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SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 30 June 2022

doi: 10.2903/j.efsa.2022.7442

Welfare of cattle during transport

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Abstract

In the framework of its Farm to Fork Strategy, the Commission is undertaking a comprehensive evaluation of the animal welfare legislation. The present Opinion deals with protection of cattle (including calves) during transport. Welfare of cattle during transport by road is the main focus, but other means of transport are also covered. Current practices related to transport of cattle during the different stages (preparation, loading/unloading, transit and journey breaks) are described. Overall, 11 welfare consequences were identified as being highly relevant for the welfare of cattle during transport based on severity, duration and frequency of occurrence: group stress, handling stress, heat stress, injuries, motion stress, prolonged hunger, prolonged thirst, respiratory disorders, restriction of movement, resting problems and sensory overstimulation. These welfare consequences and their animal-based measures are described. A variety of hazards, mainly relating to inexperienced/untrained handlers, inappropriate handling, structural deficiencies of vehicles and facilities, poor driving conditions, unfavourable microclimatic and environmental conditions, and poor husbandry practices leading to these welfare consequences were identified. The Opinion contains general and specific conclusions relating to the different stages of transport for cattle. 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 was assessed in relation to maximum journey duration. The Opinion covers specific animal transport scenarios identified by the European Commission relating to transport of unweaned calves, cull cows, the export of cattle by livestock vessels, the export of cattle by road, roll-on-roll-off ferries and 'special health status animals', and lists welfare concerns associated with these.

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Keywords: cattle, calves, animal welfare assessment, Farm to Fork Strategy, welfare consequences, animal-based measures, hazards, quantitative thresholds

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Question number: EFSA-Q-2020-00481

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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.

Acknowledgments: 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.

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.

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, de La Lama GCM, Costa LN, Thomsen PT, Ashe S, Mur L, Van der Stede Y and Herskin M, 2022. Welfare of cattle during transport. EFSA Journal 2022;20(9):7442, 121 pp. https://doi.org/10.2903/j.efsa.2022.7442

ISSN: 1831-4732

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

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Summary

In the framework of its Farm to Fork Strategy, the Commission is undertaking a comprehensive evaluation of the animal welfare legislation, including Council Regulation (EC) No 1/2005[1]. The current EU legislation on the protection of animals during transport is based on a scientific opinion adopted in 2002. Against this background, the European Commission requested the European Food Safety Authority (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 cattle (including calves) during transport.

The scientific assessment was carried out by breaking down the transport of cattle into four distinct stages, namely preparation, loading/unloading, transit and journey breaks. For road transport, which is the most common transport practice, each stage was described in terms of current practice and assessed in terms of welfare consequences, animal-based measures (ABMs) and hazards leading to the welfare consequences. In addition, recommendations to prevent hazards and to correct or mitigate welfare consequences were developed. Recommendations were also developed in relation to quantitative thresholds for microclimatic conditions within the means of transport and for spatial thresholds (minimum space allowance). 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 cattle, there are specific sections dealing with export by road, transport by roll-on-roll-off ferries and 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 unweaned calves and cull dairy cows are covered in a separate section of the Opinion.

According to TRACES, around 4.3 million cattle were transported between Member States per year in the period from 2019 to 2021, across all means of transport. Road transport constituted around 90% of total cattle transport reported in this period.

In total, 11 welfare consequences were identified as being highly relevant for the welfare of cattle 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) prolonged hunger, (vii) prolonged thirst, (viii) respiratory disorders, (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. Cattle may experience one or more negative affective states associated with these welfare consequences, including fear, pain, discomfort, frustration, fatigue and distress. Specific ABMs were identified for each of the highly relevant welfare consequences, including behavioural, clinical and physiological ABMs. A definition and interpretation for each ABM is provided in the Opinion. Some ABMs are relevant to more than one welfare consequence.

A wide variety of hazards were identified for the different welfare consequences and transport stages. These were related to factors such as inexperienced/untrained handlers, inappropriate handling, structural deficiencies of vehicles and facilities, poor driving and road 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 a few conditions leading animals to be unfit for transport, for which ABMs have been established and validated, including the establishment of thresholds. The main conditions rendering cattle 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.

The highly relevant welfare consequences during loading/unloading of cattle are: handling stress, heat stress, restriction of movement, injuries and sensory overstimulation. Across the highly relevant welfare consequences, the major hazards are inappropriate handling, unsuitable facilities, delays, high

[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.

temperatures, 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, cattle will be exposed to a number of hazards, either in isolation or in combination, leading to welfare consequences.

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

In relation to the horizontal space allowance for cattle during road transport, the available evidence suggests that a k-value of at least 0.034 in the allometric equation relating space to liveweight, is required for the animals to adjust posture in response to acceleration and other events and for all animals in a compartment to be able to lie down, as well as perform the lying-down and getting-up movements.

The vertical space in a means of transport is also important for the welfare of cattle. Regarding deck height, the available research is limited. Across cattle categories, a deck height of wither height $\times$ 1.17 + 20 cm is recommended to allow natural movements and ventilation in trucks. This recommendation corresponds to at least 40 cm above the withers for adult cattle. Research is needed to establish evidence-based thresholds.

The amount of time the animals are exposed to the hazards is dependent on the journey duration. The number and the severity of hazards that animals are exposed to during transport influence the resultant welfare consequences (continuous or semi-continuous, progressive and sporadic). On the basis of evidence on continuous welfare consequences involving stress and negative affective states, for the benefit of animal welfare, the journey duration and frequency, 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 9 h of transport; and due to practical difficulties in feeding animals on a transport physiological changes indicative of hunger can be present after 12 h 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 that they are exposed to during transit and to allow them to recover from the associated welfare consequences. Based on industry practices and the very limited available evidence allowing cattle a break on a stationary vehicle at the current commercial space allowance does not lead to the intended drinking, eating, and resting and thus does not mitigate the welfare consequences of the journey. Thus, if cattle 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 potential for exposure to hazards resulting in welfare consequences or interfering with the intended mitigation of other welfare consequences. In addition, control posts involve biosecurity risks as animals can be exposed to infectious diseases through direct or indirect contact with other animals and opportunistic pathogens. Across the categories of cattle typically transported on journeys involving journey breaks, the scientific focus on control posts has been limited. This means that whether control posts in their current state fulfil their intended function is not known.

The specific scenarios relevant to cattle that EFSA was asked by the Commission to consider were the transport of unweaned calves over long journeys, the transport of cull dairy cows to slaughterhouses, the export of cattle by road, the export of cattle in livestock vessels, the transport of cattle in roll-on-roll-off ferries and the transport of 'special health status animals', i.e. the transport of cattle where unloading them before the final destination might jeopardise their health status.

The specific concerns identified by EFSA in relation to the transport of unweaned calves were related to age at transport, immunity/provision of colostrum in the days after birth, gastroenteric disorders, handling, feeding, drinking and unloading facilities. Based on the available knowledge,

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calves should be at least 5 weeks of age and of 50 kg weight when transported. In order for all calves to be able to lie down during journeys, a k-value of at least 0.027 is required. In order to reduce the risk of the welfare consequence heat stress, the temperature inside vehicles transporting unweaned calves should not exceed 25°C. Maximum journey duration should take into account the time from last feeding. In order to allow calves to be loaded/unloaded and a 3-h post-meal rest, journeys should not exceed 8 h.

Health issues are major causes for culling dairy cows, so these animals present additional challenges when it comes to transporting them to a slaughterhouse. Therefore, the key animal welfare concern affecting the transport of cull dairy cows to slaughter is determining the fitness of the cows for transport, in order to protect their welfare during transport. Among the major health reasons for culling are reproductive diseases, lameness and mastitis. Many cull cows with these types of conditions will experience welfare consequences during transport, as they are less able to cope with the hazards associated with transport, such as getting on and off the vehicle, maintaining stability, avoiding fatigue, feed and water restriction, and microclimatic conditions. The transport of cull cows to slaughterhouses that are thin or lactating can also result in welfare consequences. If transport to slaughter involves complex journeys through for example auction markets, it may further exacerbate the welfare consequences during the subsequent journey. The Opinion contains a number of recommendations on how hazards associated with transport can be prevented, and welfare consequences from transport can be mitigated for cull dairy cows.

In the period from 2019 to 2021, 0.3–0.48 million cattle were exported from the EU by road. A number of concerns specifically related to the export of cattle to third countries by road, including long delays at border crossings when leaving the EU, and the absence of certified resting points outside of the EU, were identified in addition to the hazards described for road transport within the EU. In general, export of cattle to third countries involves journeys over several days. There are also specific health risks related to cattle being exported from the EU.

Every year, the EU exports ~ 3 million cattle by sea, mainly to the Middle East and Africa. The largest livestock vessels can carry up to 18,000 cattle. Specific concerns relating to the export of cattle by livestock vessels are presented. Overall, little is known about the possible welfare consequences of transport of cattle in livestock vessels from Europe, and research is needed to form the basis for future recommendations.

The concerns addressed for the welfare of cattle 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. More research is needed to evaluate the welfare of cattle during transport in roll-on-roll-off ferries to form the basis for future recommendations.

'Special health status animals' are cattle 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 cattle under these circumstances may present a large risk to their welfare. If, due to biosecurity concerns, cattle 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, as well as suitable microclimatic conditions. However, no scientific studies have been found that confirm that this is currently possible.

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1. Introduction

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

1.1.1. Background

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

- Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes.
- Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens.
- Council Directive 2008/119/EC of 18 December 2008 laying down minimum standards for the protection of calves.
- Council Directive 2008/120/EC of 18 December 2008 laying down minimum standards for the protection of pigs.
- Council Directive 2007/43/EC of 28 June 2007 laying down minimum rules for the protection of chickens kept for meat production.
- Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/976.
- Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing.

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

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

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

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

1.1.2. Terms of Reference

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

Free-moving animals (group 1):

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

Animals in containers (group 2):

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

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

1.1.2.1. Assessment of common transport practices

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

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

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

1.2. Interpretation of the Terms of Reference

1.2.1. General interpretation

This Scientific Opinion concerns the protection of bovines 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 Scientific Opinion focuses on *Bos taurus*, as they are the most common type of bovine in the EU. 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.

