors detect the increased osmolality and decreased extracellular volume and stimulate activation of physiological homeostatic mechanisms to conserve body water and promote thirst to motivate the sheep to drink (McKinley and Johnson, 2004).

Drinking in sheep occurs mostly in association with feeding. After transport, drinking is often not an immediate priority, but might be impaired if the animal is either in a novel environment, not familiar with the type of drinkers, due to water characteristics (water temperature, taste and cleanliness), or is influenced by social factors, e.g. aggression or dominance (Cockram et al., 1997). The susceptibility of different types of sheep to dehydration varies, with suckling lambs being at higher risk of dehydration (Jacob et al., 2006), and with breeds adapted to arid conditions likely more able to cope with periods of water deprivation, even in hot environments.

Even though methods to assess prolonged thirst through motivational tests and, e.g. operant responses have been developed and used under experimental conditions, no data for sheep transport have been found. The negative affective state of thirst (Jensen and Vestergaard, 2021), however, most likely starts before animals show signs of dehydration.

Several studies have assessed the combined effects of transport and water restriction during journeys by monitoring changes in the peripheral blood as potential indicators of dehydration and comparing these effects with non-transported sheep either with or without feed and water (as discussed by Cockram, 2019a). Cockram et al. (1996) compared post-journey water intake and packed cell volume in sheep transported for 12 h vs control sheep kept on-farm with access to water, and found higher water intake and higher packed cell volume in the transported animals. When sheep were offered water but no feed after a 15-h journey without access to water, they drank more water and drank sooner than those that had not been transported nor water deprived. Data on water intake from shorter journeys are lacking in this study, as all journeys lasted 15 h (Cockram et al., 1999). Increases in plasma protein concentration have been reported after journeys in Europe of 29 h (Messori et al., 2015b), with access to drinking water during the journey.

Knowles et al. (1996) studied physiological responses of lambs of mixed breed and sex (weight on average 36–37 kg) when exposed to journeys lasting at least 15 h combined with different resting periods, under commercial conditions. The study involved blood samples taken before and after 15 h of transport (and later) for a large number of lambs. The authors reported increased concentration of total protein, albumin and plasma osmolality, and concluded that the 15-h journey led to a measurable degree of dehydration (Figure 13).



Based on a combination of different treatments, the authors were able to sample the animals at $t = 0$ (from more than 500 lambs), and after 15 h of continuous transport and again after 24 h of transport (from 60 lambs each). The broken lines refer to a treatment involving a break, and are not relevant here. The study continued focusing on resting periods post the 24-h transport. These data are not shown.

Figure 13: Covariate adjusted means of total protein, albumin and osmolality in lambs during commercial transport without access to feed or water (Knowles et al., 1996)

Increases in plasma protein concentration were also observed in studies in Saudi Arabia after a 30-h journey (Al-Mufarrej et al., 2008), and in Australia after journeys of 30 and 48 h (Fisher et al., 2010). For the references involving journeys of more than 15 h, plasma protein concentration was not quantified during the journey, but only upon arrival, so no information from earlier time points is available.

E) Summary on journey duration

Regardless of how optimal the conditions of the journey provided are, sheep can potentially be exposed to a number of hazards during transport that might, either on their own or in combination, result in impaired animal welfare. The exposure to these hazards ends only when the journey ends and the animals are unloaded from a transport vehicle. Any aversive effects of resting problems or associated with exposure to restriction of water and feed are likely to increase with journey duration 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 WCs. The information is summarised below. Based on estimates of risk, prevalence and severity of the WCs, a table (Table 20) has been created to show the estimated journey duration after which the WCs are expected to be present. The recommendation 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 sheep 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. Motion stress is regarded as a highly relevant WC in the transit stage. The prevalence is high, as motion stress is likely to affect all animals in a moving vehicle. The duration of the WC 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. Because of the constant presence of motion stress, it is not possible to estimate a temporal cut-off for onset of this WC after initiation of the transit stage.

Summary – resting problems: Resting problems are regarded as a highly relevant WC in the transit stage. The prevalence is at least moderate, as resting problems may affect a large proportion of animals in a moving vehicle. The duration depends on journey duration, and severity is expected to increase with increasing duration, as the lack of resting becomes more problematic for the animals. Resting problems may lead to fatigue. Irrespective of the space provided, resting problems may arise due to exposure to vehicle motion associated with driving events such as accelerations, cornering and uneven roads. Such vehicle motion may disturb the lying behaviour of sheep, potentially leading to fatigue. Because of lack of knowledge, it is not possible to estimate a temporal cut-off for onset of this WC after initiation of the transit stage.

Summary – prolonged hunger: The WC prolonged hunger is regarded as highly relevant in the transit stage. The prevalence is expected to be high, as no studies have documented the successful feeding of sheep during journeys. Depending on factors such as time off feed before journey start, sheep 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 severity is expected to increase with increasing duration, as the need for feed becomes more 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. The first post-transport priority of sheep is normally to eat, then drink, then ruminant.

Summary – prolonged thirst: The WC prolonged thirst is regarded as highly relevant in the transit stage. The 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. So far, no documentation for proper intake of water, even in journeys on vehicles fitted with drinkers, is available. Depending on factors such as time off water before journey start and/or microclimatic conditions before and during the journey, sheep may not be thirsty during the initial phase of the transit stage, but thirst will develop over time if water is not freely accessible. 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 more and more

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, especially due to the lack of repeated sampling. Based on some studies of the transport of sheep, physiological changes that are likely to be associated with signs of thirst have been identified after 12 h of transport without access to water.

Other summarising considerations: In addition to the WCs 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 in sheep while they are on the farm, as they are known to not show overt signs of, for example, discomfort.

In addition, animals which did not show a health condition before the journey may get injured during the journey due to, for example falls, and the pain and discomfort from such conditions will continue, and likely worsen, until the animal can be unloaded. In this weakened state, sheep may be less able to cope with the challenges associated with transport, and their condition is likely to deteriorate with time and journey duration (Cockram, 2019b).

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.

Table 20 summarises the estimated interval from journey initiation and until presence of the WCs.

Table 20: Welfare consequences estimated to start and develop over journey time

Type of welfare consequence	Welfare consequence	Development over time	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
	Resting problems	Continuous throughout the transit stage	Severity will increase over time leading to fatigue
Progressively developing	Prolonged thirst	Available information shows that when measured after 12 h of transport without effectively accessing water, behavioural and physiological changes indicative of thirst can be present.	Severity will increase with time leading to dehydration
	Prolonged hunger	Available information shows that when measured after 12 h of transport without provision of feed, behavioural and physiological changes indicative of hunger can be present.	Severity will increase with time leading to weakness and exhaustion
Sporadic	Pain and/or discomfort from health conditions	May start any time if undetected pre-existing health conditions are present or new conditions occur during transport	If present, severity will increase with time leading to suffering

3.6. Journey breaks

At some stage during a journey, sheep will need feed, water and rest in order to avoid WCs. This can be done in two ways: (1) 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 CP). Below, the different possibilities are discussed, and hazards, WCs, preventive and corrective/mitigating measures are assessed for the use of CPs.

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

Drinking water can be provided from nipple drinkers and/or water bowls connected to a storage tank on the vehicle (Messori et al., 2015b). However, some of the sheep may not use them while the vehicle is in motion and water can spill from the drinking equipment and cause wet and slippery floor surfaces on the vehicle. In ewes transported for 29 h with access to water via nipple drinkers, the plasma total protein concentration increased and then decreased after an 8-h rest period when the sheep had access to hay, concentrates and water either on or off the vehicle (Messori et al., 2015b). Thus, despite the access to water while the vehicle was moving, the sheep appeared to need a stationary period to obtain enough water. These data come from just one study, involving only one journey, and one group of 24 sheep per experimental treatment. Research is needed to investigate whether all sheep on a moving vehicle will drink, and will drink enough.

In the study by Messori et al. (2015b), sheep were allowed to either access feed provided by clover and oats hay and 400 g of concentrate feed during an 8-h rest period on the vehicle, or after unloading in a CP for 8 h. During the stay in the stationary vehicle with a space allowance of 0.27 m²/animal, only 17% of the sheep were observed eating, whereas more than twice this number were observed eating while in the CP pen. The authors concluded that, despite the sheep having access to familiar feedstuffs, offering them feed in the stationary vehicle at commercial space allowance resulted in reduced eating. Similar results, suggesting that the sheep rested less when spending the 8-h journey break in the stationary vehicle than in a CP pen, were found (Messori et al., 2015b). Despite the very limited available evidence, these results based on 8-h journey breaks suggest that allowing sheep a break on the stationary vehicle at current commercial space allowance does not lead to the intended drinking, eating, and resting and thus does not improve the welfare of the animals. Thus, without direct comparison, these data also suggest that journey breaks on a stationary vehicle of 1 h cannot be used to provide sheep with enough feed, water and rest to an extent that protects their welfare.

3.6.2. Control posts

Small ruminant transport has become more stratified, including more intermediate steps between the main links, such as auction markets, collection centres and CPs making the logistics dynamic and complex (Miranda-de la Lama et al., 2010). These scales likely improve the efficiency of the livestock industry, but also increase the incidence of stress and accidents (Pulido et al., 2019).

Theoretically, the unloading of the sheep into a pen, allows 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 disease. Some of these can be mitigated by the duration of the stay, some by offering high-quality conditions to the animals, whereas others, such as the novelty of the surroundings and the likely mixing of unfamiliar animals as well as the inherent biosecurity risk, cannot be avoided. Any journey break needs to be long enough for each sheep to eat and then drink and rest.

CPs are specialised livestock facilities, usually private, where animals on longer journeys can be offered a journey break after reaching the maximum journey time. Currently, a stay in a CP has to be 24 h before the journey can be continued. The 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 (Schmid and Kilchsperger, 2010). Also, CPs typically offer facilities for vehicles, drivers and competent authorities. In this Scientific Opinion, the section on CPs 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, 2018).

In the last 10 years, two EU projects, funded by DG Sante of the European Commission, have been carried out to renew and promote high-quality CPs in the EU and to develop an EU-wide animal transport certification system. The core of the work was carried out by a group of academic partners who worked together in two DG Sante projects: (i) Renovation and promotion of high-quality control posts in the European Union (SANCO/2010/D5/CRPA/SI2.578062) and, (ii) Development of an EU wide

animal transport certification system and renovation of control posts (SANCO /2011/G3/CRPA/SI2.610274). 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.

3.6.2.1. Current practice

In the EU, there are 140 approved CPs (Updated 17 January 2022). Of these, 61 are approved to receive sheep. There is no list of approved CPs outside the EU, which means that it cannot be verified whether the locations outside the EU, where the animals are expected to be unloaded, have suitable conditions. Approved CPs for small ruminants are distributed in 15 of the 27 EU member states, although France (12), Italy (12), Germany (10) and Poland (10) account for 70% of those. This concentration of CP in certain countries corresponds to the fact that they are countries of obligatory transit between northern and southern Europe. The northern and north-eastern European countries export animals to international markets, mainly to North Africa and the Middle East, where there is a high demand for sheep for ritual slaughter. There are also transport flows from the Iberian Peninsula and Northern Europe to Italy and Greece to satisfy the demand for lambs and kids (Sossidou et al., 2013).

3.6.2.2. Highly relevant welfare consequences

The WCs selected as highly relevant are: group stress, handling stress, injuries, resting problems, prolonged hunger and sensory overstimulation. The WCs and the associated hazards are explained below. The presence and severity of these WCs depend on the management (e.g. cleaning and disinfection procedures, knowledge of legislation, 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. In addition to the WCs listed above, one major welfare hazard related to the use of a CP is animals developing health conditions or turning unfit for further transport. Below, this hazard is listed together with other hazards associated with the different WCs.

Animals developing health conditions or becoming unfit: The flow of animals at a CP is a major issue with respect to the spread of diseases in and outside the EU (Consortium of the Animal Transport Guides Project, 2018), with potential collateral consequences for animal welfare. Disease is always a challenge for animal welfare (as reviewed by Broom, 2006), and can lead to negative affective states and animals needing treatment or even turning unfit for further transport.

During a journey, changes in the clinical condition of animals are a significant risk to their welfare. However, no scientifically published data have documented changes in the clinical condition of sheep during and/or after journeys involving journey breaks. Therefore, it is not possible to assess the size of this risk. In addition, to the best of our knowledge, whether the physical conditions at CPs allow checks of fitness for transport or not, is not known.

- PRE: Staff should be trained on the inspection of animals at the CP to detect signs of weakness or illness on fatigued or injured animals that may compromise their fitness for re-transport (see Section 3.3.3).

Corrective/mitigating measures for animals developing health conditions

Animals showing signs of weakness or disease should be treated appropriately, preferably in a sick pen or similar. These animals may require specific handling and treatment, so contingency plans should be in place for injured and sick animals. Animals being unfit for further transport should not follow the consignment at re-loading, but be slaughtered, treated or euthanised according to the prognosis of their condition.

i) Group stress

When animals are prepared for transport, they are often regrouped with other individuals from the same farm, thereby leading to group stress (Sevi et al., 2001). In terms of transport, a social group is the group of animals coming from the same pen; these groups share management practices, previous experiences and family bonds or coexistence established on the farm of origin (Hall et al., 1998). Social mixing between animals on different floors or compartments of the same truck or between animals from different trucks is not recommended. Therefore, animals from different groups should not be mixed since they do not have an established social structure.

Mixing of animals from different groups: Sheep have the cognitive ability to visually recognise and differentiate socially familiar animals, unfamiliar animals of the same breed, animals of different

breeds and of different species (Kendrick and Baldwin, 1987; Kendrick, 1991, 1992). Social mixing of lambs in novel environments produces high levels of aggression and elevated plasma cortisol levels (Ruiz-De-La-Torre and Manteca, 1999; Miranda-de la Lama et al., 2012). Messori et al. (2015a) reported exposure to a higher number of unfamiliar animals during resting periods in the CP as a potential problem. Fighting can be exacerbated if there is limited access to dry resting areas, drinking and feeding points. Mixing of unfamiliar animals is not only associated with group stress, but it may also result in the transmission of infectious diseases between groups with no previous exposure to the opportunistic pathogens that may be present. Animals that are in the incubation period of an infection or are subclinically infected can shed pathogens, without showing clinical signs of disease. It is possible that stress related to the transport can enhance the level and duration of pathogen shedding in subclinically infected animals and thereby enhance their infectiousness.

- PRE: To prevent this hazard and consequently reduce the risk of group stress and the potential risk of exposure to different pathogens, it has earlier been recommended not to mix animals from more than two pens from the same truck in a CP (Porcelluzzi, 2013). The CP 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.

Corrective/mitigating measures of group stress

If signs of group stress, such as aggression, are observed in the CP, animals showing signs of aggression should be separated as early as possible, to allow a proper rest.

ii) Handling stress

Handling stress was selected as a highly relevant WC at the CP. The hazards contributing to it (i.e. inappropriate handling), and the preventive and corrective/mitigating measures are identical to the ones previously described in the preparation phase (Section i [handling stress] in 3.3.2 [preparation]).

iii) Injuries

Injuries was selected as a highly relevant WC at the CP. The hazards contributing to it (i.e. inappropriate handling), preventive and mitigation measures are identical to the ones previously described in the loading/unloading phase (Section iii [injuries] in 3.4.2 [loading/unloading]).

iv) Resting problems

After arrival in a novel environment, sheep can take several days before they adopt a normal sleeping pattern (Ruckebusch, 1975). Before they sleep, they pass through a state of drowsiness that is characterised by a lack of behavioural activity and a low threshold of arousal (Ruckebusch, 1975; Campbell and Tobler, 1984). During this state, their rest can easily be disturbed by activity (Tobler et al., 1991). How quickly sheep start to lie down and for how long is likely to depend on factors such as the availability of feed, the novelty of the lairage environment, their degree of fatigue, disturbance from other animals, noise and human activity, the space provided, time spent drinking and the presence of bedding or the type of floor surface (Kim et al., 1994; Jarvis and Cockram, 1995; Jongman et al., 2008). Some animals lie down immediately after arrival in a CP, but many take several hours to lie down, with the amount of lying increasing with increased duration of the stay (Cockram, 2020b).

The main hazards that can lead to resting problems in the CP are listed below, together with the preventive and mitigative measures.

Insufficient space allowance: As previously discussed during the transit stage, and below in the restriction of movement in a CP, sheep require a minimum space to allow them to rest and lie down comfortably. It is important to provide a well-drained area for sheep to rest and ruminate in a CP. They generally lie down to ruminate and the space should be large enough to accommodate all sheep in the pen to lie down at once, as sheep have a strong social motivation to synchronise activities (e.g. all feed or rest at the same time). A reduction of synchronisation of lying can be regarded as a negative indicator of welfare (Bøe et al., 2006). Lying behaviour decreases as space allowance is decreased (Jarvis and Cockram, 1995; Bøe et al., 2006). Sheep significantly reduced their lying time when lying space was decreased from 0.75 to 0.50 m² per ewe. Synchronisation of lying was also reduced with decreased lying space (Bøe et al., 2006). A space allowance of greater than 1 m² per sheep is required before most of the sheep in a group will lie down at the same time (Kim et al., 1994). Lying time is

increased in pens which have their longest side away from a corridor as opposed to pens which have their longest side along the corridor (Bøe et al., 2006).

PRE: To reduce the risk of resting problems, enough space should be allocated to animals so that they can rest comfortably lying down in the CP. Group pens should provide enough space for the behavioural needs of the sheep to be met in terms of space to rest, move away from others and express natural species-specific behaviour. A space allowance of at least 1.5 m² per adult sheep, with a minimum of 2 m² for a single sheep, should be provided to ensure adequate lying space for all sheep. Lactating ewes should be provided with at least 2.0 m² per ewe/lamb unit to ensure appropriate maintenance of air quality.