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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 cattle until the initiation of loading of the animals onto a vehicle or other means of transport. In effect, in this Opinion, the preparation of cattle 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 up until the ramp is closing. Unloading starts when the ramp is open and the first animal exits the means of transport, and ends when the last animal exits. Loading and unloading are dealt with together due to the similarities of the processes. The transit 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 2002/15/EC**
Driving • 9 h daily driving limit (can be increased to 10 h twice a week) • Max 56 h weekly driving limit • Max 90 h fortnight driving limit	**Working time (including driving)** • Working time must not exceed average of 48 h a week (no opt out)[1] • Max working time of 60 h in one week (provided average not exceeded) • Max working time of 10 h if night work performed.[2]
Breaks • 45 min break after 4.5 h driving • A break can be split into two periods, the first being at least 15 min and the second at least 30 min (which must be completed after 4.5 h driving)	**Breaks[3]** • A driver cannot work for more than 6 h without a break. A break should be at least 15 min long. • 30 min break if working between 6 and 9 h in total.[4] • 45 min break if working more than 9 h in total.
Rest • 11 h regular daily rest[5]; which can be reduced to 9 h no more than three times a week. • 45 h weekly rest, which can be reduced to 24 h, provided at least one full rest is taken in any fortnight. There should be no more than six consecutive 24 h periods between weekly rests.	**Rest** • Same rest requirements as EU' drivers.

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

This Opinion does not focus on the different types of premises defined in the transport Regulation (e.g. markets, auctions) but refers to them where appropriate. Destination is not covered, but only specified when important considerations are found. In case of animals arriving for slaughter, additional information can be found in the EFSA Opinion on Welfare of Cattle at Slaughter (EFSA AHAW Panel, 2020).

Within bovines, different animal categories exist, such as heifers and bulls. This scientific assessment focuses on non-juvenile animals, but, when relevant and when information is available, specific categories are mentioned. When specific studies are referred to, the average body weights of the animals involved are mentioned (when available) as well as the animal category involved in the study (e.g. heifer). For most of the sections of the Scientific Opinion, conclusions and recommendations concerning non-juvenile cattle of an unspecified weight, age, or horn status are drawn.

The scientific assessment carried out by the EFSA takes two forms. Firstly, for road transport practices, 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 means of transport (maximum temperature), and to 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 cattle, EFSA examines selected welfare concerns (defined as an area or a topic to which special attention should be given in order to potentially avoid 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.

In relation to the specific transport practice, the transport of 'end-of-career animals', in this Scientific Opinion, this animal category denotes 'cull animals' which is the term used in scientific literature.

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

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

For the 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 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.

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2.1.2. Data from Public Consultation

To consult interested parties and gain feedback on EFSAs interpretation of the transport mandate, a Public Consultation was launched in the period 15 April–10 June 2021. In particular, EFSA called for on 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 their work on this Opinion (See Annex A Report of the Public Consultation on the Protection of Animals during Transport, published under 'Supporting Information' in the Opinion on transport of small ruminants).

2.2. Methodologies

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

To address the terms of reference of the mandate, EFSA translated the assessment questions into more specific sub-questions. These are interrelated, meaning that the outcome of each sub-question is necessary 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.

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

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Assessment questions		Sub-questions	
iv.	**Identify the hazards leading to these welfare consequences**	***7.*** *Identify the hazards leading to the highly relevant welfare consequences*	***8.*** *Describe the hazards leading to the most relevant welfare consequences*
		Aim: The hazards leading to the most relevant welfare consequences are identified. Approach: Expert opinion via group discussion. Relationship with assessment question: this corresponds to the assessment question and is related to Sub-question 4 in which hazards are identified only for the highly 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*	***10.*** *Describe 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.	Aim: Preventive and corrective measures are described. Approach: Literature review. Relationship with assessment question: related to Sub-question 8.

ABM: animal-based measure; EKE: Expert Knowledge Elicitation.

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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 $\times$ transport stage).

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

Expert opinion is elicited in three phases:

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

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

Expert opinion was also part of the syntheses involved in the development of the quantitative recommendations for specific conditions within the means of transport (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.

The search results (general + specific) yielded a total of 1,507 (278 + 1,229) records that were exported to an EndNote library together with the relevant metadata (e.g. title, authors, abstract). Titles and abstracts were screened to remove irrelevant publications (e.g. related to species, processes

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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 329 (cattle), 141 (dairy cows) and 103 (unweaned calves) relevant records for the search concerning publication dates from 2011 to 2021. Experts screened these papers and selected 123 (cattle) + 66 (dairy cows) + 102 (unweaned calves) records. 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 topic was considered sufficient to undertake the assessment by the EFSA experts. If needed, relevant publications from before 2011 were considered.

3. Assessment

3.1. The transport of cattle within the European Union

Transport of animals between 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 within a MS (i.e. to slaughterhouses or between farms) are not recorded in this database (Rojek, 2021).

According to TRACES, around 4.3, 4.2 and 3.5 million cattle were transported between MS during 2019, 2020 and 2021, respectively, across all means of transport. Road transport constituted around 90%.

3.2. Welfare consequences associated with transport of cattle

During the last decades, several scientific reviews (e.g. Nielsen et al., 2011; Cockram, 2022), textbooks (e.g. Grandin, 2019) and international organisations (e.g. WOAH, 2011) have described and discussed the consequences of animal transport in terms of animal welfare. In general, it is agreed that animal transport can lead to severe negative animal 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 cattle, the following WCs were selected as highly relevant: group stress, handling stress, heat stress, injuries, motion stress, prolonged hunger, prolonged thirst, respiratory disorders, restriction of movement, resting problems 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 breaks
Group stress	The animal experiences stress and/or negative affective states such as pain, fear and/or frustration 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			X

WCs and definitions		Transport stages			
		Preparation	Loading/ unloading	Transit	Journey breaks
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		
Motion stress	The animal experiences motion sickness, stress and/or fatigue due to the forces exerted as a result of acceleration, braking, stopping, cornering, gear changing, vibrations and uneven road surfaces during transport.			X	
Prolonged hunger	The animal experiences craving or urgent need for food or a specific nutrient, accompanied by a negative affective state, and eventually leading to a weakened condition as metabolic requirements are not met.			X	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	X
Respiratory disorders	The animal experiences negative affective states such as discomfort, pain, air hunger and/or distress due to impaired function or lesion of the lungs or airways.			X	
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 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	

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

3.2.1. Negative affective states

The description of each WC reported in Table 3 refers to one or more negative states involving 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).
Stress[1] & 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 does not describe a negative affective state in itself, but it is mentioned and defined in the table as it is a prerequisite of distress.

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

Only a few studies have evaluated systems for the assessment of welfare during transport for livestock species, and – so far – no validated protocol for the assessment of cattle welfare before,

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during and after transport has been described. Consequently, no benchmark values documenting optimal animal welfare status during or after transport exist to inform this Scientific Opinion. In addition, benchmark values documenting good animal welfare do not exist for any of the ABMs listed in this Scientific Opinion in relation to transport.

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). 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 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 practice in question. Technological tools within this area have been used in scientific studies (e.g. Goldhawk et al., 2014), but are not yet applicable in practice. This constitutes a gap in knowledge. Until sensors and associated interpretive or alarm systems are available at a practical level, the stage of transport appears to be the biggest influence on the feasibility of any ABM during animal transport. Another aspect of transport stress that may be monitored and 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 of, 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 during transport even when the animals can be inspected (Llonch et al., 2015). Examples of these are physiological indicators requiring invasive procedures. Tables 5–14, 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 selected for the assessment of group stress in cattle during preparation and journey breaks

ABM	Definition and interpretation of the ABM
Skin lesions and wounds	**Definition:** Tissue damage such as bruises, scratches, and wounds (EFSA AHAW Panel, 2012). **Interpretation:** Skin lesions can be caused by aggressive interactions between cattle.
Fighting	**Definition:** Negative social interactions such as head-knocking and threatening, pushing at the side or chasing each other. **Interpretation**: Fighting is an aggressive behaviour occasioned by mixing of cattle from different social groups, competition for food, and lack of adequate space to respond submissively to a threat and to achieve full avoidance action and move away.

ABM: animal-based measure.

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

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

ABM	Definition and interpretation of the ABM
Heart rate (HR)	**Definition:** The number of heart beats per min (Lefcourt et al., 1999). **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. HR reflects the activity of the sympathetic branch of the autonomic nervous system and therefore it is an indicator of the stress response and of the animal's emotional reactivity. Approximately 80 beats/min is suggested to be the upper threshold of the normal heart rate in cattle (Kovács et al., 2014).
Slipping	**Definition:** Animal showing a loss of balance with a leg sliding unintendedly over a small distance (Consortium of the Animal Transport Guides Project, 2018). **Interpretation:** Cattle 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.
Falling	**Definition:** Animal showing a loss of balance causing other part(s) of the body (beside legs) to touch the floor (Consortium of the Animal Transport Guides Project, 2018). A fall is 'an unintentional loss of balance that leads to failure of postural stability'. **Interpretation:** Cattle may fall on the ramp or in the barn/truck as a result of hasty or aggressive handling or behaviour of other animals.

ABM: animal-based measure.

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

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

ABM	Definition and interpretation of the ABM
Respiration rate (RR)	**Definition:** Frequency of breathing, usually measured by counting the movements of the flank manually and converting it into breaths per minute. **Interpretation**: RR is the first visible response of cattle to heat stress and changes with thermal environment. Norris et al. (2003) found that the RR of cattle increased by 5.7 breaths per minute for every 1°C ambient temperature greater than 25°C.
Panting	**Definition:** The first phase of panting is characterised by rapid, shallow breathless 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). **Interpretation:** Panting is a response to hot environmental conditions or acute physical exercise (Lees et al., 2019).
Sweating	**Definition:** At high temperatures, evaporative cooling is the main mechanism for heat dissipation in cattle (Blackshaw and Blackshaw, 1994) and is the only form of heat loss once the ambient temperature exceeds the skin temperature (Cunningham, 2002). **Interpretation:** 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.

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ABM	Definition and interpretation of the ABM
Rectal temperature	**Definition:** Rectal measurement of core body temperature. **Interpretation:** The normal rectal temperature for cattle is between 36.7°C and 39.1°C (Cunningham, 2002). A temperature of 39.2°C is suggested to be the upper threshold of the normal core body temperature of 1-month-old dairy calves (Piccione et al., 2003). 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. Body temperature may increase during heat stress as consequence of changes in the peripheral vascular tone and blood flow on the animals' body (Godyń et al., 2019).