Light conditions of the CP: In addition to providing the necessary space for the animals to lie down and move freely, the CPs must provide the minimum conditions necessary for the animals to be physically and thermally comfortable during their stay without compromising their circadian rhythms. Piccione et al. (2008) reported that in sheep and goats housed in the same stable conditions, most activity was concentrated in the photophase of the light/dark cycle.

- **PRE:** The European manual for high-quality CP (Porcelluzzi, 2013) recommends providing adequate natural diffuse or artificial natural diffuse or artificial lighting all the way from the (un)loading area to the resting area. Care should be taken to avoid light contrasts, light reflection on metallic equipment or high luminosity, because this causes the animals to stop, and sometimes to turn around.

Bedding: Quality, type and quantity of bedding influence the resting of the animals.

- **PRE:** According to Porcelluzzi (2013) it is recommended to provide bedding in the pens at a rate of 0.5 kg/animal for ewes and between 0.20 and 0.25 kg/animal for lambs. In addition, removal of solid waste and bedding, as well as cleaning and disinfection of the pens should be completed within 24 h after a group of animals has been re-loaded for transport. Buildings and equipment should be dry before a new batch of animals can be housed again. Cleaning of barriers and flooring (pens and ways) should be done using high pressure water (40–200 bars, 25 to 70 L/min).

Human presence: The presence of handlers is associated with alert head reactions, movement and decreased lying behaviour in ewes (Kim et al., 1994).

- **PRE:** The presence of handlers should be avoided in the resting areas.

Corrective/mitigating measures of resting problems

Across the hazards listed above, if resting problems are suspected at the CP, a detailed inspection of the facilities should be performed, ensuring that the conditions described before are correct. In case that light problems are suspected, mitigation measures such as decreasing the light intensity by nets or switching off lights from certain pens of the CP should be considered.

v) Prolonged hunger

When sheep arrive at a CP, they have been deprived for feed for hours. Liveweight loss has been reported at 5.5–6% after 15 h of transport (Broom et al., 1996; Knowles et al., 1996) and at 7–8% after 24 h of transport (Knowles et al., 1995, 1996). Most of the loss occurred during the first 15 h and was due to loss of gut fill (Knowles, 1998). Following 12 h of deprivation, sheep become very eager to eat (Knowles, 1998). Cockram et al. (1997) compared the effects of transporting sheep for 24 h without feed, water or rest, with providing a mid-journey lairage/break period of either 3 h or 12 h in the home pen of the sheep. The sheep spent most of the 3-h lairage/break period and almost half of the 12-h lairage/break period standing and eating hay, but not all sheep drank during the 3-h lairage/break period (mean latency to drink was 66 min), and they only ruminated during the third hour. Similar information is not available for the 12-h lairage/break period. When sheep eat, they produce a large volume of saliva, and when feed enters the rumen, the increased osmolality can draw water into the rumen from the plasma. The net effect of this is a temporary decrease in plasma volume and increased plasma osmolality (Ternouth, 1967). If sheep do not have ready access to drinking water, they can become dehydrated after eating dry feed (Cockram et al., 1997), and they are then more susceptible to heat stress (Ghassemi Nejad and Sung, 2017).

The main hazards contributing to the prolonged hunger at a CP are:

Time off feed: The level of hunger at arrival at the CP may be exacerbated by pre-transport fasting time, physical exercise inside the truck according to the characteristics of the route, and

transport in extreme temperature conditions. In these circumstances, the animals rapidly mobilise their body energy reserves to try to maintain equilibrium and body temperature, in which case the feeding needs will be increased (Fisher et al., 2009). Short resting periods of 1 h, for example, are insufficient and may even have detrimental effects on welfare. Hall et al. (1997) studied the feeding behaviour of sheep after 14 h of deprivation and concluded that the extent to which sheep obtained food and water within the first hour was generally low. In addition, it is likely that during short resting periods sheep will not drink and the feed that they eat may lead to an increased water deficit, particularly if offered concentrates (Hall et al., 1997). Based on results of their study, Messori et al. (2015b) concluded that if the time during which sheep after transport show greater than normal eating behaviour is taken as the criterion, after a 24-h journey without feed, sheep require at least 5 h access to hay post-transport. After 24 h of transport without access to feed, raised plasma concentrations of NEFA and β -hydroxybutyrate returned to normal by about 6 h after transport. However, after a journey of 24 h, the behaviour of sheep did not return to normal until 11–15 h after unloading. Messori et al. (2017) investigated the effect of different stopover durations in a CP (24 h, 16 h, and 8 h) after a long journey (29 h) on adult sheep. The authors concluded that 16 h stop in a CP was sufficient for adult sheep to recover from a 29-h journey and to undergo a further 6-h transit without having detrimental consequences on their welfare when compared to a 24-h stop. In contrast, journey breaks of 8 h in a CP were reported to have detrimental effects on muscle indicators, possibly due to the shorter interval between unloading and re-loading procedures (Messori et al., 2017). Transport (long distance) affected the hydration of all transported groups but basal values were restored, regardless of the duration of the break. No differences in stress level were observed. It is suggested that the welfare of the ewes was not impaired by a stop reduction from 24 h to 16 h (Messori et al., 2017).

- PRE: To reduce the risk of development of the WC, prolonged hunger, the feeding and drinking points should be set up according to the number of animals to be housed per pen, and feed and water should be easily accessible in terms of quality and presentation to avoid contamination and competition between animals (i.e. appropriate feeding and drinking space/animal). Recommended trough space for sheep is $0.112 \times W^{0.33}$ m (Baxter, 1992). This means 30 cm for sheep of 20 kg bodyweight and about 34 cm for sheep of 30 kg bodyweight. If animals are not fed for *ad libitum* intake, one feeder per animal is recommended (Porcelluzzi, 2013). However, no studies supporting the latter recommendation have been found. The severity of the WCs will increase if the animals cannot eat quickly after unloading and at least in accordance with their body maintenance during the stay in the CP.

Novelty of the situation in the CP: If sheep are offered feed in a lairage or similar, the latency to feed and the amount of feed consumed might be affected by fear associated with (a) the novelty of their environment, feed and feeding equipment, and (b) unfamiliar animals in the same pen competing for limited access to feed (Boissy, 1995; Cockram, 2020a). Although most animals readily consume feed when it is offered after a journey, a novel environment can decrease feed intake (Cockram et al., 2000).

- PRE: Novelty will be generic to CPs, but the level of novelty can be reduced by, e.g. avoiding mixing unfamiliar animals, keeping the environment calm and making sure that feed and feeding equipment is of types known to most sheep.

Competition for access to feed: Feeding during resting periods may cause competition between animals, and the stronger individuals may exclude the weaker ones (Hall et al., 1997).

- PRE: It is important that feeding and drinking space is enough for all animals to have access to feed and water simultaneously.

Corrective/mitigating measures of prolonged hunger

In case the WC prolonged hunger is suspected in the CP, additional feed should be homogeneously distributed among the animals.

vi) Sensory overstimulation

Sensory overstimulation is one of the consequences for the welfare of sheep upon arrival at a CP (Porcelluzzi, 2013), mainly caused by the following hazard:

Novel stimuli at the CP: In addition to the novelty and fatigue of the journey, on arrival at the CP the animals encounter novel auditory, olfactory, visual and tactile stimuli that can provoke fear or anxiety (Wemelsfelder and Farish, 2004). This is especially important if animals from different sources are

assembled and housed together in the same facility, and with different physiological categories (i.e. males vs females, or females and lambs) (Chanvallon and Fabre-Nys, 2009; Grandin and Shivley, 2015).

- PRE: Prevention should focus on the control of the housing environment, which should be free of disturbing noises, with adequate lighting, with appropriate flooring for the animals to rest, no odours that frighten the animals (e.g. aggressive smelling cleaning products or disinfectants), well ventilated and visual separation from other groups of animals of the same or other species (as far as possible). Additional information on this WC can be found in Sections 3.4 and 3.5, as this WC was also selected as relevant during the loading/unloading of the animals and the transit stage.

Corrective/mitigating measures of sensory overstimulation

In case that sensory overstimulation is suspected in the CP, affected animals should be moved to a separate pen in calm conditions, allowing time to rest.

3.7. The transport of goats

A) Current practice

In the case of goats, 84,519 were transported between MS in 2018, 66,791 in 2019, 68,729 in 2020 and 55,018 in 2021. Road transport constituted 89%, 97%, 97% and 99% of total goats transported in 2018, 2019, 2020 and 2021, respectively. In comparison with the 3.5, 3.1 and 2.5 million of sheep transported inside the EU in 2019, 2020 and 2021, goats represent a small proportion of the small ruminant transport in Europe.

B) Concerns specific to goats

Most of the hazards, preventive, corrective and mitigating measures, as well as highly relevant WCs will be the same for sheep and goats, because they to some extent are generic to road transport. However, there are distinct differences between the two species of animals in terms of biology (AWC, 2020), and this section highlights the specific concerns for welfare of goats during transport.

Goats are more curious, bold and agile than most breeds of sheep. They are able to climb and balance and this, combined with their inquisitiveness, means they are able to escape pens that are designed for sheep. Goats are also usually taller than sheep and have longer legs, which has implications for whether the same handling methods may be used for both species.

Goats are less fearful of new experiences (neophobic) than sheep, and will explore unfamiliar surroundings and investigate objects with their prehensile upper lip and tongue. In groups, goats display less consistent herding behaviour than sheep, and they are more independent and in general less fearful of humans (AWC, 2020). Sheep usually flee from an approaching handler, whereas goats may approach or even respond aggressively to a handler.

For goats, the recognised signs of pain or discomfort are usually bruxism or teeth grinding; other signs of discomfort, stress or disease are decreased time spent eating and chewing the cud, restlessness, prolonged lying with neck and head extended and hunched back when standing (Underwood et al., 2015).

i) Fitness for transport

Similar to the other species, making sure that animals are fit for transport before departure is of utmost importance (Grandin, 2001; Cockram, 2019b). According to Miranda-de la Lama et al. (2018), conditions very similar to the ones listed above for sheep (Section 3.3.3) will leave goats unfit for transport. Also for goats, cull animals may be vulnerable to transport stress (Gautam et al., 2017). However, for goats, no list of conditions leading to animals being unfit has been developed.

ii) Handling and loading/unloading

Handling increases cortisol concentration in goats (Zimerman et al., 2011). The process of domestication of goats has substantially decreased their fear of humans. However, as explained by Miranda-de la Lama et al. (2022), goats still exhibit ancestral anti-predatory behaviour during capture and physical restraint, difficulty in adapting to unfamiliar environments and poor integration with unknown humans and animals. In addition, goats flow less easily than sheep and cattle through a handling system and they are more likely to sulk and lie down when scared.

During handling, goats are more reactive than sheep, because they are more aggressive (i.e. when they are attacked, goats tend to face the attacker, but sheep usually flee) and they exhibit more

exploratory behaviours, whereas sheep are more fearful and shy (Miranda-de la Lama, 2022). Early contact with humans and sympathetic handling can improve the human–animal relationship and result in animals that exhibit less fear and, therefore, are easier to handle during transport. Boivin and Braastad (1996) observed that gentled kids were calmer, more easily approached by humans and, when isolated, were less frightened than non-gentled kids. Additionally, Nawroth et al. (2018) described that goats are capable of predicting aversive handling events based on the facial expression of the handlers. The memory of previous experience or knowledge acquisition can last for up to 4 months (Briefer et al., 2014).

Caution should be taken in the design of the pre-shipment pens; chain-link fences are dangerous as goats tend to stand on their hind legs against fences or walls. Forelimbs and horns can be caught in the mesh (Underwood et al., 2015). It is not recommended to use dogs during handling as jumping and injuries could increase (Vincent, 2005).

Although isolation causes fear and stress in goats (increasing of vocalisations, waling and escape attempts) (Winblad von Walter et al., 2021), packing goats too tight in a holding pen also can cause increased aggression (Miranda-de la Lama, 2019). In addition, it should be noted that newly weaned kids, due to their post-weaning stress and the innate need to perform sucking behaviour, are particularly reactive to handling (Miranda-de la Lama et al., 2022).

Although goats are gregarious by nature, they tend to show less behavioural synchrony, and are more exploratory and individualistic and will not necessarily climb into the truck together immediately as sheep typically do (Miranda-de la Lama et al., 2022). As mentioned previously, extensively managed goats with little or no contact with stockpersons have more risk of slips, falls and jumps during loading (Minka and Ayo, 2007). Kids loading could be improved using adult goats to facilitate movement (Miranda-de la Lama et al., 2022).

iii) Social behaviour and the welfare consequence group stress

Goat social behaviour is quite different from sheep behaviour. Goat herds establish a stable and linear hierarchy (Barroso et al., 2000), maintained by agonistic and affiliative social interactions among individuals. Mixing unfamiliar animals alters the social hierarchy and can lead to increased aggression (Addison and Baker, 1982; Andersen and Bøe, 2007), expressed with contact (biting, bumping), or without contact, seen as threat displays, chases and escapes (Alvarez et al., 2007). The aggressive interactions continue up to 24 h after mixing (Alley and Fordham, 1994), which means that aggression can be increased in newly mixed goats, leading to more attacks and possible injuries (Ayo et al., 2006). In the case of goats, groups should thus be kept stable, repeated regrouping should be avoided, and the introduction of new individuals should be monitored closely. Horned and hornless goats should be kept separate. When goats have to be isolated for management purposes, they should be provided with olfactory, vocal and visual contact with their group members.

iv) The welfare consequence separation stress

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

v) Thermal stress

As described in SCAHAW (1999), based on data from Constantinou (1987), the TNZ for goats has an upper limit of 30°C. Studies conducted on goats transported during short journeys (between 1.5 and 3 h) in humid tropical climates (25°C to 35°C and humidity of 85–88%) showed that goats increased plasma glucose concentration and neutrophil–lymphocyte ratio, rectal temperature, plasma cortisol concentration, dopamine, adrenaline and noradrenaline (Rajion et al., 2001; Kannan et al., 2000, 2003; Zulkifli et al., 2010). Also, the hot and humid conditions during transport affected weight loss and meat quality (Kadim et al., 2006, 2014).

Goats will start panting when heat stressed, and the severity of heat stress according to panting rate is reported to be

- low: 40–60 breaths/min
- medium: 60–80,
- high: 80–120, and
- severe: > 200 (Sarangi, 2018).

Battini et al. (2016) used the following ABMs and scores to assess heat stress in dairy goats:

0 = normal respiration: the mouth is closed, the flank moves regularly (slightly visible) and the legs are frequently held near the body during lying;

1 = elevated respiration: from slightly to moderate panting with closed mouth, small amount of drool or saliva may be present, the posture is functional to heat dissipation, e.g. the neck is frequently extended, the legs may be held far from the body;

2 = panting: from heavy to severe open mouth panting, the mouth is open accompanied by protruding tongue and excessive salivation, the neck is frequently extended, the legs may be held far from the body.

In general, goats tend to tolerate heat better than other livestock species; however, depending of their characteristics, they can be more or less tolerant to heat stress. For example, goats with loose skin and floppy ears show high heat tolerance, whereas angora goats have a decreased ability to respond to heat stress as compared to sheep and other breeds of goats. Long haired goats tolerate radiant heat better than short haired goats and white or light brown goats do better than dark brown or black goats (Acharya et al., 1995). Finally, goat kids, especially suckling ones, are more likely to experience cold stress than adults.

vi) Dehydration

In situations of water deprivation, long journey time is a hazard to the welfare of goats. Polycarp al (2016) observed a decrease of haematocrit values after 7 h of transport attributed to dehydration. The authors also found that alterations in physiological parameters due transport did not return to baseline values in goats after 2 weeks post-transport. Increased urine and faeces elimination during transport and prolonged water deprivation are responsible for dehydration, haemoconcentration and increase of shrink in goats (Atkinson, 1992). These effects are exacerbated in goats under high temperatures and humidity (Kannan et al., 2000; Minka and Ayo, 2010).

vii) Respiratory problems

Signs of immunosuppression (lung's lymphocytes, neutrophils and eosinophils) have been observed after 12 h transport (Minka and Ayo, 2011; Marques et al., 2012; Zheng et al., 2019). Zheng et al. (2019) observed a morphological impairment of lung, trachea and bronchia in goats after transport stress becoming more susceptible to respiratory problems.

viii) Control posts

The CP facilities used for goats have often been designed for sheep or other species and as such may consist of pens constructed with bars rather than solid walls (AWC, 2020). Goats will tend to climb up on horizontal bars and an assessment will need to be made of the risk for the goat in trying to climb over the pen sides, so resting pens for goats should provide for possible leakage. This can be remedied by using pen walls greater than 1.50 m in height to prevent goats from jumping the fence. Chain link fences are dangerous because goats are curious and tend to stand on their hind legs against fences or walls (Miranda-de la Lama et al., 2022).

ix) Considerations for unweaned kids

Unweaned small ruminants need special attention as they are more vulnerable due to their naïve immune system than adult animals (Miranda-de la Lama et al., 2014).

3.8. The transport of unweaned lambs

A) Current practice

For lambs, depending on the production system, the weaning and slaughter ages of lambs vary according to the intensification level of sheep production, consumption habits and socio-economic factors. For example, in Mediterranean countries like Spain, Portugal and Italy, a production model is

practiced whereby lambs are being slaughtered at very early ages (30–60 days) and low live weights, and consumed as unweaned lambs (Sañudo et al., 1998).