ABM: animal-based measure.

iv) ABMs for the assessment of the welfare consequence injuries

Table 8: ABMs selected for the assessment of injuries in cattle during loading/unloading

ABM	Definition and interpretation of the ABM
Skin lesions and wounds	**Definition:** Tissue damage such as bruises, scratches, and wounds (EFSA AHAW Panel, 2012). **Interpretation:** Wounds/lesions are caused by external trauma.

ABM: animal-based measure.

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

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

ABM	Definition and interpretation of the ABM
Loss of balance	**Definition:** According to Cockram and Spence, 2012; loss of balance are losses of stability during travel that result in sudden and rapid movement of the thorax, pelvis or legs. Loss of balance does not imply actually falling to the ground (balance could be regained while still standing). **Interpretation:** The frequency of losses of balance of cattle during the journey is an indicator of the quality of the driving, the threshold of vibration received by the animals, the quality of the truck floor and the stocking density.
Bruises	**Definition:** A bruise is a lesion where tissues are crushed with a rupture of the vascular supply and an accumulation of blood and serum without discontinuity of the skin (Anderson and Horder, 1979; Knock and Carroll, 2019). Gradation of bruises can be done based on the prevalence and the location on the carcass. **Interpretation:** The frequency of bruising can increase due to the loss of balance and falls of the animals due to driving events (Garcia et al., 2019).
Skin lesions and wounds	**Definition:** Tissue damage such as bruises, scratches, and wounds (EFSA AHAW Panel, 2012). **Interpretation:** Wounds/lesions caused by trauma of falling down or slipping.
Heart rate (HR)	**Definition:** The number of heartbeats per unit of time, usually per minute. **Interpretation:** Acute stress response leads to a release of catecholamines into the serum and consequently to an increase in HR (Damián et al., 2021). However, HR is also subject to regulation by the autonomic nervous system through sympathetic nerves that increase and parasympathetic nerves that decrease HR. HR is affected by factors other than acute stress. The exercise associated with movement in response to handling and loading will also increase HR. Approximately 80 beats/min is suggested to be the upper threshold of the normal heart rate in cattle (Kovács et al., 2014).
Time spent standing	**Definition:** Cattle maintaining an upright position. **Interpretation**: Stressed animals are restless and will spend and increased time standing.

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ABM	Definition and interpretation of the ABM
Plasma CK activity	**Definition:** Concentration of creatinine kinase (CK) in plasma. **Interpretation:** CK increases above the normal range in the bloodstream as a result of muscle cell damage resulting from exertion (Warriss et al., 1995) or trauma and lesions (Tarrant, 1990).
Plasma cortisol	**Definition:** 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). **Interpretation:** Plasma cortisol concentration is an 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 cattle 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).
Falling	**Definition:** Animal showing a loss of balance causing other part(s) of the body (beside legs) to touch the floor (Consortium of the Animal Transport Guides Project, 2018). A fall is 'an unintentional loss of balance that leads to failure of postural stability'. **Interpretation:** Cattle 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.

ABM: animal-based measure.

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

Table 10: ABMs selected for the assessment of prolonged hunger in cattle in transit and during journey breaks

ABM	Definition and interpretation of the ABM
Non-esterified fatty acids in blood	**Definition**: A higher concentration of non-esterified fatty acids (NEFA)/free fatty acids in cattle is an indicator of lipolysis due to the need for its use as an alternative energy source (Knowles et al., 1995). **Interpretation**: Transport may 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).
Latency time to feed after unloading	**Definition:** A quantification of the time interval from unloading and until the animal is observed eating for the first time **Interpretation:** A short latency to eat is a reflection of a high motivation to eat. Can be affected by other factors such as fear.

ABM: animal-based measure.

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

Table 11: ABMs selected for the assessment of prolonged thirst in cattle in transit and during journey breaks

ABM	Definition and interpretation of the ABM
Plasma osmolality	**Definition**: 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 et al., 2012). **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 osmolarity (Parrott et al., 1987).

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ABM	Definition and interpretation of the ABM
Packed cell volume	**Definition:** the ratio of the volume occupied by packed red blood cells to the volume of the whole blood as measured by a haematocrit. **Interpretation:** Changes are confounded by potential splenic contraction due to stress, dehydration and loss of red blood cells (Turner and Hodgetts, 1959).
Plasma total protein concentration	**Definition**: Dehydration leads to a decrease in blood plasma volume, which increases the concentration of plastic proteins such as albumin and globulins. **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.
Latency to drink after unloading	**Definition:** Latency to drink after unloading. **Interpretation:** All other things being equal, a short latency to drink shows the transport-induced thirst (Pascual-Alonso et al., 2017).
Intake of water after unloading	**Definition:** Intake of water during the initial hours after unloading. **Interpretation:** All other things being equal, a high water intake shows the transport-induced thirst (Pascual-Alonso et al., 2017).

ABM: animal-based measure.

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

Table 12: ABMs selected for the assessment of respiratory disorders in cattle in transit

ABM	Definition and interpretation of the ABM
Respiration rate (RR)	**Definition:** Usually measured by counting the movements of the flank manually and converting it into breaths per minute (BPM). **Interpretation**: RR is increased when breathing capacity is affected in the animals.
Nasal discharge	**Definition:** Nasal discharges can be serous (thin, clear and colourless), catarrhal (grey, flocculent), purulent (thick, yellow) or haemorrhagic (red). Unilateral discharge indicates localised conditions involving the nose or sinuses, whereas bilateral discharge may indicate thoracic or systemic conditions (McGuirk, 2008; Love et al., 2014). **Interpretation:** Assigned scores range from 0 to 3 as the clinical sign progresses from normal to very abnormal. 0: normal, serous discharge; 1: small amount of unilateral, cloudy discharge; 2: bilateral, cloudy or excessive mucus; 3: copious, bilateral mucopurulent nasal discharge.
Ocular discharge	**Definition:** When serous fluid coming out of the eyes is observed. **Interpretation:** Assigned scores range from 0 to 3 as the clinical sign progresses from normal to very abnormal. 0: normal; 1: mild ocular discharge; 2: moderate bilateral ocular discharge; 3: heavy ocular discharge.
Ear position	**Definition:** When an ear drop is observed. **Interpretation:** Assigned scores range from 1 to 4 as the clinical sign progresses from normal to very abnormal. 1: upright posture; 2: forward ear posture; 3: backward ear posture; 4: hanging ear posture with a loosely ear, perpendicular to the head (Proctor and Carder, 2014).

ABM: animal-based measure.

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

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

ABM	Definition and interpretation of the ABM
Posture changes	**Definition:** Cattle actively change posture. **Interpretation:** cattle tend to change posture more often as a sign of discomfort or as a mechanism to maintain balance.

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ABM	Definition and interpretation of the ABM
Thwarted lying intentions	**Definition:** The animals show persistent movements with the intention of adopting the lying posture (Navarro et al., 2018). **Interpretation:** Tired animals show a high motivation to lie down to lessen the effects of the journey (Pascual-Alonso et al., 2017).
Falling	**Definition:** Animal showing a loss of balance causing other part(s) of the body (beside legs) to touch the floor (Consortium of the Animal Transport Guides Project, 2018). A fall is 'an unintentional loss of balance that leads to failure of postural stability'. **Interpretation:** Tired cattle may fall during the journey as a result of hasty or violent handling, sudden movements of the truck, behaviour of other animals, or slippery ground.
Plasma CK-activity	**Definition:** Concentration of creatinine kinase (CK) in plasma. **Interpretation:** CK increases in the bloodstream as a result of muscle cell damage resulting from exertion (Warriss et al., 1995) or trauma and lesions (Tarrant, 1990). The circulating blood level of creatine kinase has also been studied as a marker of muscle damage. Most investigators have found elevated creatine kinase levels upon arrival (Van de Water et al., 2003; Grigor et al., 2004; Earley et al., 2010; Bernardini et al., 2012). However, the results of another study indicated that there were no significant changes in CK activity following transport (Cafazzo et al., 2012).
Lying behaviour after journey	**Definition:** Percentage of animals lying down after journey. **Interpretation:** Increased lying behaviour, in comparison with housing conditions, after a journey might indicate a built up of lying motivation during the journey and a compensation of the energy demand to stand up during the journey (Tarrant and Grandin, 2000).

ABM: animal-based measure.

x) ABMs for the assessment of the welfare consequence restriction of movements.

Table 14: ABMs selected for the assessment of restriction of movements in cattle in transit

ABM	Definition and interpretation of the ABM
Latency to stand up after a falling event	**Definition:** Time required by the animals to regain the standing up posture after falling down. **Interpretation**: An increased latency to stand up after falling could occur due to inadequate space to adjust posture.
Overlying	**Definition:** Animals lying down (or attempting to) on other co-specifics. **Interpretation**: Overlying can occur if some animals are observed to lie down on another animal when both attempt to lie down at the same time.

ABM: animal-based measure.

3.3. Preparation of cattle for transport

Across cattle categories as well as journey types and journey durations, the preparation of cattle for transport may differ substantially. However, in terms of animal welfare, it is an important phase, as careful preparation of cattle 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 cattle until the initiation of loading of the cattle onto a vehicle or other means of transport. In effect, in this Opinion, the preparation of cattle for transport essentially involves the gathering of the animals to holding facilities and the keeping of them there prior to transport itself. Assessment of fitness for transport is included in the preparation stage. 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 can also be found in the Transport Guides. These issues will not be covered in this Scientific Opinion.

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3.3.1. Current practices

Depending on whether cattle are kept for beef or dairy, the production systems, and therefore also the preparation for transport, may differ considerably. Beef cattle may be kept under more or less intensive conditions. When intensive production takes place, the preparation phase of beef cattle can be compared to dairy cattle. Under more extensive conditions, preparation for transport is an infrequent practice, essentially involving the gathering of cattle to holding facilities and the keeping of them there prior to transport. The means of gathering and the type of premises used for holding cattle will depend on the type of journey being prepared for. The transport of cattle from one farm to another will involve the rounding up of the animals 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 cattle are being transported from one EU MS to another, they are likely to be brought by vehicle to an assembly centre from where they will be officially consigned and transported in an authorised truck. If the cattle are to be exported to third countries, they will very likely have to spend time in a quarantine premises in order to meet the requirements of the relevant health certificate. These animals have to be transported by road to the quarantine premises before the quarantine process can begin.

In most cases, dairy cattle will be loaded directly from their home pen. A few farms may have designated 'pick-up pens' where cattle, for biosecurity reasons, are housed for a short time (typically few hours) away from the main herd before loading. Likewise, only a few farms use some sort of vehicle outside the farm buildings for pick up.

The number of animals sent from a single farm differs between MS and between types of cattle. Typically, a single farm does not send full loads of cull cows for slaughter. Rather, one or a few cows are loaded on each farm. A full load, thus, typically originates from a number of different farms. Farms sending bulls or steers for slaughter may have more animals transported at the same time and may therefore send a full load – at least sometimes. The same holds for heifers (and cows) transported to other herds (nationally or in other countries) for dairy purposes. Transport may be directly from the farm of origin to the destination (slaughterhouse or another farm), or may be via an assembly centre, auction, CP or similar.

3.3.2. Highly relevant welfare consequences

In cases where cattle are kept in designated pick-up pens or vehicles before loading, the facilities and management may lead to different WCs. In cases where cattle are loaded directly from their home pen, some of these hazards and WCs may not be relevant (e.g. no/limited access to feed and water, or higher than normal animal stocking density).

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

i) Group stress

Group stress can appear during the preparation stage if animals from different origins are mixed before transport. The main hazards contributing to this risk are listed below.

Mixing unfamiliar animals: Mixing of unacquainted conspecifics is known to elicit aggression (Zayan and Dantzer, 1990) and to increase locomotory behaviour (Krohn and Konggaard, 1980; Gupta et al., 2006). Cattle are social animals and establish social bonds and hierarchy among themselves. The social hierarchy of cattle determines the priority of access to resources. Regrouping and relocation of animals commonly occurs as a husbandry practice to create homogenous groups organised by age, weight, production system (milk yield, body condition, reproduction and performance) and health state (Bøe and Færevik, 2003). Regrouping may occur by mixing animals on one occasion or repeatedly; and relocation as a change in location once or several times. Hurnik et al. (1995) defined a group 'as a collection of animals in which the animals are of the same species and the composition of the group is relatively stable over time', whereas grouping can be defined 'as the formation of a group of animals by natural means (e.g. herd formation as a result of social interaction) or by human action (e.g. allocation of a number of animals to a given pen or grouping of dairy cows according to milking performance)'. Hence, regrouping is regarded as grouping repeated once or several times (Bøe and Færevik, 2003).

The abrupt breakage of the social bond or hierarchy through regrouping and relocating may lead to social stress and an animal may respond with abnormal behaviour and consequent changes in the normal body metabolism and neuro-immuno-endocrine system.

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— PRE: Compatible groups should be selected before transport to avoid WCs. The following guidelines should be applied when assembling groups of animals; animals reared together should be maintained as a group even though they are of different sex and size; animals with a strong social bond should be transported together even though they are different sex and size; aggressive animals should be segregated.

Corrective/mitigating measure of group stress

If group stress is observed in the animals during the preparation phase, conflictive animals should be separated and given space.

ii) Handling stress

Handling stress is a highly relevant WC during the preparation stage. 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 and Estévez-Moreno, 2020). The hazards contributing to the risk of handling stress are listed below:

Inexperienced, untrained or aggressive handlers: Stockpersons and drivers of livestock vehicles handling animals should handle and load animals in a manner that reduces their fearfulness and improves their approachability. Usually, livestock drivers are the only ones present while the vehicle is on the road. How drivers operate vehicles, how much time they spend checking on animal welfare, and how well they are prepared to deal with emergency situations greatly influences the outcome of any livestock journey.

— 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 vehicle, as well as a detailed understanding of animals' behaviour and needs. Handler training should preferably be done at the loading/unloading site or similar. 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 an 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 and Estévez-Moreno, 2020). Practical teaching should 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 methods and devices: Tail-twisting, the use of devices to force animals to move, i.e. flags, electric prods and sticks; and the manner in which these devices are used, i.e. properly (with a soft touch, just to persuade), intense (stronger than before, but without damaging) or rough (rude, with excessive force, causing damage); have a great influence on the level of fear and stress of the driven animals.

— PRE: Handlers should be trained as described before in proper handling techniques (e.g. Grandin, 1999, 2008; Waiblinger et al., 2006). There are a number of handling practices, currently forbidden in the EU, such as to kick animals, to apply pressure on sensitive body parts, to drag animals by their ears, horns, legs or tail, to crush or twist their tail. Abuse includes beating animals, poking sensitive areas, dragging downed animals, deliberate slamming of gates on animals or deliberate driving animals over the top of downed animals. The use of electric prods is currently permitted under strict conditions, but less aversive handling methods are strongly recommended, as suggested by the Cattle Transport Guides (Consortium of the Animal Transport Guides Project, 2018).

Poor handling facilities: Poorly designed and poorly maintained facilities are stressors for handlers and animals. In these conditions, handlers are less efficient, tire more quickly and thus become rougher in their handling of the animals (Titterington et al., 2022). In addition, the risk of occupational accidents can increase.

— PRE: To prevent this hazard, handling facilities should be fit for purpose (Gallo et al., 2022).

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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: When possible, cattle should be habituated to handling before transport, as this will reduce the level of fear and stress of the animals. Calves having been in positive and more frequent contact with a stockperson are easier to handle and have lower heart rates during loading compared to calves that are not (Lensink et al., 2000a,b, 2001).

Corrective/mitigating measure of handling stress

Unsuitable handlers should be removed from the process and be replaced by suitable ones. Otherwise, unsuitable handlers or untrained handlers should receive training on the spot.

If the mix or aggregation of certain animals is creating too much stress, new groups should be formed to reduce the confrontations. Horned cattle require special attention, as they learn to use their horns aggressively, and are a risk to handlers and animals.

3.3.3. Fitness for transport

3.3.3.1. Introduction

Depending on the duration and quality of the journey, transport can represent significant hazards, even for healthy and physically fit cattle. These challenges are greater for cattle that are weakened or vulnerable. Those animals are most likely already experiencing WCs, even before being transported. In this condition, they are less able to cope with the hazards associated with transport, such as getting on and off the vehicle, interacting with other animals, maintaining stability, avoiding fatigue, responding to feed and water restrictions and to thermal environments. To reduce the risk of suffering during a journey, before cattle are loaded, their fitness for the intended journey must be assessed. Cattle should arrive at their destination in a similar condition to that assessed before loading with minimal deterioration during their journey. Cattle with conditions that affect their physical fitness and could result in exhaustion, debility or fatigue should not be transported and should be treated or euthanised on the farm.

Consideration of the pathophysiological implications of ill-health and injury on an animal's response to the potential physical and physiological challenges that can occur during transport can assist in identifying the welfare implications of transporting cattle that are not fit for the intended journey. Some cattle with health issues that are assessed as fit at the start of a journey can nevertheless deteriorate during the journey (Dahl-Pedersen et al., 2018a) and are more likely to die in transit, become non-ambulatory, or be euthanised on arrival than those that are healthy (Cockram, 2019).

Thus, if animals are not properly inspected, 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, 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, but some characteristics rendering animals unfit for transport do not directly relate to health, but to certain age groups or certain stages of the production cycle (Table 16).

The criteria and circumstances leading to a decision of 'unfit for transport' probably vary considerably across the different categories of cattle, thereby posing extra challenges for the professionals involved in decision-making: farmers, livestock drivers, hauliers, veterinarians and competent authorities (Dahl-Pedersen, 2022), as well as posing challenges to the welfare of cattle 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. Studies in cattle have reported doubt in the decision-making of involved professionals (Herskin et al., 2017; Dahl-Pedersen, 2022), and a comparison between and within three different professional groups (livestock drivers, veterinarians and farmers) regarding fitness for transport of dairy cows, showed at best moderate agreement (Dahl-Pedersen et al., 2018b). Hence, in order to prevent the significant welfare hazard of allowing unfit animals to enter a 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 all professionals involved are not properly educated, and if questions about responsibility are present, the risk of animals entering a 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 cattle 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.

3.3.3.2. Assessment of fitness for transport in cattle

The decision-making process for determining fitness for transport has several components and stages (Dahl-Pedersen, 2022). The initial stage is a determination/assessment and decision by the herdsman/farmer/producer on fitness. If the person is uncertain about fitness, this can be followed by a clinical veterinary examination or veterinary advice. In some situations, the recipient of the cattle at the destination is consulted. For example, a veterinarian at a slaughterhouse might be consulted to ascertain his/her opinion as to whether he/she is likely to accept the animal in a particular condition and the probability of the animal being condemned as not fit for human consumption. The final assessment before loading is made by the haulier/driver.

The fitness of cattle for transport is assessed by reference to either a list of general conditions affecting their ability to cope with transport or a list of specific conditions. If an animal is assessed as not fit for transport, the animal should not be loaded and transported. The cattle should remain on the farm, and depending on the nature of the condition, prognosis and the degree of suffering, on-farm euthanasia may be required. If the animal does not require euthanasia, transport can be delayed until fitness for transport is regained following a period of treatment or additional care if needed. In some circumstances, an animal might not be technically unfit for transport, but the ability to cope with transport, or the fitness, might be reduced. In such cases, animals can be transported on certain types of journeys and under certain conditions.

Various stakeholders have different views on the criteria for determining the severity of, for example, lameness and poor body condition that would make an animal unfit for transport (Grandin, 2016). There are potential dilemmas between the avoidance of the risk of suffering arising from a decision to not transport an animal that is not fit for transport, and the financial loss associated with on-farm euthanasia, compared with the potential return to a producer from transporting the animal for slaughter, so that it can be used for human consumption. There can be some limited options for on-farm (emergency) slaughter with transport of the carcass to a slaughterhouse, and where this option is available, it is likely to reduce some of the dilemmas associated with decisions on fitness for transport versus wastage of resources (Magalhães-Sant'Ana et al., 2017; Hultgren, 2018).

- Conditions rendering cattle unfit for transport

If, before transport, an animal has **a clinical condition that is painful**, transport will almost certainly aggravate the pain and may lead to suffering. Movement of, or pressure on, a painful area of inflammation, such as an arthritic joint, causes additional pain. Therefore, movement of body parts during loading, unloading, in response to vehicular movements or behaviour of other animals, and during postural changes, are likely to cause movement of the sensitive tissue, and result in additional pain. Animals should not be transported with a non-stabilised fracture as this will cause additional pain and may lead to suffering. Bone fractures are painful; mechanical pressure applied to the fracture site, or movement and mechanical distortion of fractured bone, causes pain (Cockram, 2019).