B) Concerns specific to unweaned lambs

Suckling length or weaning status is reported to affect carcass (Sañudo et al., 1998; Cañeque et al., 2001) and meat quality (Ekiz et al., 2012) of lambs. Ekiz et al. (2012) reported that suckling management, in which lambs were unweaned until slaughter age, resulted in higher carcass quality than those of weaned groups. The unweaned lambs had higher plasma cortisol and glucose concentration at exsanguination than those of lambs weaned at 45 days or 75 days of age suggesting that the separation from their mothers may be the results of additional stress pre-slaughter transport. In studies using lambs of different breeds, the increase in cortisol and glucose concentrations due to transport was greater in unweaned lambs than in their previously weaned counterparts (Ekiz et al., 2012; Linares et al., 2008; Sowinska et al., 2006), which may indicate that unweaned lambs exhibited more stress responses during transport. Meat from weaned lambs is generally not as juicy as meat from unweaned lambs, which has been attributed to the weaning effect on fat mobilisation (Ye et al., 2020). For additional information, EFSA will publish a Scientific Opinion on protection of cattle during transport that will include a specific section on unweaned calves and all the concerns associated to their welfare during transport.

3.9. Specific scenario: The export of sheep by road

A) Current practice

In 2019–2021, the annual number of sheep exported from EU MSs to a third country by road ranged between 113,000 and 199,000 animals. The main countries of destination for sheep are Turkey, Israel, Lebanon, Albania and Jordan but animals have been transported from the EU by road to countries as far away as the United Arab Emirates, Iran and Egypt. These very long journeys involve the repeated unloading and reloading to facilitate rest periods in CPs or CP-like facilities.

B) Concerns specific to exporting sheep by road

The WCs, ABMs, hazards, corrective and preventive measures detailed in Sections 3.2, 3.3, 3.4, 3.5 and 3.6 above are all applicable to the export of livestock by road. The following is a list of additional concerns that are particular to the export during the various export specific stages. Here, a concern is defined as an area or a topic to which special attention should be given in order to potentially avoid negative WCs.

i) Border crossing when leaving the EU

Animals leave the EU via few and busy border crossings. Therefore, problems may arise due to: the large amount of vehicles crossing, high temperatures in the summer months (i.e. temperature reaching or exceeding 30°C in the shade), and administrative challenges such as restricted opening hours and complicated administrative procedures for processing a consignment, as well as the absence of animal facilities and shaded areas (DG SANTE, 2019). Vehicles with sheep may therefore be forced to wait for a long time – often without the possibility for provision of feed or water to the animals and with high temperatures outside, and consequently inside the vehicles.

ii) Journey breaks on non-EU territory

Animal exports from the EU are allowed to depart, even though there are no EU certified resting points outside of the EU. Sheep being transported to distant third countries may potentially endure journeys that can take several days and potentially involve multiple unloading and reloading at premises.

iii) Thermoregulation

Given that most export is to countries with warm climates, heat stress is expected to be a major problem in the summer. Of the major destinations of EU livestock, Turkey and Israel have a warm Mediterranean climate, and Libya and Algeria have warm desert climates. The maximum temperature at the coast averages 30–32°C, rising to 40°C inland (ESOTC, 2021), being one of the regions of the world where global warming is occurring faster (UNEP, 2022). Mean summer temperatures for livestock at their destination are likely to be in the region of 35–40°C (daily maximum), i.e. well above the UCT for livestock.

iv) Health risks

No studies have investigated this, but it is likely that sheep can be infected by pathogens during, and develop diseases after export. Based on knowledge about ovine medicine, relevant examples are pneumonia, other respiratory diseases and salmonellosis (Phillips, 2016).

v) Handling upon arrival

EU MSs and many destination countries are bound to comply with the World Animal Health Organisation (WOAH) guidelines for the export of livestock (WOAH, 2011). These are at a lower standard than EU regulations. Application of the guidelines is left to individual member countries. The destination countries do not usually have animal welfare legislation of their own.

3.10. Specific scenario: The export of sheep in livestock vessels

A) Current practice

Every year, the EU exports approximately 3 million sheep and goats by sea, mainly to Middle East and Africa (European Commission, 2020). These animals are loaded at the premises of origin and driven to seaports, where they are unloaded from the road vehicles and subsequently loaded into the sea vessels or ships. The journey can last from an average of approximately one week but may be as long as several weeks. The livestock vessels carry large numbers of animals with the largest accommodating up to 75,000 sheep (Boada-Saña et al., 2021).

A livestock vessel is a ship adapted to carry cattle, sheep and/or goats. Vessels are either purpose-built or, more frequently, converted from ships previously used for other purposes such as car transporters. Most of the vessels used in the EU have pens for the animals in the interior of the ship (below deck) which protects the animals from the weather, but require mechanical ventilation systems. The technical/structural requirements of the livestock vessels as well as the authorisation procedures relating to seaworthiness are not covered in the present Scientific Opinion.

To operate in the EU, livestock vessels need a certificate of approval granted by a MS's competent authority or body designated by a MS. The approval is valid for a maximum of 5 years and is invalid as soon as the means of transport are modified or refitted in a way that affects the welfare of the animals. The competent authority is also required to inspect livestock vessels before any loading of animals (Council Regulation (EC) No 1/2005)¹.

The WCs, ABMs, hazards, corrective/mitigative and preventive measures detailed in Section 3.3, 3.4, 3.5 and 3.6 are all applicable to the transport of sheep by sea vessels. The following is a list of additional concerns that are particular to the sea vessel transport during the various transport stages.

B) Concerns specific to exporting sheep by livestock vessels

Animals transported in livestock vessels usually experience very long journeys, from the farm of origin to the port, the voyage in the vessels, and road transport to the final destination, including potential long waits to be loaded and unloaded from the vessel (Boada-Saña et al., 2021).

i) Waiting time at ports

Animals may need to wait several hours inside the vehicles to be unloaded and loaded in the vessels due to delays in the process, and the high number of vessels involved. When this waiting is done inside a stationary vehicle without mechanical ventilation in hot environmental conditions, the temperature inside the vehicle can increase rapidly leading to the WC of heat stress.

Proper organisation and gradual arrival of the vehicles can reduce this risk. In addition, contingency plans should be in place and ports should have some animal facilities arranged to allow the animals to be unloaded and rest until loading is permitted.

ii) Starvation

Starvation due to inappetence is an important factor in the welfare of sheep transported in livestock vessels (Boada-Saña et al., 2021). This has been widely studied in the journeys from Australia to Middle East, where approximately 43% of the sheep deaths on board were due to inappetence, mainly due to lack of adaptation to the novel feed type provided during the journey (Richards et al., 1989). However, other factors have been also identified associated with reduced feed intake such as ammonia levels, high temperatures, high stocking densities or feed access (Phillips and Santurtun, 2013).

iii) Heat stress (temperature, humidity, ventilation)

The same principles as in the road transport (Section 3.5.3.1) apply for the transport by livestock vessels. Sheep should be transported within the TCZ, so the livestock vessels should have the capacity to maintain those conditions.

However, high stocking densities, difficulties for ventilation, solar radiation and high environmental temperatures (as often these journeys are done in the warm months of the year) may result in high temperatures inside the vessels (Boada-Saña et al., 2021). The mortality rate of sheep transported from Australia to Middle East by livestock vessels increased with the wet bulb temperature in the deck and the bridge of the vessel during the hottest month of the year in the destination countries (July to September) (Carnovale and Phillips, 2020).

Depending on the design of the vessel, animals loaded in the upper deck or in outer compartments may be directly exposed to the weather changes, making them vulnerable and more frequently exposed to heat stress (Robin des Bois, 2021). In addition, these animals are more exposed to side-to-side rolling when the sea is rough.

An experiment in a sea trip of 10 days from Australia to Oman found that wet-bulb temperature and dew-point temperatures were very uniform between open-deck pens, in contrast to other variables, like ammonia, that tended to accumulate in lower ventilated pens (Pines and Phillips, 2013).

iv) Noxious gases

The accumulation of manure during the journey, especially in poorly ventilated pens, leads to increased levels of noxious gases, mostly NH₃, CO₂ and H₂S. High levels of NH₃ irritate throat, nose and eyes, which can be identified by coughing, sneezing, nasal secretion and lacrimation (Pines and Phillips, 2013). The authors have also documented the influence of the ammonia levels on the behaviour of sheep transported in livestock vessels, as animals stood longer with their heads in higher positions, spent less time ruminating and lying down and presented more conjunctivitis, in comparison with other animals in better ventilated pens with lower ammonia levels. In simulated transport conditions, exposure to high concentrations of ammonia resulted in increased sneezing and increased macrophage activity, which indicates pulmonary inflammation, as well as some behavioural changes such as less locomotion and panting (Phillips et al., 2012). Despite the evidence on the negative effects of ammonia, there is not enough evidence to propose a suitable exposure level (Phillips and Santurtun, 2013).

v) Space requirements

As previously discussed in the road transport Section 3.5.3.2, if sheep need to drink and eat onboard, which is always the case in livestock vessels, they should be provided with space to ensure they all have access to troughs and drinkers without competition. Currently, no scientific information is available to give recommendations on space allowance during journeys on livestock vessels – either in the horizontal or the vertical plane. The hazards listed in Section 3.5 for road journeys will also apply here, however taking into account that the journey duration will be much longer than for road transport.

vi) Motion stress

During journeys on livestock vessels, motion stress is a highly relevant WC, and even though weather can be forecasted, the duration of the journeys mean that rough sea cannot be prevented. Sheep exposed to simulated sea transport conditions expressed stress-related reactions (increased heart rate and changes in heart rate variability) when the moving platform rolled (side to side), leading to constant changes in posture (Santurtun et al., 2015). Further experiments using the same moving platform designed specifically for these studies confirmed that irregular movements of roll and pitch, which is similar to the movement experienced when waves are irregular during severe weather conditions, were stressful for the sheep, that showed heart rate increase and heart rate variability changes. It seems evident that sea travel in general can result in negative welfare for the animals. However, no scientific studies were found on the effects of real sea swell on the animals.

vii) Handling upon arrival

The same concerns as described for export by road (Section 3.9) apply here.

3.11. Specific scenario: Transport of sheep on roll-on-roll-off ferries

A) Current practice

Roll-on-roll-off vessels are ferries designed to carry trucks, on which livestock trucks can travel. Unlike livestock vessels, RO-RO ferries do not require inspection and approval before they are used to carry animals. Typically, a truck or trailer arrives in the port before ferry departure, and some stationary time will take place. During the sea voyage, the animals are kept in the vehicle.

B) Concerns specific to RO-RO ferries

Typically, the WCs, hazards, preventive and corrective/mitigative measures explained before in the transport by road (Section 3.1) also apply here. In addition, the transport by RO-RO ferry present certain concerns that are addressed below. No studies have been found focusing on the welfare of sheep during RO-RO journeys. Hence, this assessment is based on expert opinion and general knowledge about RO-RO ferries and animal transport. The main welfare concerns related to transport of sheep on RO-RO ferries are:

i) Exceeding the maximum journey time

Vehicles typically have to wait before they can board the ferry and before the ferry leaves. Some commonly used sea journeys may take longer than the recommended journey time, especially when the waiting time before boarding and the (un)boarding time are included. As a general consideration of the journey to the ferry, time taken while waiting to board the ferry and onward to the destination or CP should be taken into account.

ii) Weather disruptions

Rough weather may cause ferry services to be postponed or cancelled. This can result in animals having to wait for a long time at the port or in having to return to the port.

iii) Inadequate ventilation, heat or cold stress

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 ferry, the animals stay in their TCZ. Ventilation is also crucial. Airflow around and through the vehicle's animal compartment, including removal of exhaust fumes, must be sufficient to ensure that a suitable environment is maintained within the vehicle.

Vehicles stowed on open decks will generally benefit from better airflow than those in enclosed decks. However, open decks mean increased risk of overheating if located in sunlight, particularly when little air is moving across the deck. Strong cold winds could have an adverse effect, particularly on young animals.

Attention needs to be given by multi-tier vehicles as the low vertical space allowance can pose hazards to the ventilation (Section 3.5.3.2).

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.

v) Motion stress

In addition to the motion stress involved in road transport, RO-RO ferries involve additional problems if the sea is rough and/or vehicles are not properly secured against movement in any direction in the ferry. Therefore, during journeys on RO-RO ferries, motion stress is even more relevant than during road transport.

3.12. Specific scenario: The transport of sheep by air

A) Current practice

The transport of livestock by air happens at low frequency when compared to road transport but it can still be part of industry practice, involving especially breeding animals. According to TRACES, in 2019, 2020 and 2021 8,864, 1,812, and 1,724 sheep were transported by air, between MS and exported to third countries.

The transport of sheep by air starts with a journey by road from a farm or assembly centre to an airport where the sheep are unloaded from the road transport vehicle and loaded into transport crates. These crates are then loaded into the aircraft. Upon arrival at the airport of destination the crates are unloaded from the airplane and brought to an area where the sheep are removed from the crates and loaded into road transport vehicles to continue their journey.

B) Concerns specific to sheep travelling by air

The WCs, hazards, preventive and corrective/mitigative measures explained in the transport by road (Section 3.3, 3.4, 3.5 and 3.6) also apply here. In addition, transport by air presents certain concerns that are addressed below. No studies have been found focusing on the welfare of sheep during air transport, though. Hence, this assessment is based on expert opinion and general knowledge about air transport and animal transport.

Among the welfare concerns identified for air transport of sheep are high density confinement in crates, lengthy waiting times, extended periods of water and feed deprivation, variation in microclimatic conditions and potential exposure to noxious gases. The main concerns identified by stakeholders in the industry are: food and water deprivation times particularly when the aircraft is delayed, the access to and management of crated animals in transit especially in hot and humid climates, and the adequacy of ventilation. Other factors that may cause animal discomfort are motion stress and loud noises (Collins et al., 2018, 2020).

3.13. Specific scenario: The transport of sheep by rail

A) Current practice

Rail is the means of transport used the least for sheep. According to TRACES, in 2020 and 2021, 1,081 and 501 sheep were transported by rail between MS. The transport of sheep by rail starts with a journey by road from a farm or assembly centre to a train station where the sheep are unloaded from the road transport vehicle and loaded onto the train. Upon arrival at the train station of destination, the sheep are unloaded from the train and loaded into road transport vehicles to continue their journey.

B) Concerns specific to sheep transported by rail

No scientific literature pertaining to the transport of sheep by rail were found. However, based on expert opinion and general knowledge about train transport and animal transport, the following welfare concerns were identified.

The WCs and hazards identified in Sections 3.3, 3.4 and 3.5 are all applicable to the transport of sheep by rail. The unloading of sheep from a vehicle at a railway station with the subsequent loading onto a railway carriage is a procedure requiring care. Similarly, the unloading of a railway carriage and the loading of a vehicle requires equal care. The animal handling facilities present in a railway station are of particular importance. This includes races/chutes and pens that will allow the sheep to be safely moved from the ramp of a vehicle to the loading ramp of the railway carriage. Among other welfare concerns identified for rail transport of sheep are high density confinement in railway carriages, lengthy waiting times, extended periods of water and feed deprivation, variation in microclimatic conditions, potential exposure to noxious gases.

3.14. Specific scenario: 'Special health status animals'

A) Current practice

In some cases, sheep are transported from a herd (or region/country) with a higher health status than the general animal population, through an area with a lower health status, to a new herd (or region/country) with a higher health status. In such cases, the herd (or region/country) receiving the sheep does not want them exposed to the general sheep population during the course of any given journey. Therefore, journeys taking place without unloading the sheep during the journey may be advantageous. The focus here is on the transport of sheep on long journeys by road without unloading them before their final destination.

B) Concerns specific to sheep where they would not be unloaded from the truck

The WCs selected as highly relevant for sheep during the transit stage are heat stress, prolonged thirst, prolonged hunger, motion stress, restriction of movement, resting problems and sensory

overstimulation, and have already been dealt with in Section 3.5.2. Recommendations have been made in Section 3.5.2 that attempt to prevent hazards and/or mitigate WCs. However, not unloading sheep before their final destination presents a greater challenge than when they can be unloaded, with respect to the welfare of the animals in question. If, due to biosecurity concerns, sheep are not unloaded to provide required rest, feed and water, facilities must be available on the vehicle to provide the necessary resting, feeding and drinking, and a suitable microclimatic environment. As explained in Section 3.6, Messori et al. (2015b) suggested that, despite having access to water while the truck was moving, sheep appear to need a stationary period to obtain enough water. In addition, when sheep were allowed to either access feed during an 8-h rest period on the truck with a space allowance of 0.27 m²/animal, only 17% of the sheep were observed eating, whereas more than twice this number were observed eating while offloaded in a CP pen (Messori et al., 2015b). The authors concluded that, despite the sheep having access to familiar feedstuffs, offering them feed in the stationary truck at commercial space allowance resulted in reduced eating. Similar findings, suggesting that the sheep rested less when spending the 8-h journey break in the stationary truck than in a CP pen, were found.

No scientific studies have been found that can demonstrate the effective feeding and watering of sheep on a vehicle during journeys. Air and bedding quality are other important issues that have not been studied in such a context.

The impact of rest periods has not been studied either, nor has the impact of repeated driving and rest periods and where to put a limit on such a journey.

3.15. 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 – which may be measured in a qualitative or quantitative way – and hazards causing these WCs. Based on this identification and listing of these 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: 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 highly 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 to be selected.
Literature search – Publication type	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 off.	Underestimation of the published relevant studies.

Source of uncertainty	Nature or cause of the uncertainty	Impact of the uncertainty on the assessment
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 sheep 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.
Limited available knowledge on goats	The scientific focus on goat transport has been limited, both in terms of available studies and available experts.	Reduced level of detail in the assessment of goat welfare during transport.
Husbandry practices and sheep breeds and categories of the studies retrieved in the extensive literature search	The studies retrieved may have involved husbandry practices and sheep 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.

Source of uncertainty	Nature or cause of the uncertainty	Impact of the uncertainty on the assessment
Time and resource allocation	The time and resources allocated to this opinion 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. 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.

WC: welfare consequence; ABM: animal-based measure.

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

4. Conclusions

4.1. General conclusions on transport of sheep

- There are published protocols to assess animal welfare on farm and at slaughter, but no validated protocols are available to assess welfare of sheep during transport. Only checklists to assess welfare of sheep at unloading have been proposed.
- ABMs for all highly relevant WCs along the transport stages are available based on expert opinion (see Section 3.2.2). None of these, however, has been documented to be useful during transport in terms of feasibility, sensitivity or specificity.
- The use of ABMs in animal transport is hampered by the reduced accessibility of animals during the transit stage, but may be more feasible during other transport stages (e.g. loading/unloading). In general, validity and reliability of ABMs is much less documented under transport conditions than in on-farm situations.
- Technological developments such as artificial intelligence-based cameras or motion sensors, may increase the possibility to record and/or monitor ABMs during the entire transport process. However, such systems are not yet available in practice.
- This Scientific Opinion has identified many hazards (see examples below for each transport stage) during the transport of sheep and the consequences of exposure to them throughout the transport stages (See Sections 3.3, 3.4, 3.5 and 3.6).
- 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 WCs 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 has 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) 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 the preparation of sheep 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 WCs.
- 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 re-loading of the animals.
- Handling stress and predation stress are the highly relevant WCs during the preparation of sheep for transport, and for both, education and training of handlers and their dogs are among the most important preventive measures.
- If water and feed are not accessible during the preparation phase, animals will be predisposed to the WCs of prolonged hunger and prolonged thirst during subsequent transport stages. In this case, the interval from the initiation of the journey until hunger and thirst are present, will be shortened.

A) Fitness for transport

- The assessment of fitness for transport (Section 3.3.3) before departure is of utmost importance in the protection of animal welfare. However, currently, no 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 WCs during later transport stages, and potentially leading to negative affective states such as discomfort, pain and suffering.
- Characteristics leading to animals being 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. A list of conditions rendering animals unfit for transport has been provided in this Scientific Opinion, of which some still require scientific validation (Section 3.3.3).
- At present, thresholds for ABMs as indicators of animals being unfit for transport have most often not been established or validated. If sheep are to be fully protected from the consequences of being transported while in reality unfit for transport, knowledge about the risk associated with transport of animals with a number of conditions potentially leading to negative affective states (e.g. lameness, wounds, mastitis), 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 to be added to the list provided in this Scientific Opinion.
- Successful assessment of fitness for transport requires well-educated staff (including professional groups such as veterinarians, farmers, herdsman 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 WCs during transport. There is consensus across different available guidelines that sheep should not be transported in the last 10% of their pregnancy. However, scientific evidence to substantiate this threshold is lacking, and the risk of reduced animal welfare may be present earlier in the pregnancy.

4.3. Conclusions for loading/unloading of sheep during road transport

- The highly relevant WCs during loading/unloading of sheep are: heat stress, handling stress, injuries, sensory overstimulation and predation stress.
- Across the highly relevant WCs, the major hazards are inappropriate handling, unsuitable facilities and high effective temperatures.
- Due to lack of habituation to handling, most sheep are expected to experience handling stress, which may be associated with fear and may lead to distress. The main preventive measures are establishment and maintenance of proper facilities, education and training of handlers and their dogs as well as habituating sheep to handling.

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

- The highly relevant WCs for sheep during the transit stage are heat stress, motion stress and sensory overstimulation, prolonged hunger, prolonged thirst, resting problems and restriction of movement.
- Among the major hazards for animal welfare during the transit stage are high temperatures, insufficient space allowance, reduced intake of water, time off feed and vehicle movements.
- In this Scientific Opinion, preventive and corrective/mitigating measures have been suggested (see Section 3.5.2). Several of the highly relevant WCs of the transit stage (e.g. motion stress, resting problems, restriction of movement) cannot be fully prevented.
- Albeit based on few studies, the evidence suggests that sheep drink less during transport, even on journeys when vehicles are equipped with water drinkers, than when kept on-farm.
- No studies have documented successful feeding of sheep during the transit stage.
- The severity of WCs during the transit stage of transport will depend on the exact conditions pertaining to an individual journey (e.g. microclimatic conditions, space allowance and road conditions). These hazards potentially interact. The exposure to these hazards will continue at least as long as the journey continues.

A) Microclimatic conditions

- If the temperature in the transport vehicle remains below the upper limit of the TCZ, sheep will most likely not experience stress or the negative affective states associated with heat stress during transport.
- The WC heat stress may start when sheep are no longer in their TCZ, and the risk and severity of heat stress is high when the thermal conditions reach the UCT.
- Not only the temperature in the vehicle, but also other environmental conditions influence heat load placed on sheep during transport, such as relative humidity, thermal radiation, temperature of surrounding surfaces and wind speed. These will all influence the microclimatic conditions experienced by sheep and should in theory, all be taken into account when microclimatic conditions of sheep during transport are evaluated. However, humidity is considered the most important of these to take into consideration.
- The available information has allowed for estimates of thresholds for TCZ (C), and for upper critical temperature (D) for sheep (Table 22). For lambs, less information is available.

Table 22: Estimates of upper thermal thresholds for the thermal comfort zone (C) and the upper critical temperature (D) in dry bulb temperature (°C)

	Upper threshold of thermal comfort zone (C)	UCT (D)
Fleeced sheep		28°C
Shorn sheep	25°C	32°C

- 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 that influence heat load placed on sheep 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 TCZ and UCT will be, when measured as a dry temperature only.

B) Space requirements

- The allometric equation ($A = k \times W^{2/3}$) (where A is area in m² per animal and W is liveweight in kg) can be used to calculate the physical space requirements of any category of sheep.
- The minimum space allowance is set by the first limiting factor reducing the ability of sheep to undertake biological functions during transport. The available evidence suggests that a k-value of at least 0.037 is required. Providing the different sheep categories this space during transport will allow them to adjust posture in response to acceleration and other events related to driving, and to rest in a lying position. The suggestion with respect to space to lie down has, however, not been validated during transport.

- The table below gives estimations of the minimum space allowance suggested for sheep during transport (Table 23).

Table 23: Estimates of minimum space allowance suggested for sheep of different weights

Approximate weight	Area (m ² /animal)
12 kg	0.19
23 kg	0.29
40 kg	0.43
55 kg	0.54

- The vertical space in a means of transport is important for animal 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 WCs such as heat stress and restriction of movement.
- No studies have established a proper deck height for sheep during transport, but the space above the highest point of the animals has been recommended to be at least 15 cm in vehicles with mechanical ventilation and 30 cm in naturally ventilated vehicles. Establishment of evidence-based thresholds constitutes a gap in knowledge.

C) Journey times

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 WCs 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 WCs 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 that are **progressive** (i.e. may not be present at the beginning of the journey but develop progressively as it continues), and those that are **sporadic** (i.e. problems in individual animals which may be an exacerbation of a pre-existing condition or may occur spontaneously at any point in the journey and whose WCs will continue thereafter). These are summarised in Table 24.

Table 24: Summary of conclusions on welfare consequences during journey

Type of welfare consequence	Welfare consequence	Development over time	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
	Resting problems	Continuous throughout the transit stage	Severity will increase over time leading to fatigue
Progressively developing	Prolonged thirst	Available information shows that when measured after 12 h of transport without effectively accessing water, both behavioural and physiological changes indicative of thirst can be present	Severity will increase with time leading to dehydration
	Prolonged hunger	Available information shows that when measured after 12 h of transport without provision of feed, both behavioural and physiological changes indicative of hunger can be present	Severity will increase with time leading to weakness and exhaustion
Sporadic	Pain and/or discomfort from health conditions	May start any time if undetected pre-existing health conditions are present or new conditions occur during transport	If present, severity will increase with time leading to suffering

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

- Motion stress and sensory overstimulation start as soon as a vehicle starts moving, and continue while the vehicle is moving, affecting all transported animals. Animals experience stress potentially leading to fatigue and negative affective states such as fear and distress;
- 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;
- The prevalence of resting problems is at least moderate, as it may affect a large proportion of animals in a moving vehicle. Duration depends on journey duration and the severity is expected to increase with increasing duration and may lead to fatigue;
- The prevalence of prolonged thirst may be high, even in vehicles with fitted drinkers. The severity is expected to increase with increasing journey duration. Prolonged thirst may lead to dehydration and negative affective states. Behavioural and physiological changes that are likely associated to thirst can be identified after 12 hours of transport;
- The prevalence of prolonged hunger is expected to be high due to practical difficulties in feeding animals while on a transport vehicle. Severity is expected to increase with increasing duration. Behavioural and physiological changes indicative of hunger can be present after 12 hours of transport.

To conclude, during transport sheep will be exposed to a number of hazards, either on their own or in combination, leading to WCs. The amount of time the animals are exposed to these hazards is dependent on the journey duration.

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 allow them to recover from the associated WCs.
- No studies have documented successful feeding of sheep during the transit stage, and it is currently not considered practically possible. This constitutes a gap in knowledge.
- There is insufficient published literature to document that it is possible for sheep to drink and rest according to their needs, in a journey break on a stationary vehicle. This constitutes a gap in knowledge.
- If sheep are to recover from the WCs experienced during transit they need to be unloaded from the vehicle for a sufficiently long period of time.
- The highly relevant WCs for sheep during the CP stage are group stress, handling stress, injuries, resting problems, prolonged hunger and sensory overstimulation.
- Among the major hazards for animal welfare during the CP stage are inappropriate handling, mixing of unfamiliar animals and exposure to novel stimuli.
- When CPs are used, animals must be unloaded and reloaded. These procedures involve hazards potentially leading to WCs such as heat stress, handling stress, injuries, sensory overstimulation, isolation stress and predation stress (Section 3.4).
- In addition, CPs 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 sheep typically transported on journeys involving journey breaks, the scientific focus on CPs has been limited. This means that whether CPs in their current state fulfil their intended function is not known, and there is a (presently unquantified) risk that even though CPs conform to the current regulation, their use may be associated with animal WCs.
- The science underlying the duration of a journey break necessary to protect the welfare of sheep is limited. This constitutes a gap in knowledge. The available evidence suggests that the welfare of sheep is not impaired by a break reduction from 24 h to 16 h. Setting the required period will, however, depend on the journey experienced. It will also depend on the state of the animals, and depend on whether sheep are required to recover from feed and water deprivation, or to acquire normal rest or sleep patterns after arrival in a novel environment. A short journey break for one hour is unlikely to allow all animals to feed, drink, and rest adequately.

4.6. Conclusions on welfare concerns during transport of goats

- The number of goats transported between MS and exported from the EU is considerably smaller than the number of sheep. In recent years, road transport has constituted around or more than 90% of total goats transported.
- Most of the hazards, preventive, corrective and mitigating measures, as well as highly relevant WCs will be the same for sheep and goats, because they to some extent are generic to road transport of small ruminants.
- Although sheep and goats are often considered together in relation to welfare during transport, there are distinct differences between the two species of animals in terms of their biology (e.g. goats are usually taller with more tendency to climb). Understanding of these species-specific differences will help to improve operators' safety and animal welfare during transport.
- Making sure that goats are fit for transport before departure is of utmost importance. Conditions very similar to the ones listed above for sheep (Section 3.3.3) will most likely leave goats unfit for transport. However, for goats no list of conditions leading to animals being unfit have been developed.

4.7. Conclusions on welfare concerns during transport of unweaned lambs

- For unweaned lambs, procedures of unweaning and prolonged transport immediately after are stressful and exhaust body reserves. From an animal welfare point of view, weaning lambs sometime before transport would be advantageous.

4.8. Conclusions on specific scenarios

A) Export by road

- Typically, the WCs, hazards, preventive and corrective measures explained in the transport by road (Sections 3.3, 3.4 and 3.5) also apply here.
- Although based on limited scientific studies, it is clear that export of sheep by road involves risks for the welfare of the transported animals, some of them difficult or impossible to control such as delays in leaving the EU, handling and journey breaks outside of the EU. Thus, export by road likely involves more hazards and higher exposure to hazards than intra-EU transport.
- These exports usually involve long journeys due to the long distance destination with all the implication stated before in Section 3.5.3.3 (Thresholds for journey time).
- Other hazards can be presumed such as high environmental temperature and lack of plans made to mitigate extreme external temperatures.

B) Export by livestock vessels

- Typically, the WCs, hazards, preventive and corrective/mitigative measures explained in the transport by road (Sections 3.3, 3.4 and 3.5) also apply here.
- Transport of sheep in livestock vessels increases risks for the welfare of the animals, as they are exposed to additional hazards (such as waiting times at ports, starvation, handling upon arrival) as compared to road transport within the EU.
- Among the concerns for animal welfare are microclimatic conditions during the waiting time in ports and during the journey, motion stress arising from sea conditions and post-journey handling.

C) Roll-on-roll-off ferries

- Limited studies have been found focusing on the welfare of sheep during RO-RO journeys. Hence, this assessment is mostly based on expert opinion and general knowledge about RO-RO ferries and animal transport.
- Typically, the WCs, hazards, preventive and corrective/mitigating measures explained in the 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 sheep 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 potentially 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 to attend to animals and unload in case of emergencies.

D) Transport by air and rail

- For sheep, air transport involves only a small fraction of transported animals, and mainly breeding animals. Rail is the mode of transport used the least for sheep.
- The WCs, hazards, preventive and corrective/mitigating measures explained in the transport by road (Section 3.1) also apply here, but there will be additional concerns for sheep welfare during air or rail transport due to the complexity of the journeys.
- The available evidence to evaluate the welfare of sheep when transported by air or train is very scarce. This constitutes a gap in knowledge.

E) Special health status

- Typically, the WCs, hazards, preventive and corrective/mitigating measures explained in the transport by road (Sections 3.3, 3.4 and 3.5) also apply here, with some extra additions due to the lack of possibility to unload the animals.
- The available evidence to evaluate the welfare of sheep during journeys where animals cannot be unloaded is very scarce. This constitutes a gap in knowledge.

5. Recommendations

5.1. General recommendations on the transportation of sheep

- Protocols for the assessment of welfare of sheep during transport should be developed and validated, preferably involving aspects of pre-and post-transport housing or production systems, e.g. if animals are transported to/from intensive or extensive husbandry 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 output more than input.

5.2. Recommendations for preparation of sheep before transport

- Protocols to assess animal welfare during the preparation phase, including scenarios characterised by repeated re-loading of animals, should be developed and validated.
- In order to avoid WCs 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.

A) Fitness for transport

- In order to avoid WCs, 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, dyspnea, ataxia, disorientation/abnormal behaviour, abnormal navel, wounds, aspect, demeanour, swollen joints, abscess, in grown horns, hernias, rectal and vaginal prolapse, late pregnancy, bone fractures, body condition score, and eyesight deficiency.
- Risk associated with transport of animals with a number of conditions potentially leading to negative affective states, such as pregnancy, should be examined.
- In order to avoid doubt and mis-classification of animals in relation to fitness for transport, the concept should be properly defined, professional groups (including farmers, stockpersons, drivers, hauliers, inspectors and veterinarians) should be well-educated, and questions on responsibility between the groups should be clarified.

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

- In order to minimise handling stress and other WCs during loading and unloading, handlers (and their dogs) should be properly educated and trained in the use of non-coercive methods and tools.

- Loading and unloading facilities should be fit for purpose in order to avoid WCs such as injuries.
- Delays in loading may lead to WCs such as heat stress, and may further predispose sheep to WCs such as heat stress during the journey, and should thus be avoided.

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

A) Microclimatic conditions

- Among the environmental factors affecting the heat load placed on sheep during transport, it is recommended to take dry bulb temperature and at least relative humidity into account when sheep 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, sheep should be transported in their TCZ, the upper threshold of which is estimated to be 25°C. In order to reduce the risk of WCs due to exposure to high effective temperatures, the temperature inside vehicles transporting sheep should not exceed the UCT estimated to be 28°C for fleeced sheep, and 32°C for shorn sheep.
- 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 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 TCZ. Technical issues (e.g. accuracy, maintenance, placement, reliability and calibration) relating to this improved approach will need to be addressed.
- Future research should be carried out to support informed decision-making on the protection of the welfare of sheep during transport, within:
 - Development of systems to maintain the microclimatic conditions in stationary as well as moving vehicles across different compartments and deck heights by, e.g. air conditioning.

B) Space requirements

- In order to protect the welfare of sheep during transport, it is recommended to use the allometric equation $A = kW^{2/3}$ to calculate the minimum space allowance.
- In order for the sheep to be able to adjust to acceleration events, and lie down during journeys, it is recommended that the minimum space allowance should be based on a k-value of at least 0.037.
- Research should establish evidence-based thresholds for vertical height. The space above the highest point of the animals is recommended to be at least 15 cm in vehicles with mechanical ventilation and 30 cm in naturally ventilated vehicles.
- Future research is recommended in the following areas in order to support informed decision-making on the protection of the welfare of sheep during transport:
 - Determine the effect of different space allowances, as based on the biological functions of sheep during transport and the minimum space requirement to perform these functions as determined by k-values, on the behaviour and pathophysiological condition of sheep during transport. In particular, research should explore the ability of sheep to lie, to stabilise themselves and to thermoregulate during journeys of long and short duration.
 - Determine the space required for sheep to consume water during journeys, including the location of drinkers, the drinker type, the sheep to drinker ratio, and the behaviour of sheep when accessing drinkers.
 - Determine vehicle design components, including the deck height and ventilation capacities to protect the welfare of sheep.