Lameness is common in cattle (Garvey, 2022) and can be detected using locomotion scoring (Schlageter-Tello et al., 2014; Tunstall et al., 2020). When an animal appears lame or is reluctant to walk, it is most likely experiencing pain. Prolonged standing and efforts to maintain stability when the vehicle or other animals move, 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 animals that remain standing. To be fit for transport, an animal must be able to stand, bear weight on all legs, and be able to adjust footing to maintain balance during the journey. The animal must also be able to walk up and down ramps at the start and the end of the journey.

ABMs for locomotion scoring are available for cattle (Schlageter-Tello et al., 2014; Tunstall et al., 2020), and have been established and validated as part of welfare assessment of cattle on farm (Welfare Quality®, 2009). However, even though all the existing guidelines for the assessment of fitness for transport of cattle involve lameness (Table 16), none of them recommend the use of locomotion scoring to establish thresholds of fitness for transport. For example, in the Danish

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guidelines (DVFA, 2019) it is clearly explained that the assessment of fitness for transport of lame animals should take into account the planned journey as well as the general condition of the animals, and therefore should, according to these guidelines, per definition be case-based.

Some conditions can make an animal unfit for transport, because they **reduce the ability to perform an important physiological function**, e.g. pneumonia can reduce exercise tolerance and capacity to deal with heat. Calves with pneumonia often have clinical signs of fever, increased frequency of respiration, and decreased tidal volume. The severity of these physiological effects is related to the pathological changes in the lungs, such as the constriction of airways, accumulation of mucus within the lumen of airways, oedema and thickening of the mucous membranes (Reinhold et al., 2002). Calves in this condition have reduced lung function that can result in hypoxia from impaired oxygen supply (Linden et al., 1995). If pneumonia affects a large proportion of the lung (Lowie et al., 2022), calves may not receive sufficient oxygen for muscular activity associated with handling and transport, resulting in reduced exercise tolerance.

The association between transport and the occurrence of the bovine **respiratory disease** (BRD) complex has long been recognised. Many hypotheses regarding this association have been declared through the past decades, and it is agreed upon by most researchers that the multiple stressors that calves experience during transport result in an overall immunosuppression that allows the respiratory tract to be invaded by numerous opportunistic pathogens (Buckham-Sporer et al., 2008; Sporer et al., 2008; Earley et al., 2017).

Most **cull cattle** should be considered vulnerable, as they are at a greater risk of deteriorating during the journey (Dahl-Pedersen et al., 2018a) than other cattle types. See Section 3.8 for examination of concerns for animal welfare in cull dairy cows during transport. In general, cattle that are emaciated or otherwise weakened may have reduced ability to obtain feed and water and respond to external events such as vehicle motion or physical interactions with other animals. Weak animals are more likely to fall and be unable to get back up again and regain their footing. An animal in a poor body condition has limited fat reserves and is likely to be more susceptible to the combined effects of fasting and cold exposure.

Another vulnerable group of cattle is **lactating females.** During lactation, the udder fills with milk and becomes painfully engorged if the pressure is not relieved by milking or suckling by offspring (Bertulat et al., 2013). If the udder has become painfully engorged, animals may appear reluctant to lie down, have inflamed mammary glands (swollen, painful, warm or red) and may appear lame. The risk of udder engorgement in a lactating animal destined for transport should be reduced by making arrangements for the lactating cow to be milked before the start of a journey and at regular intervals during the journey, or even better – if possible – to dry-off several weeks before the journey.

A special case of cattle transport is the export of **early lactation dairy cows** from EU MS. All existing guidelines for the fitness of transport of cattle mention that females should not be transported shortly after birth, and different thresholds are mentioned (e.g. 48 h or 7 days; Table 16). However, at least until 3 weeks post-calving, dairy cows are in the so-called transition period, as recently reviewed by Redfern et al. (2021). During this period, important physiological, metabolic and nutritional changes take place, and it is during this period, that most metabolic disorders occur (Mulligan and Doherty, 2008; Mezzetti et al., 2021), and dairy cows experience high rates of metabolic diseases that are detrimental to animal welfare (Suthar et al., 2013; Macrae et al., 2019). Around calving, the suspension of the pedal bones weakens due to hormonal changes, and the risk of hoof horn lesions increases (Tarlton et al., 2002). Efforts to maintain balance during transport may be associated with an increased risk of developing hoof horn lesions in cattle transported shortly after calving. No studies relating the characteristics of transition dairy cows to fitness for transport have been found, which constitutes a gap in knowledge, as these animals are likely to be more prone to WCs, especially during long and complicated journeys, as compared to the average animal.

Another concern for animal fitness for transport is **pregnancy** (Velarde et al., 2021). Pregnant heifers are exported from EU MS as breeding animals. In addition, pregnant cows may be sent to slaughter. Sending cows to slaughter in the final third of gestation is not uncommon (EFSA AHAW Panel, 2017; Nielsen et al., 2019).

The concern for animal welfare in relation to transport of pregnant females is two-fold, and includes the pregnant female as well as the fetus/newborn:

Concerns for the welfare of the pregnant female relates to:

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

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

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

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

Table 15: Increases in fetal fluids (mL), placenta (g) and metabolisable energy requirements (MJ) for pregnancy during gestation in cattle

		Gestation stage (month)										Source
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	
Total volume of fetal fluids	mL	58	170	844	3,573	4,544	5,653	9,229	10,050	15,125	20,072	Arthur (1957)
	% of final stage	< 1	1	4	18	23	28	46	50	75	100	
Allantoic fluid volume	mL	4	94	333	476							Eley et al. (1979)
Amniotic fluid volume	mL		11	109	149							
Placental membrane weight (g)	g	1	21	154	443							
Metabolisable energy requirement for a 600 kg pregnant cow	MJ/day	51	52		53		55		60	72	89	Moe and Tyrrell (1972)
	% of final stage	57	58		60		62		67	81	100	
Metabolisable energy requirement for pregnancy	MJ/day				3	4	6	9	14	22	33	Sguizzato et al., (2020)
	% of final stage				9	12	18	27	40	67	100	

A cow is pregnant for approximately 9 months (283 days) (Carthy et al., 2014; Vieira-Neto et al., 2021), and pregnancy does not in itself make a cow unfit for transport (Lay Jr et al., 1996). Table 15 shows the increases in foetal fluids, placenta and metabolisable energy requirements for pregnancy during gestation.

Price et al. (2015) did not identify increasing effects of gestation length (between 60 and 140 days of gestation) on stress and blood metabolite responses (glucose and NEFA) of cows to repeated 2-h journeys. However, in late pregnancy, there are physical and physiological changes that increase the challenges experienced by a cow during transport. Cows and heifers transported on long journeys in late pregnancy (especially the final 2 months of gestation) are at increased risk of metabolic disorders due to the effects of fasting and motion stress (Hepple et al., 2010). The placenta and developing fetus utilise glucose, and this places increasing demands on the energy metabolism of the cow (Bell and Bauman, 1997). To reduce the risk of ketosis and fatty liver, a pregnant cow requires an adequate supply of nutrient energy during the last 2–3 weeks before calving (Gerloff, 1988) rather than the fasting period that may accompany longer duration transport (Warnock et al., 1978). Fisher et al. (1999) reported that non-lactating dairy cows that were pregnant for 183–192 days showed increased serum β-hydroxybutyrate (BHB) concentration and reduced serum Mg concentration after 3–4 days of road and sea transport. However, Lambooy and Hulsegge (1988) did not identify an increased plasma BHB concentration after pregnant heifers (about 150 days gestation) had been transported without feed and water for 18 h. Warnock et al. (1978) reported that fasted dairy cows that were more than 8 months pregnant had lower serum Ca concentration than cows that were not pregnant or were less than 6 months pregnant. Although pregnant cows are capable of exercise during the last 2 months before calving (Lamb et al., 1981), pregnancy is associated with changes in the cardiovascular system that could reduce the exercise capacity of a cow. The blood flow to the

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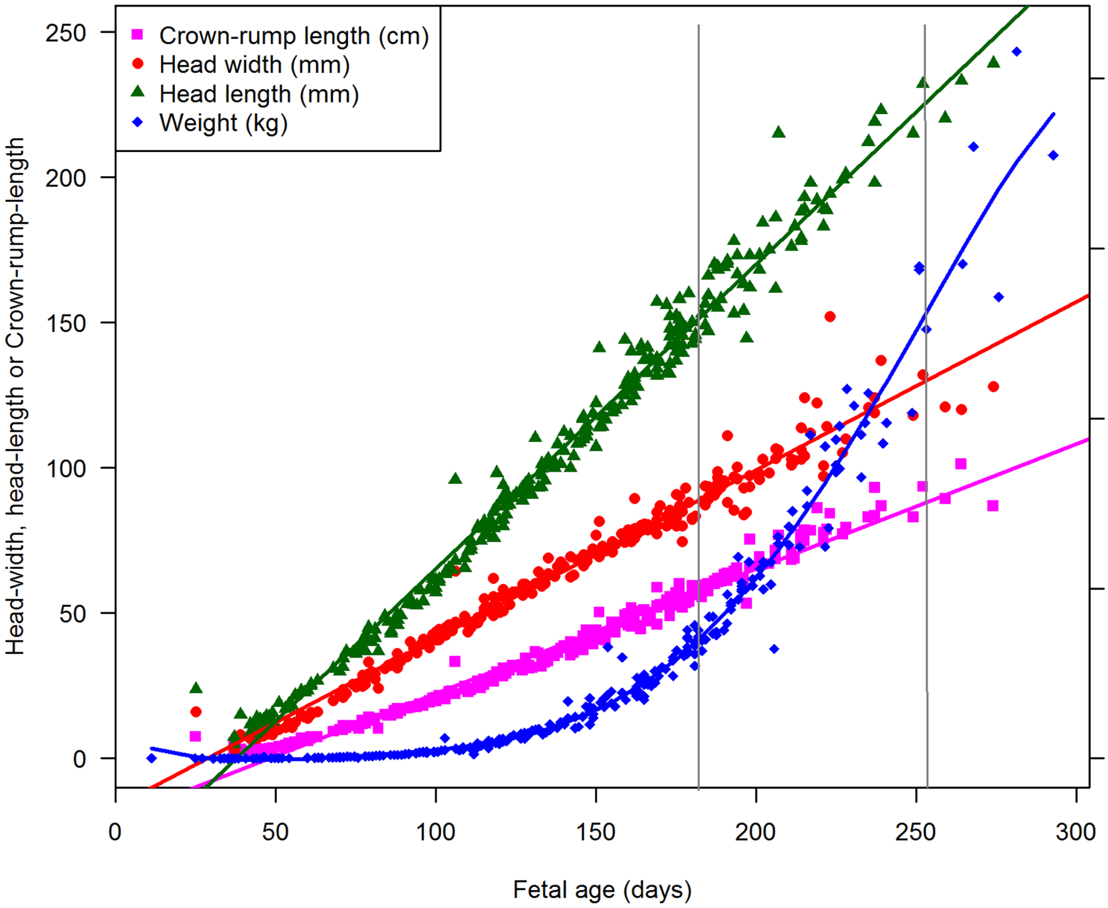
pregnant uterus increases significantly already from day 25 of gestation (Ford, 1982). Pregnant cows are at increased risk of heat stress with increased gestation (Fabris et al., 2019). The increase in the volume of the allantoic and amniotic fluid compartments with gestation (Bongso and Basrur, 1976), especially after 80 days of pregnancy, and increased udder size will affect the locomotion of the cow (Chapinal et al., 2009). In addition, the stress of transport can cause abortion/premature birth (Nagel et al., 2019).