C) Journey times during road transport of sheep

- The number and the severity of hazards that animals are exposed to during transport influence the resultant WCs, but on the basis of evidence on continuous WCs 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 journey potentially leading to fatigue, fear and distress;
 - The pain and/or discomfort due to health conditions might be relatively rare but when it happens, the consequences can be severe, and will worsen over time;
 - Resting problem severity is expected to increase with increasing duration and may lead to fatigue;
 - Physiological changes that are likely to be associated with thirst have been identified after 12 hours of transport;
 - Physiological changes indicative of hunger can be present after 12 hours of transport.
- Future research is recommended in the following areas:
 - Investigation of relationships between journey time, journey conditions and ABMs considered to reflect affective states of sheep for all animal categories, including knowledge about the progressively developing WCs 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 for journey breaks and control posts

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

- Sheep should be unloaded from the vehicle to effectively provide food, water and rest.
- Avoid mixing sheep from different vehicle compartments in a CP.
- Keep the microclimatic conditions in the CP in a way where the sheep can only experience their TCZ.
- Feed should be provided for *ad libitum* intake and all sheep should be able to eat simultaneously and have free access to drinkers and fresh water at all times.
- Animals showing signs of weakness or disease should be inspected and treated accordingly. Animals unfit for further transport should not follow the consignment at re-loading, 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 CP (including unloading and reloading) leads to WCs, it is recommended that the number of times that sheep stay in a CP should be as low as possible.
- Future research in the following areas are recommended in order to support informed decision-making on the protection of the welfare of sheep during transport:
 - Examine whether CPs fulfil their function, and how they should be designed and managed in order to protect sheep welfare.
 - Scientifically define an appropriate duration of stays at CPs.

5.6. Recommendations for transport of goats

- When goats are transported, focus should be given to the prevention of the hazards that are generic to transport, and thereby to prevent that goats experience WCs such as resting problems, heat stress, group stress, prolonged hunger, prolonged thirst, motion stress and handling stress.
- Handling and restraining systems should be fit for goats, and handlers should be educated and trained in goat handling.
- Repeated regrouping should be avoided and horned and hornless goats should be kept separated.
- In order to reduce the risk of WCs due to exposure to high effective temperatures, the temperature inside vehicles transporting goats should not exceed the UCT estimated to be 30°C.
- Until evidence-based thresholds are established for goats, alignment with the suggested space allowance and journey time for sheep is recommended.

5.7. Recommendations for specific scenarios

A) Export by road

- Due to the similarity in the WCs and hazards alignment with the recommendations for road transport (see Sections 3.3, 3.4, 3.5 and 3.6) is recommended.

B) Export by livestock vessel

- Until evidence-based thresholds are established for livestock vessels, alignment with the recommendations made for microclimatic conditions and space allowance for sheep are recommended.
- Sufficient ventilation on the deck where the animals are located should be ensured.
- Transporters must ensure that they have contingency plans in case of emergencies e.g., disease outbreaks, fire, refusal to unload at port of destination.
- Animals should not be shipped when the effects of weather conditions anticipated for the voyage are likely to cause them injury or suffering.
- Research to be able to evaluate the welfare of sheep when transported in livestock vessels is recommended.

C) Roll-on-roll-off ferries

- 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 they have 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 stabilized.
- 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.

D) Transport by air and rail

- Until evidence-based thresholds are established for these means of transport, alignment with the recommendations for road transport is recommended.

E) Special health status

- 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, except for journey breaks. Research is needed to develop trucks and procedures, including stationary resting periods, to ensure that the welfare of sheep during this type of transport is protected.

References

- AAWSG (Australian Animal Welfare Standards and Guidelines), 2012. Land Transport of Livestock. Available online: <https://www.animalwelfarestandards.net.au/files/2021/06/Land-transport-of-livestock-Standards-and-Guidelines-Version-1.-1-21-September-2012.pdf>
- Acharya RM, Gupta UD, Sehgal JP and Singh M, 1995. Coat characteristics of goats in relation to heat tolerance in the hot tropics. *Small Ruminant Research*, 18, 245–248.
- Addison WE and Baker E, 1982. Agonistic behavior and social organization in a herd of goats as affected by the introduction of non-members. *Applied Animal Ethology*, 8, 527–535.
- Akin PD, Yilmaz, A and Ekiz B, 2018. Effects of stocking density on stress responses and meat quality characteristics of lambs transported for 45 minutes or 3 hours. *Small Ruminant Research*, 169, 134–139. <https://doi.org/10.1016/j.smallrumres.2018.08.009>
- Alberta Farm Animal Care Association and Alberta Lamb Producers, 2019. Humane handling guidelines for sheep. Standards for the care of compromised and unfit animals. High River, Alberta, Canada. Available online: https://www.afac.ab.ca/wp-content/uploads/2019/01/HHG_Sheep-low-res.pdf
- Alexander G, 1974. 9 - Heat loss from sheep. In: Monteith JL and Mount LE (eds.). *Heat Loss from Animals and Man*. Butterworth-Heinemann. London, England. pp. 173–203.

- Alley JC and Fordham RA, 1994. Social events following the introduction of unfamiliar does to a captive feral goat (*Capra hircus* L.) herd. *Small Ruminant Research*, 13, 103–107.
- Al-Mufarrej S, Al-Haidary I, Al-Kraidees M, Hussein MF and Metwally HM, 2008. Effect of chromium dietary supplementation on the immune response and some blood biochemical parameters of transport-stressed lambs. *Asian-Australasian Journal of Animal Sciences*, 21, 671–676.
- Alvarez L, Zarco L, Galindo F, Blache D and Martin GB, 2007. Social rank and response to the “male effect” in the Australian Cashmere goat. *Animal Reproduction Science*, 102, 258–266.
- Andanson S, Boissy A and Veissier I, 2020. Conditions for assessing cortisol in sheep: the total form in blood v. the free form in saliva. *Animal*, 14, 1916–1922.
- Andersen IL and Bøe KE, 2007. Resting pattern and social interactions in goats—The impact of size and organisation of lying space. *Applied Animal Behaviour Science*, 108, 89–103.
- Aoyama M, Negishi A, Abe A, Maejima Y and Sugita S, 2003. Sex differences in stress response to transportation in goats: effect of gonadal hormones. *Animal Science Journal*, 74, 511–519.
- Aradom S, 2013. *Animal transport and welfare with special emphasis on transport time and vibration* (Vol. 2013, No. 2013: 98).
- Ashkenazy S and DeKeyser Ganz F, 2019. The differentiation between pain and discomfort: a concept analysis of discomfort. *Pain Management Nursing*, 20, 556–562.
- Asplund JM and Pfandes WH, 1972. Effects of water restriction on nutrient digestibility in sheep receiving fixed water: feed ratios. *Journal of Animal Science*, 35, 1271–1274.
- Atkinson PJ, 1992. Investigation of the effects of transport and lairage on hydration state and resting behaviour of calves for export. *The Veterinary Record*, 130, 413–416.
- AWC (Animal Welfare Committee), 2020. Opinion on the welfare of goats at the time of killing. Available online: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/903438/AWC_Opinion_on_the_welfare_of_goats_at_the_time_of_killing.pdf
- Ayo JO, Minka NS and Mamman M, 2006. Excitability scores of goats administered ascorbic acid and transported during hot-dry conditions. *Journal of Veterinary Science*, 7, 127–131.
- Baile CA and Della-Fera M, 1981. Nature of hunger and satiety control systems in ruminants. *Journal of Dairy Science*, 64, 1140–1152.
- Barroso FG, Alados CL and Boza J, 2000. Social hierarchy in the domestic goat: effect on food habits and production. *Applied Animal Behaviour Science*, 69, 35–53.
- Battini M, Barbier S, Fioni L and Mattiello S, 2016. Feasibility and validity of animal-based indicators for on-farm welfare assessment of thermal stress in dairy goats. *International Journal of Biometeorology*, 60, 289–296.
- Baxter MR, 1992. The Space Requirements of Housed Livestock. In: Phillips C and Piggins D (eds.). *Farm Animals and the Environment*. CAB International, Wallingford. pp. 67–81.
- Beatty DT, Barnes A, Fleming PA, Taylor E and Maloney SK, 2008. The effect of fleece on core and rumen temperature in sheep. *Journal of Thermal Biology*, 33, 437–443.
- Beausoleil NJ, Stafford KJ and Mellor DJ, 2005. Sheep show more aversion to a dog than to a human in an arena test. *Applied Animal Behaviour Science*, 91, 219–232.
- Blackshaw JK and Blackshaw AW, 1994. Heat stress in cattle and the effect of shade on production and behaviour: a review. *Australian Journal of Experimental Agriculture*, 34, 285–295.
- Blaxter KL, 1962. The fasting metabolism of adult wether sheep. *The British Journal of Nutrition*, 16, 615–626.
- Blaxter KL, Graham NM, Wainman FW and Armstrong DG, 1959a. Environmental temperature, energy metabolism and heat regulation in sheep. II. The partition of heat losses in closely clipped sheep. *The Journal of Agricultural Science*, 52, 25–40.
- Blaxter KL, Graham NM and Wainman FW, 1959b. Environmental temperature, energy metabolism and heat regulation in sheep. III. The metabolism and thermal exchanges of sheep with fleeces. *The Journal of Agricultural Science*, 52, 41–49.
- Boada-Saña M, Kulikowska K, Baumgärtner I, Romańska M and Dronijc T, 2021. Research for ANIT Committee – Animal welfare on sea vessels and criteria for approval of livestock authorisation, European Parliament, Policy Department for Structural and Cohesion Policies, Brussels. Available online: https://www.europarl.europa.eu/meetdocs/2014_2019/plmrep/COMMITTEES/ANIT/DV/2021/05-25/Report_Boada_Animalwelfareonseavessels_EN.pdf
- Bøe KE, Berg S and Andersen IL, 2006. Resting behaviour and displacements in ewes—effects of reduced lying space and pen shape. *Applied Animal Behaviour Science*, 98, 249–259.
- Bohmanova J, Misztal I and Cole JB, 2007. Temperature-humidity indices as indicators of milk production losses due to heat stress. *Journal of Dairy Science*, 90, 1947–1956.
- Boissy A, 1995. Fear and fearfulness in animals. *The Quarterly Review of Biology*, 70, 165–191.
- Boivin X and Braastad BO, 1996. Effects of handling during temporary isolation after early weaning on goat kids’ later response to humans. *Applied Animal Behaviour Science*, 48, 61–71.
- Braastad BO, 1998. Effects of prenatal stress on behaviour of offspring of laboratory and farmed mammals. *Applied Animal Behaviour Science*, 61, 159–180.

- Bracke MBM, Herskin MS, Marahrens M, Gerritzen MA and Spoolder HAM, 2020. Review of climate control and space allowance during transport of pigs. EU Reference Center for Animal Welfare (EURCAW). Pigs. Available online: <https://edepot.wur.nl/515292>
- Briefer EF, Haque S, Baciadonna L and McElligott AG, 2014. Goats excel at learning and remembering a highly novel cognitive task. *Frontiers in Zoology*, 11, 1–12.
- Broom DM, 2006. Behaviour and welfare in relation to pathology. *Applied Animal Behaviour Science*, 97, 73–83.
- Broom DM, Goode JA, Hall SJG, Lloyd DM and Parrott RF, 1996. Hormonal and physiological effects of a 15 hour road journey in sheep: comparison with the responses to loading, handling and penning in the absence of transport. *The British Veterinary Journal*, 152, 593–604. [https://doi.org/10.1016/S0007-1935\(96\)80011-X](https://doi.org/10.1016/S0007-1935(96)80011-X)
- Burnard CL, Pitchford WS, Edwards JH and Hazel SJ, 2015. Facilities, breed and experience affect ease of sheep handling: the livestock transporter's perspective. *Animal*, 9, 1379–1385.
- Cain KPR, Rosenstock SS and Turner JC, 2006. Mechanisms of thermoregulation and water balance in desert ungulates. *Wildlife Society Bulletin*, 34, 570–581.
- Caldeira RM, Belo AT, Santos CC, Vazques MI and Portugal AV, 2007. The effect of body condition score on blood metabolites and hormonal profiles in ewes. *Small Ruminant Research*, 68, 233–241. <https://doi.org/10.1016/j.smallrumres.2005.08.027>
- Campbell SS and Tobler I, 1984. Animal sleep - a review of sleep duration across phylogeny. *Neuroscience and Biobehavioral Reviews*, 8, 269–300.
- Campo MM, Mur L, Fugita CA and Sañudo C, 2016. Current strategies in lamb production in Mediterranean areas. *Animal Frontiers*, 6, 31–36.
- Cañeque V, Velasco S, Díaz M, Pérez C, Huidobro F, Lauzurica S, Manzanares C and Gonzalez J, 2001. Effect of weaning age and slaughter weight on carcass and meat quality of Talaverana breed lambs raised at pasture. *Animal Science*, 73, 85–95.
- Carnovale F and Phillips CJ, 2020. The effects of heat stress on sheep welfare during live export voyages from Australia to the Middle East. *Animals*, 10, 694 pp.
- Caulfield MP, Cambridge H, Foster SF and McGreevy PD, 2014. Heat stress: a major contributor to poor animal welfare associated with long-haul live export voyages. *The Veterinary Journal*, 199, 223–228.
- Chanvallon A and Fabre-Nys C, 2009. In sexually naive anestrus ewes, male odour is unable to induce a complete activation of olfactory systems. *Behavioural Brain Research*, 205, 272–279.
- Cockram MS, 2007. Criteria and potential reasons for maximum journey times for farm animals destined for slaughter. *Applied Animal Behaviour Science* 106, 234–243.
- Cockram MS, 2019a. Sheep transport. In: Grandin T (ed). *Livestock handling and transport*. CABI. pp. 239–253.
- Cockram MS, 2019b. Fitness of animals for transport to slaughter. *Canadian Veterinary Journal*, 60, 423–429.
- Cockram M, 2020a. Welfare issues at slaughter. In: Grandin T and Cockram M (eds). *The Slaughter of Farmed Animals: Practical ways of enhancing animal welfare*. CABI Publishing, Wallingford, Oxon, UK. pp. 5–34.
- Cockram M, 2020b. Condition of animals on arrival at the abattoir and their management during lairage. In: Grandin T and Cockram M (eds.). *The Slaughter of Farmed Animals: Practical ways of enhancing animal welfare*. CABI Publishing, Wallingford, Oxon, UK. pp. 49–77.
- Cockram MS and Hughes BO, 2018. Chapter 8. Health and Disease. In: Appleby MC, Olsson IAS, Galindo F (eds.), *Animal Welfare*, 3rd Edition. CABI, Wallingford, UK. pp. 141–159.
- Cockram MS and Mitchell MA, 1999. Role of research in the formulation of 'rules' to protect the welfare of farm animals during road transportation. *Occasional Publication - British Society of Animal Science*, 23, 43–64.
- Cockram MS and Velarde A, 2022. Sheep. In: Faucitano L (ed.). *Preslaughter Handling of Livestock*, 1st Edition. Wageningen Academic Publishers, Wageningen, the Netherlands. pp. 267–310.
- Cockram MS, Kent JE, Goddard PJ, Waran NK, McGilp IM, Jackson RE, Muwanga GM and Prytherch S, 1996. Effect of space allowance during transport on the behavioural and physiological responses of lambs during and after transport. *Animal Science*, 62, 461–477.
- Cockram MS, Kent JE, Jackson RE, Goddard PJ, Doherty OM, McGilp IM, Fox A, Studdert-Kennedy T, McConnell TI and O'Riordan T, 1997. Effect of lairage during 24 h of transport on the behavioural and physiological responses of sheep. *Animal Science*, 65, 391–402.
- Cockram MS, Kent JE, Waran NK, McGilp IM, Jackson RE, Amory JR, Southall EL, O'Riordan T, McConnell TI and Wilkins BS, 1999. Effects of a 15h journey followed by either 12 h starvation or ad libitum hay on the behaviour and blood chemistry of sheep. *Animal Welfare* 8, 135–148.
- Cockram MS, Kent JE, Goddard PJ, Waran NK, Jackson RE, McGilp IM, Southall EL, Amory JR, McConnell TI, O'Riordan T and Wilkins BS, 2000. Behavioural and physiological responses of sheep to 16 h transport and a novel environment post-transport. *Veterinary Journal*, 159, 139–146. <https://doi.org/10.1053/tvj.1999.0411>
- Cockram MS, Baxter EM, Smith LA, Bell S, Howard CM, Prescott RJ, Mitchell MA, 2004. Effect of driver behaviour, driving events and road type on the stability and resting behaviour of sheep in transit. *Animal Science*, 79, 165–176.
- Cockram MS, Murphy E, Ringrose S, Wemelsfelder F, Miedema HM and Sandercock DA, 2012. Behavioural and physiological measures following treadmill exercise as potential indicators to evaluate fatigue in sheep. *Animal*, 6, 1491–1502.

- Collins T, Stockman CA, Barnes AL, Miller DW, Wickham SL and Fleming PA, 2018. Quali-tative behavioural assessment as a method to identify potential stressors during commercial sheep transport. *Animals*, 8, 209 pp.
- Collins T, Stockman C, Hampton JO and Barnes A, 2020. Identifying animal welfare impacts of livestock air transport. *Australian Veterinary Journal*, 98, 197–199.
- Consortium of the Animal Transport Guides Project, 2018. Guide to good practices for the transport of sheep. Available online <https://animaltransportguides.eu/wp-content/uploads/2016/05/EN-Guides-Sheep-final.pdf>
- Constantinou A, 1987. Goat housing for different environments and production systems. Proceedings, Fourth International Conference on Goats, Brasilia, Brazil, EMBRAPA, 1, 241–268.
- Cozar A, Rodriguez AI, Garijo P, Calvo L and Vergara H, 2016. Effect of space allowance during transport and fasting or nonfasting during lairage on welfare indicators in merino lambs. *Spanish Journal of Agricultural Research*, 14. pp. e0501–e0501. <https://doi.org/10.5424/sjar/2016141-8313>
- Cunningham JG, 2002. Textbook of Veterinary Physiology, Third ed. Edited by JG Cunningham, Saunders, Philadelphia. The Veterinary Journal, 1, 90–91.
- da Cunha Leme TM, Titto EAL, Titto CG, Amadeu CCB, Neto PF, Vilela RA and Pereira AMF, 2012. Influence of transportation methods and pre-slaughter rest periods on cortisol level in lambs. *Small Ruminant Research*, 107, 8–11.
- Da Silva RG, LaScala N, Lima Filho A and Catharin M, 2002. Respiratory heat loss in the sheep: a comprehensive model. *International Journal of Biometeorology*, 46, 136–140.
- Dahl-Pedersen K, 2022. Danish cattle farmers' experience with fitness for transport—A questionnaire survey. *Frontiers in Veterinary Science*, 9.
- Dahl-Pedersen K, Foldager L, Herskin MS, Houe H and Thomsen PT, 2018. Lameness scoring and assessment of fitness for transport in dairy cows: agreement among and between farmers, veterinarians and livestock drivers. *Research in Veterinary Science*, 119, 162–166.
- Dalla Costa FA, Lopes LS and Dalla Costa OA, 2017. Effects of the truck suspension system on animal welfare, carcass and meat quality traits in pigs. *Animals*, 7, 5 pp. <https://doi.org/10.3390/ani7010005>
- Dalmáu A, Di Nardo A, Realini CE, Rodríguez P, Llonch P, Temple D, Velarde A, Giansante D, Messori S and Villa PD, 2014. Effect of the duration of road transport on the physiology and meat quality of lambs. *Animal Production Science*, 54, 179–186. <https://doi.org/10.1071/AN13024>
- Damián JP, de Soto L, Espindola D, Gil J and van Lier E, 2021. Intranasal oxytocin affects the stress response to social isolation in sheep. *Physiology and Behavior*, 230, 113282
- de Araujo IET, Kringelbach ML, Rolls ET and McGlone F, 2003. Human cortical responses to water in the mouth, and the effects of thirst. *Journal of Neurophysiology*, 90, 1865–1876. <https://doi.org/10.1152/jn.00297.2003>
- des Bois R, 2021. 78 EU-approved livestock carriers Animal Welfare Foundation, Tierschutzbund Zuerich. Available online: https://www.animal-welfare-foundation.org/files/downloads/78_EU_livestock_carriers_June_2021_RobindesBois_AWF_TSB-1.pdf
- de Castro Júnior SL and Silva IJOD, 2021. The specific enthalpy of air as an indicator of heat stress in livestock animals. *International Journal of Biometeorology*, 65, 149–161.
- D'Eath RB, Tolkamp BJ, Kyriazakis I, Lawrence AB, 2009. 'Freedom from hunger' and preventing obesity: the animal welfare implications of reducing food quantity or quality. *Animal Behaviour*, 77, 275–288. <https://doi.org/10.1016/j.anbehav.2008.10.028>
- Department for Transport, United Kingdom, 2014. Simplified guidelines. Simplified guidance on European Union (EU) rules on drivers' hours and working time. Available online: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/856360/simplified-guidance-eu-drivers-hours-working-time-rules.pdf
- DG SANTE (Directorate General for Health and Food Safety), 2019. Overview report on welfare of animals exported by road. Available online: https://ec.europa.eu/food/audits-analysis/overview_reports/act_getPDF.cfm?PDF_ID=1520
- Dikmen S, Orman A and Ustuner H, 2011. The effect of shearing in a hot environment on some welfare indicators in Awassi lambs. *Tropical Animal Health and Production*, 43, 1327–1335.
- Dos Santos DDS, Klauck V, Campigotto G, Alba DF, Dos Reis JH, Gebert RR, Souza CF, Baldissera MD, Schogor ALB, Santos ID and Wagner R, 2019. Benefits of the inclusion of açai oil in the diet of dairy sheep in heat stress on health and milk production and quality. *Journal of Thermal Biology*, 84, 250–258.
- Duvaux-Ponter C, Roussel S, Tessier J, Sauviant D, Ficheux C and Boissy A, 2003. Physiological effects of repeated transport in pregnant goats and their offspring. *Animal Research*, 52, 553–566.
- Dwyer CM, 2008. Environment and the sheep. In *The welfare of sheep* (pp. 41–79). Springer, Dor-drecht.
- Dwyer CM, 2009. Welfare of sheep: Providing for welfare in an extensive environment. *Small Ruminant Research*, 86, 14–21.
- Dwyer C, 2017. Reproductive management (including impacts of prenatal stress on offspring development). *Advances in Sheep Welfare*. Woodhead Publishing, Duxford, UK. pp. 131–152.

- EFSA (European Food Safety Authority), 2004. Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to the Standards for the microclimate inside animal road transport vehicles. *EFSA Journal* 2004;122, 1–25 pp. <https://doi.org/10.2903/j.efsa.2004.122>
- EFSA AHAW Panel (EFSA Panel on Animal Health and Animal Welfare), More S, Bicout D, Botner A, Butterworth A, Calistri P, Depner K, Edwards S, Garin-Bastuji B, Good M, Gortazar Schmidt C, Michel V, Miranda MA, Saxmose Nielsen S, Velarde A, Thulke H-H, Sihvonen L, Spoolder H, Stegeman JA, Raj M, Willeberg P, Candiani D and Winckler C, 2017. Scientific Opinion on the animal welfare aspects in respect of the slaughter or killing of pregnant livestock animals (cattle, pigs, sheep, goats, horses). *EFSA Journal* 2017; 15(5):4782, 96 pp. <https://doi.org/10.2903/j.efsa.2017.4782>
- EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare Panel), 2012. Guidance on risk assessment for animal welfare. *EFSA Journal* 2012;10(1):2513, 30 pp. <https://doi.org/10.2903/j.efsa.2012.2513>
- EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2011. Scientific Opinion concerning the welfare of animals during transport. *EFSA Journal* 2011;9(1):1966, 125 pp. [10.2903/j.efsa.2011.1966](https://doi.org/10.2903/j.efsa.2011.1966)
- EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), Nielsen SS, Alvarez J, Bicout DJ, Calistri P, Depner K, Drewe JA, Garin-Bastuji B, Gonzales Rojas JL, Gortazar Schmidt C, Michel V, Miranda Chueca MA, Roberts HC, Sihvonen LH, Spoolder H, Stahl K, Velarde A, Viltrop A, Candiani D, Van der Stede Y and Winckler C, 2020. Scientific Opinion on the welfare of cattle at slaughter. *EFSA Journal* 2020;18(11):6275, 107 pp. <https://doi.org/10.2903/j.efsa.2020.6275>
- EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), Nielsen SS, Alvarez J, Bicout DJ, Calistri P, Canali E, Drewe JA, Garin-Bastuji B, Gonzales Rojas JL, Gortazar Schmidt C, Herskin M, Miranda Chueca MÁ, Padalino B, Pasquali P, Roberts HC, Spoolder H, Stahl K, Velarde A, Viltrop A, Winckler C, Candiani D, Rapagnà C, Van der Stede Y and Michel V, 2021. Scientific Opinion on the welfare of sheep and goats at slaughter. *EFSA Journal* 2021;19(11):6882, 111 pp. <https://doi.org/10.2903/j.efsa.2021.6882>
- EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), Nielsen SS, Alvarez J, Bicout DJ, Calistri P, Canali E, Drewe JA, Garin-Bastuji B, Gonzales Rojas JL, Gortazar Schmidt C, Herskin M, Miranda Chueca MA, Michel V, Padalino B, Pasquali P, Roberts HC, Spoolder H, Stahl K, Velarde A, Viltrop A, Edwards S, Sean A, Candiani S, Fabris C, Lima E, Mosbach-Schulz O, Rojo Gimeno C, Van der Stede Y, Vitali M and Winckler C, 2022a. Scientific Opinion on the methodological guidance for the development of animal welfare mandates in the context of the Farm To Fork Strategy. *EFSA Journal* 2022;20(7):7403, 45 pp. <https://doi.org/10.2903/j.efsa.2022.7403>
- EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), Nielsen SS, Alvarez J, Bicout DJ, Calistri P, Canali E, Drewe JA, Garin-Bastuji B, Gonzales Rojas JL, Gortazar Schmidt C, Herskin M, Miranda Chueca MA, Michel V, Padalino B, Pasquali P, Roberts HC, Spoolder H, Stahl K, Velarde A, Viltrop A, Winckler C, Mitchell L, Vinco LJ, Voslarova E, Candiani D, Mosbach-Schulz O, Van der Stede Y and Velarde A, 2022b. Welfare of domestic birds and rabbits transported in containers. *EFSA Journal* 2022;7441, 187 pp. <https://doi.org/10.2903/j.efsa.2022.7441>
- EFSA Scientific Committee, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Younes M, Craig P, Hart A, Von Goetz N, Koutsoumanis K, Mortensen A, Ossendorp B, Germini A, Martino L, Merten C, Mosbach-Schulz O, Smith A and Hardy A, 2018. Scientific Opinion on the principles and methods behind EFSA's Guidance on Uncertainty Analysis in Scientific Assessment. *EFSA Journal* 2018;16(1):5122, 235 pp. <https://doi.org/10.2903/j.efsa.2018.5122>
- Ekiz B, Ekiz EE, Yalcintan H, Kocak O and Yilmaz A, 2012. Effects of suckling length (45, 75 and 120 d) and rearing type on cortisol level, carcass and meat quality characteristics in Kivircik lambs. *Meat Science*, 92, 53–61.
- Erhard HW, Fàbrega E, Stanworth G and Elston DA, 2004. Assessing dominance in sheep in a competitive situation: level of motivation and test duration, *Applied Animal Behaviour Science*, 85, 277–292. <https://doi.org/10.1016/j.applanim.2003.09.013>
- ESOTC (European State of the Climate), 2021. Mediterranean summer extremes. Copernicus Climate Change Service (C3S). Available online: <https://climate.copernicus.eu/esotc/2021/mediterranean-summer-extremes>
- European Commission, 2020. Welfare of animals transported by sea. Overview report. <https://doi.org/10.2875/47273>
- Faurie AS, Mitchell D and Laburn HP, 2004. Peripartum body temperatures in free-ranging ewes (*ovis aries*) and their lambs. *Journal of Thermal Biology*, 29, 115–122.
- Fisher A and Matthews L, 2001. The social behaviour of sheep. In: Keeling LJ and Gonyou HW (ed.). *Social Behaviour in Farm Animals*. CABI International, Wallingford, Oxfordshire, UK, pp. 211–245.
- Fisher AD, Stewart M, Tacon J and Matthews LR, 2002. The effects of stock crate design and stocking density on environmental conditions for lambs on road transport vehicles. *New Zealand Veterinary Journal*, 50, 148–153.
- Fisher AD, Colditz IG, Lee C and Ferguson DM, 2009. The influence of land transport on animal welfare in extensive farming systems. *Journal of Veterinary Behavior: Clinical Applications and Research*, 4, 157–162. <https://doi.org/10.1016/j.jveb.2009.03.002>
- Fisher AD, Niemeyer DO, Lea JM, Lee C, Paull DR, Reed MT and Ferguson DM, 2010. The effects of 12, 30, or 48 hours of road transport on the physiological and behavioral responses of sheep. *Journal of Animal Science*, 88, 2144–2152.

- Franchi GA, Herskin MS and Jensen MB, 2019. Dairy cows fed a low energy diet before dry-off show signs of hunger despite ad libitum access. *Scientific Reports*, 9, 1–9.
- Gardner GE, McGilchrist P and Pethick DW, 2014. Ruminant glycogen metabolism. *Animal Production Science*, 54, 1575–1583.
- Gautam M, Stevenson MA, Lopez-Villalobos N and McLean V, 2017. Risk factors for culling, sales and deaths in New Zealand dairy goat herds, 2000–2009. *Frontiers in Veterinary Science*, 4, 191.
- Ghassemi Nejad J and Sung K, 2017. Behavioral and physiological changes during heat stress in Corrie-dale ewes exposed to water deprivation. *Journal of Animal Science and Technology* 59(1), 1–6. <https://doi.org/10.1186/s40781-017-0140-x>
- González LA, Schwartzkopf-Genswein K, Bryan M, Silasi R and Brown F, 2012. Factors affecting body weight loss during commercial long haul transport of cattle in North America. *Journal of Animal Science*, 90, 3630–3639. <https://doi.org/10.2527/jas.2011-4786>
- Goodwin SD, 1998. Comparison of body temperatures of goats, horses, sheep measured with a tympanic infrared thermometer, an implantable microchip transponder, and a rectal thermometer. *Contemporary Topics in Laboratory Animal Science*, 37, 51–55.
- Government of Canada, 2020. Health of Animals Regulations: Part XII. Interpretive Guidance for Regulated Parties. Available online: <https://inspection.canada.ca/animal-health/humane-transport/health-of-animals-regulations-part-xii/eng/1582126008181/1582126616914>
- Government of Canada (CHAR), 2022. Health of Animals Regulations (CRC, c. 296). Available online: https://www.laws-lois.justice.gc.ca/eng/regulations/C.R.C.,_c._296/index.html
- Grandin T, 2001. Perspectives on transportation issues: The importance of having physically fit cattle and pigs. *Journal of Animal Science*, 79, E201-E207.
- Grandin T, 2019. *Livestock Handling and Transport*. 5th Edition. CABI Publishers. Wallingford, Oxfordshire, UK| Boston, MA.
- Grandin T and Shivley C, 2015. How farm animals react and perceive stressful situations such as handling, restraint, and transport. *Animals (Basel)*.
- Gregory NG, Benson T, Smith N and Mason CW, 2009. Sheep handling and welfare standards in livestock markets in the UK. *The Journal of Agricultural Science*, 147, 333–344.
- Hales JRS and Webster MED, 1967. Respiratory function during thermal tachypnoea in sheep. *The Journal of Physiology*, 190, 241–260.
- Hall SJG, Schmidt B and Broom DM, 1997. Feeding behaviour and the intake of food and water by sheep after a period of deprivation lasting 14 hours. *Animal Science*, 64, 105–110.
- Hall SJG, Kirkpatrick SM and Broom DM, 1998. Behavioural and physiological responses of sheep of different breeds to supplementary feeding, social mixing and taming, in the context of transport. *Animal Science*, 67, 475–483.
- Herbut P, Angrecka S and Walczak J, 2018. Environmental parameters to assessing of heat stress in dairy cattle—a review. *International Journal of Biometeorology*, 62, 2089–2097.
- Herrtage ME, Saunders RW and Terlecki S, 1974. Physical examination of cull ewes at point of slaughter. *Veterinary Record*, 95, 257–260. <https://doi.org/10.1136/vr.95.12.257>
- Herskin MS, Hels A, Anneberg I and Thomsen PT, 2017. Livestock drivers' knowledge about dairy cow fitness for transport—A Danish questionnaire survey. *Research in Veterinary Science*, 113, 62–66.
- Herskin MS, Aaslyng MD, Anneberg I, Thomsen PT, Gould LM and Thodberg K, 2020. Significant variation in the management of cull sows before transport for slaughter: results from a survey of Danish pig farmers. *Veterinary Record*, 186, 185 pp.
- Herskin MS, Gerritzen MA, Marahrens M, Bracke MBM and Spoolder HAM, 2021. Review of fitness for transport of pigs. EU Reference Center for Animal Welfare (EURCAW). Pigs. Available online: <https://edepot.wur.nl/546057>
- Horton GMJ, Baldwin JA, Emanuele SM, Wohlt JE and McDowell LR, 1996. Performance and blood chemistry in lambs following fasting and transport. *Animal Science*, 62, 49–56.
- Houpt KA, 2005. *Domestic Animal Behavior for Veterinarians and Animal Scientists*. 4th edn. Blackwell Publishing, Ames, IA, USA.
- Humane slaughter association, 2022. Transport of Livestock. Online guide. Available online: <https://www.hsa.org.uk/transport-of-livestock-introduction/introduction-8>
- Jackson RE, Waran NK and Cockram MS, 1999. Methods for measuring feeding motivation in sheep. *Animal Welfare*, 8, 53–63.
- Jacob RH, Pethick DW, Ponnampalam E, Speijers J and Hopkins DL, 2006. The hydration status of lambs after lairage at two Australian abattoirs. *Australian Journal of Experimental Agriculture*, 46, 909–912. <https://doi.org/10.1071/EA05327>
- Jarvis AM and Cockram MS, 1995. Some factors affecting resting behaviour of sheep in slaughterhouse lairages after transport from farms. *Animal Welfare*, 4, 53–60.
- Jensen MB and Vestergaard M, 2021. Invited review: Freedom from thirst—Do dairy cows and calves have sufficient access to drinking water?. *Journal of Dairy Science*, 104, 11368–11385.
- Jones TA, Look A, Guise HJ and Lomas MJ, 2002. Head height requirements, and assessing stocking density, for sheep in transit. *The Veterinary Record*, 150, 49–50.