From Table 16, it is clear that there is consensus among different guidelines from e.g. industry (e.g. MLA (Meat and livestock Australia) (2019)) but also governmental (CHAR, 2022)) and intergovernmental sources (WOAH, 2011) to recommend that pregnant cows are not transported beyond 90% of pregnancy. However, no evidence to support these guidelines have been found, as, for example illustrated from Figure 1, showing the fetal growth curve of calves.



Pregnancy is on average 283 days. Points are observations, whereas the lines represent the modelled curves (linear models for all but the 5-degree polynomial model for the fetal weight). The two vertical lines denote the last trimester and the 90% threshold.

Figure 1: Fetal growth curve (in blue) and other fetal measurements in relation to fetal age in bovine Holstein fetuses. Source: Krog et al. (2018)

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. Prenatal stress in cattle has been reviewed by Merlot et al. (2013) and by Arnott et al. (2012), concluding that prenatal stress in beef and dairy cattle has implications for offspring welfare and performance, but that there are considerable gaps in knowledge about the effects of prenatal stress in cattle. Thus, it cannot be ignored that transport stress experienced while *in utero* does affect calves. Following *in utero* heat stress in late gestation, calves have been reported to have lower circulating immunoglobulin G (IgG) concentrations relative to calves gestated in dams that received heat stress abatement (Ahmed et al., 2021).

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During late pregnancy colostrum is formed when mammary cells are proliferating and differentiating in preparation for lactation; this process is called colostrogenesis (Baumrucker et al., 2010). Colostral immunoglobulins arise from systemic and local sources (Hurley and Theil, 2011). Bovine IgG_1 is specifically transported by a process of transcytosis across the mammary epithelial cells during colostrogenesis by an IgG_1-specific receptor, FcRn (Hurley and Theil, 2011). The decrease in blood IgG_1 concentrations prepartum, in the last month of gestation, is much greater in dairy (Guy et al., 1994) and beef × dairy (McGee et al., 2005; Murphy et al., 2005) cows compared to beef breed cows, implying that more IgG_1 is transferred into colostrum for the dairy and dairy crossbred genotypes. Indeed, heat stress during late gestation is reported to decrease the concentration of IgG and IgA and the percentage of total protein in colostrum (Nardone et al., 1997), which may compromise passive immune transfer in calves born from heat-stressed cows (Fabris et al., 2019; Almoosavi et al., 2020).

Kukharenko and Fedorova (2018) examined calves born from heifers transported by road (N = 100; journey duration 4 days) from neighbouring countries to Russian regions during the hot summer period. The transport conditions or stage of pregnancy were not described. The authors reported the occurrence of abortions (3/100) and stillborn calves (6/100).

EFSA AHAW Panel (2017) concluded that livestock fetuses in the last third of gestation have the anatomical and neurophysiological structures required to experience negative affect. However, regarding welfare of fetuses during transport, EFSA AHAW Panel (2017) concluded, with 66–99% likelihood, that the neurophysiological situation of 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 most likely are 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. To summarise, advanced pregnancy is associated with increased risks of WCs during transport.

Young calves have a reduced capacity to withstand the challenges of transport. See Section 3.9 for examination of concerns for animal welfare in unweaned calves during transport.

Conditions affecting fitness for transport are listed in several regulations (Government of Canada, 2022; Council Regulation (EC) No 1/2005)[1], and there are several guidance documents and decision trees available to assist in the assessment of the fitness of cattle (Eurogroup for Animals et al., 2015; MLA, 2019; Farm Animal Council, 2010; WOAH, 2011). These are summarised in Table 16. Meat and Livestock Australia and LiveCorp (2021) have also produced guidance on the assessment of the fitness of cattle before live export by sea.

Table 16: List of conditions that can make cattle unfit for transport

General condition	Specific condition	Reference
Sickness/illness	Not specified further	CHAR, WOAH
	Pathological processes	CR2005
	Laboured breathing	CHAR, EGA
	Congestive heart failure	OFAC
	Generalised nervous system disorder	CHAR, OFAC
	Shock or dying	CHAR
	Fever	CHAR, OFAC
	Pneumonia (unresponsive with fever)	OFAC
	Infected navel	CHAR
	Gangrenous udder	CHAR, EGA, OFAC
	Acute mastitis	MLA, OFAC
	Bloated to the extent that it exhibits signs of discomfort or weakness	CHAR, EGA, OFAC
	Massive purulent discharge	EGA
	Actinomycosis (lumpy jaw)	MLA, OFAC
	Extensive cancer/leukosis	OFAC
	Ketosis	OFAC

General condition	Specific condition		Reference
	Orchitis		MLA
	Swollen penis		MLA
	Multiple abscesses		OFAC
	Peritonitis		OFAC
	Urinary calculi causing abdominal distention		OFAC
Pathophysiological state	Weakness		CR2005, EGA, DVFA, MLA, OFAC, WOAH
	Emaciation		AAWSG, CHAR, EGA, MLA, OFAC
	Fatigue/exhaustion		CHAR, OFAC, WOAH
	Dehydration		AAWSG, CHAR, OFAC, MLA
	Distress		AAWSG
	Hypothermia		CHAR
	Hyperthermia	Not specified further	CHAR
		Heat stress	MLA
	Engorged udder		MLA
Eye lesion	Blind in both eyes		AAWSG, EGA, MLA, WOAH
	Severe squamous cell carcinoma		CHAR, MLA, OFAC
Injured	Not specified further		AAWSG, CHAR, CR2005, WOAH
	Severe open wound or a severe laceration		CHAR, CR2005, EGA
	Disabled/infirmity		CHAR, WOAH
	Unhealed wounds after recent surgery		EGA, WOAH
	Severe haemorrhage		EGA, OFAC
	Has sustained an injury and is hobbled to aid in treatment		CHAR
	Ingrown horn, broken horns if bone tissue is affected or animal appears depressed		MLA, DVFA
Prolapse	Prolapsed uterus or a severe rectal or severe vaginal prolapse		CHAR, CR2005, EGA, OFAC
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, OFAC
Experiencing pain	Cannot be moved/transported without causing additional suffering		AAWSG, CHAR, WOAH
	Experience pain when moving		EGA
	Fracture that impedes mobility or causes pain or suffering		CHAR, OFAC
Lameness	Unable to bear weight on each leg		AAWSG, CHAR, DVFA, EGA, MLA, OFAC, 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, DVFA, OFAC
	Unable to walk as fast as a brisk human pace (cannot keep up with the healthy herd)		EGA
	Likely to lose balance during transport		EGA
	Arthritis in multiple joints		OFAC
Non-ambulatory	Not specified further		OFAC, CHAR, CR2005, EGA, MLA, WOAH

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General condition	Specific condition		Reference
Reproductive state	Pregnancy	Final 10% of their gestation period	CHAR, CR2005, MLA, WOAH
		Last month of pregnancy	EGA
		Within 2 weeks of calving	AAWSG
	Recent calving	Given birth within the previous 48 h	CHAR, OFAC, WOAH
		Given birth within previous week	CR2005, EGA
		Given birth within the previous 14 days	DVFA
		Visible placenta	EGA
Newborn	Unhealed navel		CHAR, CR2005, WOAH
	< 1 week old		CR2005, OFAC

Material has where appropriate been modified for consistency and clarity

- AAWSG: Australian Animal Welfare Standards and Guidelines (2012).
- CHAR: Government of Canada (2022).
- CR2005: European Council (2005).
- DVFA: The Danish Veterinary and Food Administration guide (2019).
- EGA: Eurogroup for Animals et al. (2015).
- MLA: Meat & Livestock Australia (2019).
- OFAC: Ontario Farm Animal Council (2010).
- WOAH: World Organisation of Animal Health (2011).

3.3.3.3. Transport of animals with reduced fitness

Some guidelines and regulations list conditions that would consider cattle vulnerable, but fit for transport as long as mitigation measures are used, e.g.

a) the animal is isolated;
b) the animal is individually loaded and unloaded without having to negotiate any ramps inside the conveyance;
c) measures are taken that are necessary to prevent the animal's suffering, injury or death during loading, confinement, transport and unloading; and
d) the animal is transported directly to the nearest place, other than an assembly centre, where care or euthanasia can be applied.

Examples of additional measures that could be used to reduce the risk of suffering by animals of reduced fitness during transport include: increased contingency planning, reducing journey duration, adjusting ventilation, increased 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 an animal with reduced fitness is questionable. There are also differing opinions on the types of conditions that would make vulnerable cattle fit for transport, even when additional mitigation measures are used. If animals of reduced fitness are transported, they are likely to continue to experience pain and discomfort, there is a risk of deterioration of the animal during the journey, and pre-existing conditions are likely to be aggravated by transport (Dahl-Pedersen et al., 2018a; Cockram, 2019). This constitutes a gap in knowledge.