- Jones TA, Waitt C and Dawkins MS, 2010. Sheep lose balance, slip and fall less when loosely packed in transit where they stand close to but not touching their neighbours. *Applied Animal Behaviour Science*, 123, 16–23.
- Jongman EC, Edge MK, Butler KL and Cronin GM, 2008. Reduced space allowance for adult sheep in lairage for 24 hours limits lying behaviour but not drinking behaviour. *Australian Journal of Experimental Agriculture*, 48, 1048–1051. <https://doi.org/10.1071/EA08039>
- Joy A, Dunshea FR, Leury BJ, Clarke IJ, DiGiacomo K and Chauhan SS, 2020. Resilience of small ruminants to climate change and increased environmental temperature: a review. *Animals*, 10, 867 pp.
- Jubb T and Perkins NR, 2015. *Veterinary Handbook for Cattle, Sheep and Goats*. Australia. Available online: <https://www.veterinaryhandbook.com.au/>
- Kadim IT, Mahgoub O, Al-Kindi A, Al-Marzooqi W and Al-Saqri N, 2006. Effects of transportation at high ambient temperatures on physiological responses, carcass and meat quality characteristics of three breeds of Omani goats. *Meat Science*, 73, 626–634.
- Kadim IT, Mahgoub O and Khalaf S, 2014. Effects of the transportation during hot season and electrical stimulation on meat quality characteristics of goat *Longissimus dorsi* muscle. *Small Ruminant Research*, 121, 120–124.
- Kaler J and Green LE, 2008. Recognition of lameness and decisions to catch for inspection among sheep farmers and specialists in GB. *BMC Veterinary Research*, 4, 1–9 pp.
- Kaler J and Green LE, 2009. Farmers' practices and factors associated with the prevalence of all lameness and lameness attributed to interdigital dermatitis and footrot in sheep flocks in England in 2004. *Preventive Veterinary Medicine*, 92, 52–59. <https://doi.org/10.1016/j.prevetmed.2009.08.001>
- Kannan G, Terrill TH, Kouakou B, Gazal OS, Gelaye S, Amoah EA and Samake S, 2000. Transportation of goats: effects on physiological stress responses and live weight loss. *Journal of Animal Science*, 78, 1450–1457.
- Kannan G, Terrill TH, Kouakou B, Gelaye S and Amoah EA, 2002. Simulated preslaughter holding and isolation effects on stress responses and live weight shrinkage in meat goats. *Journal of Animal Science*, 80, 1771–1780.
- Kannan G, Kouakou B, Terrill TH and Gelaye S, 2003. Endocrine, blood metabolite, and meat quality changes in goats as influenced by short-term, preslaughter stress. *Journal of Animal Science*, 81, 1499–1507.
- Karaca S, Erdoğan S, Kor D, Kor A, 2016. Effects of pre-slaughter diet/management system and fasting period on physiological indicators and meat quality traits of lambs. *Meat Science*, 116, 67–77. <https://doi.org/10.1016/j.meatsci.2016.01.014>
- Katsu Y and Iguchi T, 2016. Cortisol. *Handbook of Hormones*. Academic Press, 533–e95D-2.
- Katz ML and Bergman EN, 1969. Hepatic and portal metabolism of glucose, free fatty acids, and ketone bodies in the sheep. *American Journal of Physiology-Legacy Content*, 216, 953–960.
- Kearnton TR, Doughty AK, Morton CL, Hinch GN, Godwin R and Cowley FC, 2020. Core and peripheral site measurement of body temperature in short wool sheep. *Journal of Thermal Biology*, 90, 102606 pp. <https://doi.org/10.1016/j.jtherbio.2020.102606>
- Kendrick KM, 1991. How the sheep's brain controls the visual recognition of animals and humans. *Journal of Animal Science*, 69, 5008 pp.
- Kendrick KM, 1992. Cognition. In: Phillips C and Piggins D (eds.), *Farm Animals and the Environment*. CAB International, Wallingford, UK. 209 pp.
- Kendrick KM and Baldwin BA, 1987. Cells in temporal cortex of conscious sheep can respond preferentially to the sight of faces. *Science*, 236, 448 pp.
- Kim FB, Jackson RE, Gordon GDH and Cockram MS, 1994. Resting behaviour of sheep in a slaughterhouse lairage. *Applied Animal Behaviour Science*, 40, 45–54. [https://doi.org/10.1016/0168-1591\(94\)90086-8](https://doi.org/10.1016/0168-1591(94)90086-8)
- Kingma BR, Frijns AJ, Schellen L and van Marken Lichtenbelt WD, 2014. Beyond the classic thermoneutral zone: including thermal comfort. *Temperature*, 1, 142–149.
- Knowles TG, 1998. A review of the road transport of slaughter sheep. *Veterinary Record*, 143, 212–219.
- Knowles TG, Warriss PD, Brown SN and Kestin SC, 1994. Long distance transport of export lambs. *The Veterinary Record*, 134, 107–110.
- Knowles TG, Brown SN, Warriss PD, Phillips AJ, Dolan SK, Hunt P, Ford JE, Edwards JE and Watkins PE, 1995. Effects on sheep of transport by road for up to 24 hours. *The Veterinary Record*, 136, 431–438.
- Knowles TG, Warriss PD, Brown SN, Kestin SC, Edwards JE, Perry AM, Watkins PE and Phillips AJ, 1996. Effects of feeding, watering and resting intervals on lambs transported by road and ferry to France. *The Veterinary Record*, 139, 335–339. <https://doi.org/10.1136/vr.139.14.335>
- Knowles TG, Warriss PD, Brown SN and Edwards JE, 1998. Effects of stocking density on lambs being transported by road. *The Veterinary Record*, 142, 503–509.
- König U, Nyman AJ and de Verdier K, 2011. Prevalence of footrot in Swedish slaughter lambs. *Acta Veterinaria Scandinavica*, 53, 27–31.
- Lees AM, Lees JC, Sejian V and Gaughan J, 2017. Management Strategies to Reduce Heat Stress in Sheep. *Sheep Production Adapting to Climate Change*. Springer, Singapore. pp. 349–370.
- Lees AM, Sullivan ML, Olm JCW, Cawdell-Smith AJ and Gaughan JB, 2019. A panting score index for sheep. *International Journal of Biometeorology*, 63, 973–978.
- Ley SJ, Livingston A and Waterman AE, 1989. The effect of chronic clinical pain on thermal and mechanical thresholds in sheep. *Pain*, 39, 353–357.

- Li J, Wijffels G, Yu Y, Nielsen LK, Niemeyer DO, Fisher AD, Ferguson DM and Schirra HJ, 2011. Altered fatty acid metabolism in long duration road transport: an NMR-based metabolomics study in sheep. *Journal of Proteome Research*, 10, 1073–1087.
- Linares MB, Bórnez R and Vergara H, 2008. Cortisol and catecholamine levels in lambs: effects of slaughter weight and type of stunning. *Livestock Science*, 115, 53–61.
- Llonch P, King EM, Clarke KA, Downes JM and Green LE, 2015. A systematic review of animal based indicators of sheep welfare on farm, at market and during transport, and qualitative appraisal of their validity and feasibility for use in UK abattoirs. *The Veterinary Journal*, 206, 289–297.
- Losada-Espinosa N, Miranda-de la Lama GC and Estévez-Moreno LX, 2020. Stockpeople and animal welfare: compatibilities, contradictions, and unresolved ethical dilemmas. *Journal of Agricultural and Environmental Ethics*, 33, 71–92.
- Lotgering FK, Gilbert RD and Longo LD, 1983. Exercise responses in pregnant sheep: oxygen consumption, uterine blood flow, and blood volume. *Journal of Applied Physiology*, 55, 834–841. <https://doi.org/10.1152/jappl.1983.55.3.834>
- Lovatt FM, 2010. Clinical examination of sheep. *Small Ruminant Research*, 92, 72–77.
- Marai IFM, El-Daraway AA, Fadiel A and Abdel-Hafez MAM, 2007. Physiological traits as affected by heat stress in sheep—a review. *Small Ruminant Research*, 71, 1–12. <https://doi.org/10.1016/j.smallrumres.2006.10.003>
- Marques RS, Cooke RF, Francisco CL and Bohnert DW, 2012. Effects of twenty-four hour transport or twenty-four hour feed and water deprivation on physiologic and performance responses of feeder cattle. *Journal of Animal Science*, 90, 5040–5046.
- Mason GJ and Burn CC, 2011. Behavioural Restriction. *Animal Welfare*, 2nd Edition. Appleby MC, Mencha JA, Olsson IA and Hughes BO (Eds). CAB International, 344 pp.
- McKinley MJ and Johnson AK, 2004. The physiological regulation of thirst and fluid intake. *News in Physiological Sciences*, 19, 1–6.
- McLaren A, McHugh N, Lambe NR, Pabiou T, Wall E and Boman IA, 2020. Factors affecting ewe longevity on sheep farms in three European countries. *Small Ruminant Research*, 189, 106145 <https://doi.org/10.1016/j.smallrumres.2020.106145>
- McMillan FD, 2020. What Is Distress? A Complex Answer to a Simple Question — Franklin D. McMillan. In: McMillan FD (ed.). *Mental Health and Well-being in Animals*, 2nd Edition. CAB International: Wallingford, UK.
- Menchetti L, Nanni Costa L, Zappaterra M and Padalino B, 2021. Effects of reduced space allowance and heat stress on behavior and eye temperature in unweaned lambs: a pilot study. *Animals*, 11, 3464 pp. <https://doi.org/10.3390/ani11123464>
- Messori S, Pedernera-Romano C, Magnani D, Rodriguez P, Barnard S, Dalmau A, Velarde A and Dalla Villa P, 2015b. Unloading or not unloading? Sheep welfare implication of rest stop at control post after a 29h transport. *Small Ruminant Research*, 130, 221–228. <https://doi.org/10.1016/j.smallrumres.2015.07.012>
- Messori S, Sossidou E, Buonanno M, Mounaix B, Barnard S, Vousdouka V, Dalla Villa P, De Roest K and Spooler H, 2015a. A pilot study to develop an assessment tool for sheep welfare after long journey transport. *Animal Welfare*, 24, 407–416. <https://doi.org/10.7120/09627286.24.4.407>
- Messori S, Pedernera-Romano C, Rodriguez P, Barnard S, Giansante D, Magnani D, Dalmau A, Velarde A and Dalla Villa P, 2017. Effects of different rest-stop durations at control posts during a long journey on the welfare of sheep. *Veterinaria Italiana*, 53, 121–129. <https://doi.org/10.12834/VetIt.316.1483.3>
- Minka NS and Ayo JO, 2007. Physiological responses of transported goats treated with ascorbic acid during the hot-dry season. *Animal Science Journal*, 78, 164–172.
- Minka NS and Ayo OJ, 2010. Serum biochemical activities and muscular soreness in transported goats administered with ascorbic acid during the hot-dry season. *European Journal of Translational Myology*, 20, 193–203.
- Minka NS and Ayo JO, 2011. Modulating effect of ascorbic acid on transport-induced immunosuppression in goats. *International Scholarly Research Notices*, 2011.
- Miranda-de la Lama GC, 2019. Goat handling and transport. *Livestock Handling and Transport*. 271 pp.
- Miranda-de la Lama GC, Villarroel M, Olleta JL, Alierta S, Sañudo C and María GA, 2009. Effect of the pre-slaughter logistic chain on meat quality of lambs. *Meat Science*, 83, 604–609.
- Miranda-de la Lama GC, Villarroel M, Liste G, Escós J and María GA, 2010. Critical points in the pre-slaughter logistic chain of lambs in Spain that may compromise the animal's welfare. *Small Ruminant Research*, 90, 174–178.
- Miranda-de la Lama GC, Monge P, Villarroel M, Olleta JL, García-Belenguer S and María GA, 2011. Effects of road type during transport on lamb welfare and meat quality in dry hot climates. *Tropical Animal Health and Production*, 43, 915–922.
- Miranda-de la Lama GC, Villarroel M and María GA, 2012. Behavioural and physiological profiles following exposure to novel environment and social mixing in lambs. *Small Ruminant Research*, 103, 158–163.
- Miranda-de la Lama GC, Villarroel M and María GA, 2014. Livestock transport from the perspective of the pre-slaughter logistic chain: a review. *Meat Science*, 98, 9–20.

- Miranda-de la Lama GC, Rodríguez-Palomares M, Cruz-Monterrosa RG, Rayas-Amor AA, Pin-heiro RSB, Galindo FM and Villarroel M, 2018. Long-distance transport of hair lambs: effect of location in pot-belly trailers on thermo-physiology, welfare and meat quality. *Tropical Animal Health and Production*, 50, 327–336.
- Miranda-de la Lama GC, Mamani-Linares W and Estevez-Moreno LX, 2022. Chapter 9: Species destined for nontraditional meat production: 2. Goats and south American domestic camelids. In: L Faucitano (ed). *Preslaughter handling and slaughter of meat animals*. Wageningen Academic Publishers, Netherlands. pp. 349–389.
- Mitchell MA, 2006. Using physiological models to define environmental control strategies. In: Gous EM, Morris TR and Fisher C (eds). *Mechanistic Modelling in Pig and Poultry Production*. CABI International, Wallingford, Oxfordshire, UK. pp. 209–228.
- Mitchell MA and Kettlewell PJ, 2008. Engineering and design of vehicles for long distance road transport of livestock (ruminants, pigs and poultry). *Veterinaria Italiana*, 44, 201–213.
- MLA (Meat & Livestock Australia and LiveCorp), 2011. Management of unfit-to-load transport. Sydney, Australia. 17 pp. Available online: <https://futurebeef.com.au/wp-content/uploads/2019/01/Management-of-unfit-to-load-livestock.pdf>
- MLA (Meat & Livestock Australia), 2019. Is the animal fit to load? A national guide to the pre-transport selection and management of livestock. Available online: <https://www.mla.eu/articles/animal-welfare/fit-to-load/mlas-updated-livestock-transport-guidance-for-2019/>
- Morris BK, Benjamin Davis R Brokesh E, Flippo DK Houser TA Najjar-Villarreal F, Turner KK Williams JG, Stelzleni JM and Gonzalez JM. 2021. Measurement of the three-axis vibration, temperature, and relative humidity profiles of commercial transport trailers for pigs. *Journal of Animal Science*, 99, 1–14. <https://doi.org/10.1093/jas/skab027>
- Mount LE, 1974. The role of the evaporative heat loss in thermoregulation of animals. *Contemporary Biology*, Edwards Arnold.
- Napolitano F, De Rosa G, Girolami A, Scavone M and Braghieri A, 2011. Avoidance distance in sheep: test–retest reliability and relationship with stockmen attitude. *Small Ruminant Research*, 99, 81–86.
- Navarro G, Col R and Phillips CJC, 2018. Effects of space allowance and simulated sea transport motion on behavioural and physiological responses of sheep. *Applied Animal Behaviour Science*, 208, 40–48.
- Nawroth C, Albuquerque N, Savalli C, Single MS and McElligott AG, 2018. Goats prefer positive human emotional facial expressions. *Royal Society Open Science*, 5, 180491
- Nielsen BL, Dybkjær L and Herskin MS, 2011. Road transport of farm animals: effects of journey duration on animal welfare. *Animal*, 5, 415–427.
- Norris AL and Kunz TH, 2012. Effects of solar radiation on animal thermoregulation. In: EB Babatunde (ed). *Solar radiation*. IntechOpen, Rijeka, Croatia. pp. 195–220.
- Nowak R, Porter RH, Lévy F, Orgeur P and Schaal B, 2000. Role of mother-young interactions in the survival of offspring in domestic mammals. *Reviews of Reproduction*, 5, 153–163.
- Parrott RF, Thornton SN, Forsling ML and Delaney CE, 1987. Endocrine and behavioural factors affecting water balance in sheep subjected to isolation stress. *Journal of Endocrinology*, 112, 305–310.
- Parrott RF, Misson BH, De La Riva CF, 1994. Differential stressor effects on the concentrations of cortisol, prolactin and catecholamines in the blood of sheep. *Research in Veterinary Science*, 56, 234–239.
- Parrott RF, Hall SJG, Lloyd DM, Goode JA and Broom DM, 1998. Effects of a maximum permissible journey time (31 h) on physiological responses of fleeced and shorn sheep to transport, with observations on behaviour during a short (1 h) rest-stop. *Animal Science*, 66, 197–207. <https://doi.org/10.1017/S1357729800008961>
- Pascual-Alonso M, Miranda-de la Lama GC, Aguayo-Ulloa L, Villarroel M, Mitchell M and María GA, 2017. Thermophysiological, haematological, biochemical and behavioural stress responses of sheep transported on road. *Journal of Animal Physiology and Animal Nutrition*, 101, 541–551.
- Petherick JC and Phillips CJC, 2009. Space allowances for confined livestock and their determination from allometric principles. *Applied Animal Behaviour Science*, 117, 1–12.
- Petherick JC and Baxter SH, 1981. Modelling the static spatial requirements of livestock. *Modelling, design and evaluation of agricultural buildings*, 75.
- Phillips C, 2016. The welfare risks and impacts of heat stress on sheep shipped from Australia to the Middle East. *The Veterinary Journal*, 218, 78–85.
- Phillips CJ and Santurtun E, 2013. The welfare of livestock transported by ship. *The Veterinary Journal*, 196, 309–314.
- Phillips CJC, Pines MK, Latter M, Muller T, Petherick JC, Norman ST and Gaughan JB, 2012. Physiological and behavioral responses of sheep to gaseous ammonia. *Journal of Animal Science*, 90, 1562–1569.
- Phythian CJ, Michalopoulou E and Duncan JS, 2019. Assessing the validity of animal-based indicators of sheep health and welfare: do observers agree? *Agriculture*, 9, 88 pp.
- Piccione G, Grasso F, Fazio F, Giudice E and Pennisi P, 2006. Evaluation of some physiological and haematological parameters in the ewes: influence of shearing and sheltering. *Folia Veterinaria*, 50, 13–16.
- Piccione G, Giannetto C, Casella S and Caola G, 2008. Circadian activity rhythm in sheep and goats housed in stable conditions. *Folia Biol (Krakow)*, 56, 133–7. https://doi.org/10.3409/fb.56_3-4.133-137
- Pillai SM, Jones AK, Hoffman ML, McFadden KK, Reed SA, Zinn SA and Govoni KE, 2017. Fetal and organ development at gestational days 45, 90, 135 and at birth of lambs exposed to under- or over-nutrition during gestation. *Translational Animal Science*, 1, 16–25.