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 (as recently reviewed by Gallo et al. (2022) and

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Cockram (2022)). 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. 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 transport (Miranda-de La Lama et al., 2014). Loading may be preceded by potentially stressful practices such as social separation, mixing and physical restraint that all may impact cattle, and the response of animals to unloading depends on the cumulative effect of, for example, journey duration, road conditions and microclimatic conditions (Lee et al., 2017).

The infrastructure for loading/unloading tends to be heterogeneous in terms of design and manufacture, being highly dependent on the degree of innovation of the logistics chain, although in general it can be classified into three categories: (1) fixed ramps or docks, (2) mobile ramps or docks, and (3) ramp systems and/or automated lifts. The first is usually part of the equipment present in cattle 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 WCs selected as highly relevant during the loading/unloading of cattle were: handling stress, heat stress, injuries, and sensory overstimulation. The selected ABMs for the assessment of these WCs are shown in Section 3.2.2. Below, hazards, preventive and corrective/mitigating measures are described.

i) Handling stress

Handling stress is caused by physical exertion, noise, and the contact with people during handling (Trunkfield and Broom, 1990). Most of the hazards contributing to the risk of handling stress during loading and unloading of the animals are identical to the ones present during the preparation stage (i.e. inexperienced handlers, inadequate handling and physical force/instruments; Section 3.3). In addition, the hazards specific for handling stress during loading and unloading, and their preventive measures, are:

Inappropriate facilities: If the access of the vehicle to the loading point is difficult, it can increase the handling stress of the animals during loading. In addition, slippery floors or inappropriate ramps and side protections may result in increased interventions by the handlers and cause fear and loss of balance in the animals.

— PRE: Loading facilities should be properly designed including non-slippery materials. A maximum ramp angle of 20° is appropriate for cattle, providing it has non-slip floors and appropriate cleats at 30 cm intervals.

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 in this matter; animals reared together should be maintained as a group even though they are of different sex and size; animals with a strong social bond should be transported together even though they are of different sex and size; aggressive animals should be segregated.

Corrective/mitigating measure of handling stress

As suggested by the Consortium of the Animal Transport Guides Project (2018), if an animal stops and refuses to move, the following procedure should be applied: behave calmly, and let the animal calm down, check that the animal is not showing signs of sickness, is wounded, or in other ways unfit for transport. If this is the case, remove the animal from the race and decide on the course of action. Corrective measures include removal of the specific person performing inappropriate handling or provision of assistance to him/her on the spot.

ii) Heat stress

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

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information on the influence of high temperature on the welfare of cattle during transport can be found in Section 3.5.3.1 dealing with heat stress during the transit stage.

High effective temperature: In cattle, heat stress is mainly due to high air temperature but intensified by high humidity (e.g. from animals emitting water vapour), solar radiation (lack of shade), low air movement and by metabolic heat. Solar radiation is a major factor in heat stress and increases heat gain (Berman and Horovitz, 2012). High ambient temperature accompanied by high air humidity may cause discomfort and enhances the stress level (Ganaie et al., 2013; Gonzalez-Rivas et al., 2020).

- PRE: To prevent high temperatures, avoid loading cattle during the hottest hours of the day. This is particularly relevant if the vehicle is not equipped with a mechanical ventilation system. Shading can help to protect the animals from solar radiation and from high ambient temperatures.

Pre-transport insufficient water provision: If animals have not been provided water during the preparation phase, there is an increased risk of heat stress during later stages of transport.

- PRE: Animals should be given access to water until the moment of loading.

High stocking density: Loading cattle in bigger groups with small space can increase the risk of heat stress during the process.

- PRE: Under hot climatic conditions, lowering stocking density at loading will help prevent heat stress, especially when no mechanical ventilation is provided.

Delays to loading/unloading: Delays in loading and unloading can happen (e.g. due to the facilities in the premises, lack of organisation, handler untrained). Any delay in the time of loading/unloading in high temperatures and humidity will increase the probability of heat stress. Moreover, internal temperature increases in a stationary vehicle if not ventilated.

- PRE: Loading/unloading should be well planned in advance and should include informing staff at the destination of the time of arrival such that the facilities are prepared, and thus avoiding any unnecessary delays. The loading order of the animals and standardised protocols should be ready beforehand, avoiding any unnecessary delays.

Corrective/mitigating measure of heat stress

After the loading process has started, any heat stressed animal should not be loaded. Affected animals should be moved to a place with shade and ventilation (fan) and water should be provided with no restrictions. Animals can be cooled down using water sprinklers, showers, or equivalent, but caution should be taken as these procedures may themselves increase the risk of heat stress due to the increased humidity. 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 cattle are associated with the facilities and the handling of the animals.

Inappropriate handling methods and devices: Wrong use of guillotine doors at the rear of a trailer can cause injury and bruising, especially in the back and rump zones of the cattle. Most of the bruising is the result of physical blows from driving instruments like sticks, projecting objects in facilities and vehicles, and animals falling (Chambers et al., 2004).

- PRE: As described in the preparation stage, handlers should be trained in proper handling techniques, and pay special attention to the use of guillotine doors. Lesions during loading and unloading can be reduced by training people in animal behaviour and low stress handling methods (Grandin, 2008).

Inadequate facilities: The presence of sharp or protruding surfaces on ramps, vehicles and equipment, as well as slippery floors highly contribute to the risk of injuries during loading. Bruises are usually caused by impact with blunt objects such as sticks, horns of other animals, farm structures or vehicle structures (Mendonça et al., 2016).

- PRE: Loading facilities should be adequate and properly maintained. To minimise slipping and soiling, non-slip flooring is necessary, since it is impossible to handle large animals safely when they are slipping on the floor or panicked because they are losing their footing (Šímová

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et al., 2016) and it may cause lesions. Stairsteps are recommended on concrete ramps. Stairsteps are easier to walk on after the ramp becomes worn or dirty. The steps should be grooved to provide a non-slip surface (Šímová et al., 2016). Future focus on injury prevention by padding or similar of the inside of vehicles, being able to withstand the requirements for cleaning and disinfection (due to biosecurity concerns), is recommended.

Corrective/mitigating measure of injuries

In severe cases when lesions are observed, the affected animal should receive veterinary treatment and their fitness for transport be re-evaluated.

iv) Sensory overstimulation

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

Noise, odours and light reflections: Exposure to sensory stressors of a simultaneous and changing nature at varying intensities and frequencies is closely related to the risk of stress and restlessness in animals. Common examples during loading are exposure to dust, odours and noises from the vehicle engine, as well as contrasting light.

- PRE: If the loading and unloading areas are congested, ventilation is required and any unnecessary noise (motor, yelling, dogs barking) should be stopped. Lighting during loading/unloading should be considered to avoid strong contrast between bright light and shadows. At night, lighting should be positioned to give even illumination over ramps, races, yards, inside the vehicle, personnel access areas, and should not shine into the eyes of livestock moving in the desired direction. In the morning and evening, walking cattle directly towards bright light (artificial or sun) should be avoided.

Shouting: Waynert et al. (1999) reported that sound made by people during handling increased the heart rate and the reactivity of the animals more so than the banging of gates, whereas Pajor et al. (2000) found that shouting was aversive, and especially at high pitch frequencies (Lanier et al., 2000).

- PRE: Reducing sound during handling would reduce cattle fear levels. Low pitch frequencies are recommended when orally signalling to cattle and seem to calm down the animals (Arave, 1996). Cattle are able to identify the human voice, and changes in tones can change the behaviour of the animals (Waynert et al., 1999).

Corrective/mitigating measure of sensory overstimulation

Animals should be provided with enough time to recover in a calm environment limiting the exposure to the mentioned hazards.

3.5. Transit stage

3.5.1. Current practice

There is a diverse range of vehicles used to transport cattle, from small trailers to specialised double-decker trucks (see, e.g. Figures 7 and 15).

Throughout the transit stage, even under favourable conditions, cattle 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 stress, injuries and fatigue (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.

3.5.2. Highly relevant welfare consequences

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

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i) Heat stress

Some of the hazards contributing to the risk of heat stress during the transit stage are identical to the ones present during the preparation stage (i.e. high effective temperature and pre-transport insufficient water provision; Section 3.3). Additional hazards that contribute to heat stress together with their preventive, mitigative and corrective measures are described below. More detailed information on the microclimatic conditions recommended for the transit stage is included in Section 3.5.3.1.

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 cattle during the hottest period of the day. Avoid stopping transport vehicle (unless air-conditioned), only transport the cattle in colder part of day (night if necessary). In the longer term, the roofs and walls of vehicles could be insulated and/or constructed of radiation-reflective material.

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

— PRE: To reduce the risk of high effective temperatures inside the vehicle, 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 cattle that can be loaded onto a vehicle and the amount of metabolic heat and moisture that they produce will increase.

— PRE: Theoretically, increasing space allowance can help to reduce the risk of heat stress. However, as described in detail in Section 3.5.3.2, the proportion of animals needed to be reduced to achieve this benefit is very important.

Corrective/mitigating measure of heat stress

When animals show signs of heat stress, and available ventilation is not sufficient to mitigate the heat stress, they should be unloaded immediately, moved to a place with shade and ventilation (fan) and provided with water. Animals can be cooled down using water sprinklers, showers or equivalent. Proper design and location of water sprinklers, and showers are essential. The addition of water without the use of ventilation fans will lead to increased humidity and heat stress in holding pens. Fans in sprinklered systems may be susceptible to fouling and need to be cleaned frequently.

If unloading cannot be done immediately, it is recommended to complete the journey as soon as possible, increasing ventilation and providing convective air cooling (when available). Avoid stopping the vehicle when ventilation is restricted.

ii) Motion stress and sensory overstimulation

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 of cattle. The main hazards leading to these WCs in cattle during the journey are listed below, and the ABMs have been described in Section 3.2.2. During transport, animals are exposed to vertical, lateral and horizontal vibrations. Unpaved roads or roads with strong wind currents transmit a more significant amount of vibrations. An animal's sensitivity increases after long standing periods (Gebresenbet et al., 2011), causing fatigue and displacement of their centre of gravity, which leads to falls and injuries (Bulitta et al., 2015). Additionally, animals make a more considerable effort seeking for a place to lean on in the vehicle during braking (Broom, 2007). The more tired and unsteady the animals become, the more likely they are to slip and fall, which can result in injury (Schuetze et al., 2017). In addition, cattle are exposed to numerous unfamiliar stimuli such as sound, visual and olfactory cues that makes transport a strange, aversive and very physically demanding procedure for animals (Miranda-de La Lama et al., 2014). Transport motion research has focused on road transport and there is, thus, limited research on sea or air transport motion, even though it has potential to stimulate the vestibular system and produce body instability, both precursors of motion sickness. The vestibular system detects motion and gravity and initiates movements to maintain balance and orientation. Further research is needed to investigate physiological and behavioural responses to determine responses to this condition, leading to a better understanding of how motion sickness could affect livestock during transport (Santurtun and Phillips, 2014).