- Pines MK and Phillips CJ, 2013. Microclimatic conditions and their effects on sheep behavior during a live export shipment from Australia to the Middle East. *Journal of Animal Science*, 91, 4406–16.
- Polycarp TN, Obukowho EB and Yusoff SM, 2016. Changes in haematological parameters and oxidative stress response of goats subjected to road transport stress in a hot humid tropical environment. *Comparative Clinical Pathology*, 25, 285–293.
- Porcelluzzi A, 2013. Handbook for High Quality Control Posts for cattle, pigs and sheep. Available online: <https://www.flipsnack.com/andpor/quality-control-post-handbook-english.html>
- Proctor GB, 2000. The physiology of salivary secretion. *Periodontology*, 70, 11–25. <https://doi.org/10.1111/prd.12116>
- Pulido MA, Estévez-Moreno LX, Villarreal M, Mariezcurrena-Berasain MA and Miranda-de la Lama GC, 2019. Transporters knowledge toward preslaughter logistic chain and occupational risks in Mexico: an integrative view with implications on sheep welfare. *Journal of Veterinary Behavior*, 33, 114–120.
- Raja SN, Carr DB, Cohen M, Finnerup NB, Flor H, Gibson S, Keefe FJ, Mogil JS, Ringkamp M, Sluka KA, Song XJ, Stevens B, Sullivan MD, Tutelman PR, Ushida T and Vader K, 2020. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. *Pain*, 161, 1976–1982. <https://doi.org/10.1097/j.pain.0000000000001939>
- Rajion MA, Mohamed I, Zulkifli I and Goh YM, 2001. The effects of road transportation on some physiological stress measures in goats. *Asian-Australasian Journal of Animal Sciences*, 14, 1250–1252.
- Randall JM and Patel R, 1994. Thermally induced ventilation of livestock transporters. *Journal of Agricultural Engineering Research*, 57, 99–107.
- Rashamol VP, Sejian V, Pragna P, Lees AM, Bagath M, Krishnan G and Gaughan JB, 2019. Prediction models, assessment methodologies and biotechnological tools to quantify heat stress response in ruminant livestock. *International Journal of Biometeorology*, 63, 1265–1281.
- Renaudeau D, Collin A, Yahav S, De Basilio V, Gourdine JL and Collier RJ, 2012. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Animal*, 6, 707–728.
- Reynolds R, Garner A and Norton J, 2019. Sound and vibration as research variables in terrestrial vertebrate models. *ILAR Journal*, 60, 159–174.
- Richards RB, Norris RT, Dunlop RH and McQuade NC, 1989. Causes of death in sheep exported live by sea. *Australian Veterinary Journal*, 66, 33–38.
- Richardson C, 2002. Lowering stress in transported goats. Ontario Ministry of Agriculture and Food–Livestock Technology Branch, Northern Ontario Regional Office.
- Ridgway M, 2021. Herding Dogs. *Veterinary Clinics: Small Animal Practice*, 51, 975–984.
- Ridler AL and West DM, 2010. Examination of teeth in sheep health management. *Small Ruminant Research; Special Issue: Sheep Diagnostic Medicine* 92, 92–95. <https://doi.org/10.1016/j.smallrumres.2010.04.014>
- Robin des Bois, 2021. 78 EU-approved livestock carriers Animal Welfare Foundation, Tierschutzbund Zuerich. Available from: https://www.animal-welfare-foundation.org/files/downloads/78_EU_livestock_carriers_June_2021_RobindesBois_AWF_TSB-1.pdf
- Roche JR, Blache D, Kay JK, Miller DR, Sheahan AJ and Miller DW, 2008. Neuroendocrine and physiological regulation of intake with particular reference to domesticated ruminant animals. *Nutrition Research Reviews*, 21, 207–234. <https://doi.org/10.1017/S0954422408138744>
- Rodrigues VC, da Silva IJO, Vieira FMC and Nascimento ST, 2011. A correct enthalpy relationship as thermal comfort index for livestock. *International Journal of Biometeorology*, 55, 455–459.
- Rojek, 2021. Protection of animals during transport: Data on live animals transport. European Parliamentary Research Service report. Available online: [https://www.europarl.europa.eu/RegData/etudes/BRIE/2021/690708/EPRS_BRI\(2021\)690708_EN.pdf](https://www.europarl.europa.eu/RegData/etudes/BRIE/2021/690708/EPRS_BRI(2021)690708_EN.pdf)
- Romo-Barron C, Diaz D, Portillo-Loera J, Romo-Rubio J, Jimenez-Trejo F and Montero-Pardo A, 2019. Impact of heat stress on the reproductive performance and physiology of ewes: a systematic review and meta-analyses. *International Journal of Biometeorology*, 63, 949–962. <https://doi.org/10.1007/s00484-019-01707-z>
- Ross S and Berg J, 1956. Stability of food dominance relationships in a flock of goats. *Journal of Mammalogy*, 37, 129–131.
- Roussel S, Hemsworth PH, Leruste H, White C, Duvaux-Ponter C, Nowak R and Boissy A, 2006. Repeated transport and isolation during pregnancy in ewes: effects on the reactivity to humans and to their offspring after lambing. *Applied Animal Behaviour Science*, 97, 172–189.
- Ruckebusch Y, 1975. The hypnogram as an index of adaptation of farm animals to changes in their environment. *Applied Animal Ethology*, 2, 3–18.
- Ruiz-De-La-Torre JL and Manteca X, 1999. Effects of testosterone on aggressive behaviour after so-cial mixing in male lambs. *Physiology and Behavior*, 68, 109–113.
- Ruiz-De-La-Torre JL, Velarde A, Manteca X, Diestre A, Gispert M, Hall SJG and Broom DM, 2001. Effects of vehicle movements during transport on the stress responses and meat quality of sheep. *Veterinary Record*, 148, 227–229.
- Saba N, Burns KN, Cunningham NF, Hebert CN and Patterson DSP, 1966. Some biochemical and hormonal aspects of experimental ovine pregnancy toxæmia. *The Journal of Agricultural Science*, 67, 129–138. <https://doi.org/10.1017/S0021859600067666>

- Santurtun E, Moreau V, Marchant-Forde JN and Phillips CJ, 2015. Physiological and behavioral responses of sheep to simulated sea transport motions. *Journal of Animal Science*, 93, 1250–7.
- Sañudo C, Nute GR, Campo MM, Maria G, Baker A, Sierra I, Enser ME and Wood JD, 1998. Assessment of commercial lamb meat quality by British and Spanish taste panels. *Meat Science*, 48, 91–100.
- Sapolsky RM, 2002. Endocrinology of the stress-response. In: Becker JB, Breedlove SM, Crews D and McCarthy MM (eds). *Behavioral Endocrinology*. 2nd edn. MIT Press, Cambridge, MA. pp. 409–450.
- Sarangi S, 2018. Adaptability of goats to heat stress: a review. *The Pharma Innovation Journal*, 7, 1114–1126.
- SCAHAW (Scientific Committee on Animal Health and Animal Welfare), 1999. Standards for the microclimate inside animal transport road vehicles. Report of the Scientific Committee on Animal Health and Animal Welfare, European Commission.
- SCAHAW (Scientific Committee on Animal Health and Animal Welfare), 2002. The welfare of animals during transport (details for horses, pigs, sheep and cattle). European Commission, Report of the Scientific Committee on Animal Health and Animal Welfare. 130 p.
- Schlader ZJ, Simmons SE, Stannard SR and Mündel T, 2011. The independent roles of temperature and thermal perception in the control of human thermoregulatory behavior. *Physiology and Behavior*, 103, 217–24. <https://doi.org/10.1016/j.physbeh.2011.02.002>
- Schmid O and Kilchsperger R, 2010. Overview of animal welfare standards and initiatives in selected EU and third countries. Final Report Deliverable, 1, 1–260.
- Schoenian S, 2019. Heat Stress in Small Ruminants. The Ohio State University. Available online: <https://u.osu.edu/sheep/2019/05/21/heat-stress-in-small-ruminants/>
- Scott PR, 2007. *Sheep Medicine*. London: Manson Publishing Ltd. Cardiovascular system; pp. 161–164.
- Scott PR and Gessert ME, 1998. Ultrasonographic examination of the ovine thorax. *The Veterinary Journal*, 155, 305–310. [https://doi.org/10.1016/S1090-0233\(05\)80027-9](https://doi.org/10.1016/S1090-0233(05)80027-9)
- Sevi A, Taibi L, Albenzio M, Muscio A, Dell'Aquila S and Napolitano F, 2001. Behavioral, adrenal, immune, and productive responses of lactating ewes to regrouping and relocation. *Journal of Animal Science*, 79, 1457–1465.
- Shorthose WR and Shaw FD, 1977. Plasma constituents of “downer” sheep slaughtered at an abattoir. *Australian Veterinary Journal*, 53, 330–333. <https://doi.org/10.1111/j.1751-0813.1977.tb00242.x>
- Silanikove N, 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livestock Production Science*, 67, 1–18.
- Smith RF, French NP, Saphier PW, Lowry PJ, Veldhuis JD and Dobson H, 2003. Identification of stimulatory and inhibitory inputs to the hypothalamic-pituitary-adrenal axis during hypoglycaemia or transport in ewes. *Journal of Neuroendocrinology*, 15, 572–585.
- Sossidou EN, Messori S, Mounaix B, Ouweltjes W, Pedernera C, Voudoula V and Spooler H, 2013. assessment protocol for measuring and monitoring sheep welfare in long distance transportation. Proceedings of the EAAP Annual Meeting 2013, Nantes, France. August.
- Sowińska J, Brzostowski H, Tański Z and Lisowska J, 2006. Stress reaction of lambs to weaning and short transport to slaughterhouse with regards to the breed and age. *Medycyna Weterynaryjna*, 62(8), 946–948.
- Tataranni PA, Gautier J, Chen K, Uecker A, Bandy D, Salbe AD, Pratley RE, Lawson M, Reiman EM and Ravussin E, 1999. Neuroanatomical correlates of hunger and satiation in humans using positron emission tomography. *Proceedings of National Academy Science USA*, 96, 4569 pp. <https://doi.org/10.1073/pnas.96.8.4569>
- Teixeira DL, Miranda-de la Lama GC, Villarroel M, Escós J and María GA, 2014. Lack of straw during finishing affects individual and social lamb behavior. *Journal of Veterinary Behavior*, 9, 1558–7878. <https://doi.org/10.1016/j.jveb.2014.02.008>
- Teke B, Ekiz B, Akdag F, Ugurlu M, Ciftci G and Senturk B, 2014. Effects of stocking density of lambs on biochemical stress parameters and meat quality related to commercial transportation. *Annals of Animal Science*, 14, 611–621. <https://doi.org/10.2478/aoas-2014-0012>
- Ternouth JH, 1967. Post-prandial ionic and water exchange in the rumen. *Research in Veterinary Science*, 8, 283–293.
- Thodberg K, Gould LM, Støier S, Anneberg I, Thomsen PT and Herskin MS, 2020. Experiences and opinions of Danish livestock drivers transporting sows regarding fitness for transport and management choices relevant for animal welfare. *Translational Animal Science*, 4, 1070–1081.
- Thom EC, 1959. The discomfort index. *Weatherwise*, 12, 57–61.
- Thompson GE, 1985. Respiratory system. In: Young MK (ed.). *Stress Physiology in Livestock*. CRC Press, Inc., Boca Raton, Florida, USA. pp. 155–162.
- Tobler I, Jaggi K, Arendt J and Ravault J, 1991. Long-term 24-hour rest activity pattern of sheep in stalls and in the field. *Experientia*, 47, 744–749.
- Turner AW and Hodgetts VE, 1959. The dynamic red cell storage function of the spleen in sheep I. relationship to fluctuations of jugular haematocrit *Australian Journal of Experimental Biology and Medical Science*, 37, 399–420.
- Turnpenny JR, Wathes CM, Clark JA and McArthur AJ, 2000. Thermal balance of livestock: 2. Applications of a parsimonious model. *Agricultural and Forest Meteorology*, 101, 29–52.

- Underwood WJ, Blauwiekel R, Delano ML, Gillesby R, Mischler SA and Schoell A, 2015. Biology and diseases of ruminants (sheep, goats, and cattle). In: Fox JG (ed). *Laboratory Animal Medicine*. Elsevier, St.-Louis, IA. pp. 623–694.
- United Nations Environment Program (UNEP), 2022. Climate change in the Mediterranean. Factsheet. Available online: <https://www.unep.org/unepmap/resources/factsheets/climate-change>
- Vaintrub MO, Levit H, Chincarini M, Fusaro I, Giammarco M and Vignola G, 2020. Precision livestock farming, automats and new technologies: possible applications in extensive dairy sheep farming. *Animal*, 100143
- Van Nuffel A, Zwertvaegher I, Pluym L, Van Weyenberg S, Thorup VM, Pastell M, Sonck B and Saeys W, 2015. Lameness detection in dairy cows: Part 1. How to distinguish between non-lame and lame cows based on differences in locomotion or behavior. *Animals*, 5, 838–860.
- Vidić B, Savić-Jevdendić S, Grgić Ž, Bugarski D and Maljković M, 2007. Infectious abortion in sheep. *Biotechnology in Animal Husbandry*, 23, 383–389.
- Vincent B, 2005. *Farming Meat Goats: Breeding, Production and Marketing*. Landlinks Press, Collingwood, Australia. 330 p.
- Visser K, 2014. Note on minimum space allowance and compartment height for cattle and pigs during transport. Report 764. Wageningen UR Livestock Research, the Netherlands. Available online: <https://edepot.wur.nl/330021>
- Walz PH and Taylor D, 2012. Fluid therapy and nutritional support. *Sheep and Goats Medicine*. 2nd Edition. Saunders, Elsevier, Missouri, pp. 50–61.
- Wang X, Gao H, Gebremedhin KG, Bjerg BS, Van OJ, Tucker CB and Zhang G, 2018. A predictive model of equivalent temperature index for dairy cattle (ETIC). *Journal of Thermal Biology*, 76, 165–170.
- Warriss PD, 1998. Choosing appropriate space allowances for slaughter pigs transported by road: a review. *The Veterinary Record*, 142, 449–454. <https://doi.org/10.1136/vr.142.17.449>
- Warriss PD, Bevis EA, Brown SN and Ashby JG, 1989. An examination of potential indices of fasting time in commercially slaughtered sheep. *The British Veterinary Journal*, 145, 242–248.
- Wemelsfelder F and Farish M, 2004. Qualitative categories for the interpretation of sheep welfare: a review. *Animal Welfare*, 13, 261–268.
- Whay HR, 2002. Locomotion scoring and lameness detection in dairy cattle. *In Practice*, 24, 444–449.
- Wickham SL, Collins T, Barnes AL, Miller DW, Beatty DT, Stockman CA, Blache D, Wemelsfelder F and Fleming PA, 2015. Validating the use of qualitative behavioral assessment as a measure of the welfare of sheep during transport. *Journal of Applied Animal Welfare Science*, 18, 269–286.
- Winblad von Walter L, Forkman B, Högberg M and Hydbring-Sandberg E, 2021. The effect of mother goat presence during rearing on kids' response to isolation and to an arena test. *Animals*, 11, 575 pp.
- Winter AC, 2008. Lameness in sheep. *Small Ruminant Research*, 76, 149–153.
- Wintour EM, Laurence BM and Lingwood BE, 1986. Anatomy, physiology and pathology of the amniotic and allantoic compartments in the sheep and cow. *Australian Veterinary Journal* 63, 216–221. <https://doi.org/10.1111/j.1751-0813.1986.tb02999.x>
- WOAH (World Organisation for Animal Health), 2011 Terrestrial animal health code. Chapter 7.3. Transport of animals by land. Available online: https://www.woah.org/fileadmin/Home/eng/Health_standards/tahc/current/chapitre_aw_land_transpt.pdf
- Ye Y, Schreurs NM, Johnson PL, Corner-Thomas RA, Agnew MP, Silcock P, Eyres GT, MacLennan G and Realini CE, 2020. Carcass characteristics and meat quality of commercial lambs reared in different forage systems. *Livestock Science*, 232, 103908.
- Zhang M, Feng H, Luo H, Li Z and Zhang X, 2020. Comfort and health evaluation of live mutton sheep during the transportation based on wearable multi-sensor system. *Computers and Electronics in Agriculture*, 176, 105632. AVA (Australian Veterinary Association), 2018. Heat Stress Risk Assessment (HotStuff): Issues Paper.
- Zheng W, Liu B, Hu W and Cui Y, 2021. Effects of transport stress on pathological injury and main heat shock protein expression in the respiratory system of goats. *Journal of Animal Physiology and Animal Nutrition*, 105, 1–13.
- Zhong RZ, Liu HW, Zhou DW, Sun HX and Zhao CS, 2011. The effects of road transportation on physiological responses and meat quality in sheep differing in age. *Journal of Animal Science*, 89, 3742–3751.
- Zimmerman M, Grigioni G, Taddeo H and Domingo E, 2011. Physiological stress responses and meat quality traits of kids subjected to different pre-slaughter stressors. *Small Ruminant Research*, 100, 137–142.
- Zulkifli I, Bahyuddin N, Wai CY, Farjam AS, Sazili AQ, Rajion MA and Goh YM, 2010. Physiological responses in goats subjected to road transportation under the hot, humid tropical conditions. *International Journal of Agricultural Biology*, 12, 840–844.

Abbreviations

ACTH	adrenocorticotrophic hormone
ABM	animal-based measure
AET	apparent equivalent temperature
CP	control post

DOA	dead-on-arrival
ECI	Enthalpy Comfort Index
EFSA AHAW Panel	EFSA Panel on Animal Health and Welfare
EKE	expert knowledge elicitation
LCT	lower critical temperature
MS	Member State
NEFA	non-esterified fatty acids
PRE	preventive measures
RH	relative humidity
RO-RO	roll-on-roll-off
TCZ	thermal comfort zone
THI	temperature–humidity index
TNZ	thermoneutral zone
TRACES	TRAdE Control and Expert System
Twb	wet-bulb temperature
UCT	upper critical temperature
WC	welfare consequence
WOAH	World Animal Health Organisation

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

Name		Example		
Animal species		Example		
Animal type		Example		
Husbandry 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)				