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 cattle (as reviewed by Tarrant, 1990).

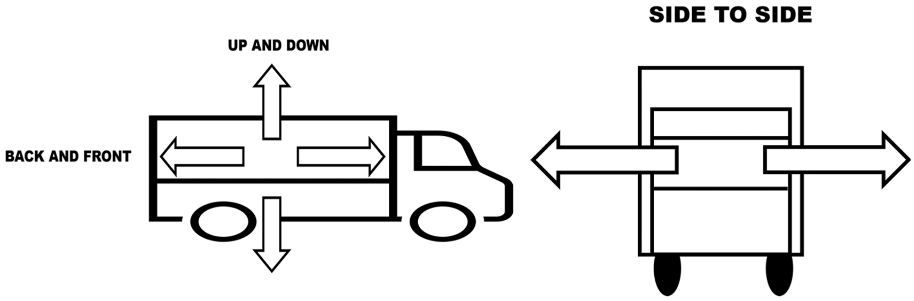

Figure 2: Schematic drawing showing the three planes of vibratory movements animals are exposed to during transport by road. Adapted from Humane Slaughter Association, 2022

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. Duration depends on journey duration and onset of vehicle 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 vehicle accidents. These are not covered specifically in this Scientific Opinion but may have severe consequences in terms of animal welfare. See Section 3.5.3.3A [Motion stress and sensory overstimulation] for additional information on sensory overstimulation and motion stress and how the WCs develop 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 cattle are to some extent exposed to motion stress. The stress is increased during acceleration events (including braking and accelerating) and turns. Rural roads are a mixture of paved, unpaved and surfaced roads. The latter two increase the transmission of vibration to the animals compared to larger roads and, when waterlogged, can cause the vehicle to lose stability and the animals can lose their balance.

- PRE: Among the preventive measures for this hazard are planning the journeys on motorways, and driven by experienced and skilled drivers. In addition, vehicle vibration can be reduced through 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 (Kehler et al., 2022). Low inflation of the tires and air suspension systems have been recommended by some authors versus leaf spring systems (Aradom, 2013).

Poor driving: The driver's ability to control the vehicle 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., 2012b).

- 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.

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Condition of the vehicle: When tires are over-inflated, this increases the vibration in the trailer, and may increase the stress of cattle (Stevens and Camp, 1979). Inadequate suspension systems are considered very stressful factors during animal transport (Gebresenbet, 2003).

— PRE: Use a vehicle that is serviced and avoid overinflated tyres.

Slippery floors: Wet vehicle floors can be a risk element for animals due to leaking water from water troughs or nipples, excess slurry, and no or little bedding material. Inappropriate surfaces or non-properly maintained floors can also contribute to the risk of slips and falls.

— PRE: Non-slippery floors are essential, and they should be properly maintained to avoid edges and cavities that can lead to falls and injuries. Additional bedding can be added in the case of slippery floors. This may also reduce resting problems. It is important to consider that 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, cattle need space in order to be able to balance and adjust their posture to 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. Intensive noises from other road vehicles, trains, industrial areas and other conspecifics, new odours from engine fumes, dust exposure and the fast-changing sight due to the movement of the vehicle can lead to sensory overstimulation in the animals.

— 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 measure of motion stress and sensory overstimulation

In cases of severe motion stress and/or sensory overstimulation, the driver can stop the vehicle, unload the animals and give them some rest. Otherwise, the best option would be to finish the journey as soon as possible.

iii) 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 cattle during journeys. Depending on factors such as time off feed before the journey starts, cattle 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 of feed while transported, 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. 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, see Section 3.5.3.3 for an assessment of the development of the WC over time. To prevent too long feed deprivation, the feed should not be withdrawn prior to transport. Careful planning of the transport time, scheduling and prioritising the slaughter of the animals (if destined for slaughter) should be done to avoid prolonged periods of feed withdrawal.

Corrective/mitigating measure of prolonged hunger

The most effective measure to correct hunger is to offer feed by stopping the vehicle or unloading the animals to allow cattle to feed. It is vital that the animals are familiar with the type of feed

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provided, and this is especially important in animals coming from extensive systems (little experience with pellet feeding).

iv) 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 if they are not able to obtain enough water (e.g. due to 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, cattle may not be thirsty during the initial phase of the transit stage, but thirst will develop over time, if they are not able to drink as much as they need. The duration of thirst depends on time off water, and severity is expected to increase with increasing journey duration and heat, as the need for water becomes more problematic for the animals. Prolonged thirst may lead to dehydration, discomfort, and suffering. Additional information on this WC is included in Section 3.5.3.3. The main hazards, preventive and corrective/mitigating measures are:

Time off water: The absence of water during the journey is a risk for cattle, leading to electrolyte imbalance in plasma measurements. These effects can be exacerbated if the journeys are in hot environments, if they involve high stocking densities, or if they are associated with exacerbated motion stress. There is no demonstration of advantage in water deprivation prior to transport (Sparke et al., 2001; Earley et al., 2006).

- 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.

Insufficient/inadequate 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. Water supply systems may become inadequate during very hot weather when demand is high. Low intake of water during journeys will be evident on arrival with animals being highly motivated to drink, and they may show signs of dehydration despite having water during the transit stage.

- PRE: To prevent this hazard, cattle should not be transported in vehicles with inadequate/insufficient drinking devices. In addition, in vehicles equipped with drinking devices, periodic checks should be carried out to ensure the functionality of the drinkers including the water flow rate.

Reduced intake of water: Technical failures may lead to inaccessible, inefficient or completely failing water systems. Only a few studies have examined the intake of water during transport. Results from studies by Earley et al. (2010, 2013) (reviewed in Section 3.5.3.3D) suggest that, if cattle are provided with access to drinking water during the transit stage, they may not drink, and if they do, their consumption may be reduced compared with non-transported cattle. Thus, it is possible, that cattle, even when water is available on vehicles during the transit stage, and they are familiar with the equipment, for some reason (i.e. potential difficulties to access the drinkers) are reluctant to drink in transit.

- PRE: If it is correct that cattle 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 vehicles. 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, cattle should be watered there.

High effective temperature in vehicle: Previous experiments suggested that water needs can increase from 1.2- to 2-folds in case of heat stress (Beatty, 2005).

- PRE: To prevent this hazard, temperatures inside the vehicle should be kept inside the thermal comfort zone, as described in detail in Section 3.5.3.1.

Space allowance: If cattle 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.

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- PRE: Previously, a space allowance corresponding with a k-value of 0.0315 was recommended for cattle 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. See Section 3.5.3.2 for examination of space required for cattle to drink onboard transport vehicles.

Corrective/mitigating measure of prolonged thirst

If cattle are suspected to be experiencing the WC prolonged thirst during the transit stage, the vehicle should be stopped, and all animals given access to drinking water, providing enough time for this.

v) Respiratory disorders

Respiratory disorders, such as BRD, are caused by different bacterial and viral pathogens (Grissett et al., 2015) and have been associated with cattle of most ages (Earley et al., 2017; Cirone et al., 2019). Although studies of respiratory microbiota associated to BRD are actively ongoing, only a limited number of studies have been performed to investigate the longitudinal changes of the microbiota of calves diagnosed with BRD compared to healthy calves or its evolution after antibiotic therapy (Timsit et al., 2016; Zeineldin et al., 2020).

Respiratory disorders are regarded as a highly relevant WC during the transit stage. Across the different cattle categories, the prevalence may not be high, but the duration of the respiratory disorders will often outlast the duration of the journey itself. The diagnosis of BRD remains a challenge due to the lack of a validated accurate ante-mortem diagnostic method meaning that delayed and under-detection of BRD is a significant problem. Evaluation of clinical respiratory signs (cough, nasal discharge, eye discharge, ear drooping or elevated rectal temperature) is widely used at farm level for BRD diagnosis (Buczinski and Pardon, 2020). Different respiratory scoring charts have been developed, especially for use in pre-weaned calves (Buczinski et al., 2015). These BRD scoring systems are one way to standardise diagnosis that can be easily applied by stockpersons (Love et al., 2014). The one most frequently applied, with some modifications, is the Wisconsin calf respiratory scoring chart (McGuirk and Peek, 2014). This scoring system classifies rectal temperature, presence of cough, appearance of nasal and eye discharges, and ear position with scores ranging from 0 to 3 (from normal to very abnormal), and the sum of all scores of each clinical sign defines the total clinical respiratory score. However, the diagnostic accuracy of these scoring systems is only moderate, and between-observer agreement of clinical scoring for BRD remains rather low (Buczinski et al., 2016). Thoracic ultrasonography (TUS) is a rapid, non-invasive, on-site diagnostic and prognostic tool for detection of lung abnormalities (Lowie et al., 2022) and it has been evidenced as the most accurate diagnostic test for pneumonia in calves (Lowie et al., 2022). TUS has greater accuracy for BRD diagnosis compared to conventional methods such as auscultation or clinical scoring criteria in dairy (Cramer and Ollivett, 2019; Cuevas-Gómez et al., 2021; Rhodes et al., 2021; Lowie et al., 2022) and beef calves (Cuevas-Gómez et al., 2020). Indeed, few studies have used clinical scoring of signs which are suitable to differentiate animals with pneumonia from animals with only an upper respiratory tract infection. TUS has the advantage of detecting sub-clinical cases of BRD that are undetected by clinical respiratory signs alone. Different abnormalities that can be detected using TUS are the presence of pleural fluid accumulation, abscesses or lung consolidation. Transrectal probes are already widely used by bovine veterinarians and are suitable for TUS. Large size calves can be restrained in a chute to perform the technique, whereas restraining in a chute is not required for small size calves.

Respiratory disorders may lead to discomfort and pain. The main hazards, preventive measures, and corrective/mitigating measures associated with respiratory disorders during the transit stage are listed below.

Mixing animals: during the transit stage, the contact among animals increases, as space is reduced and also ventilation, including animals from different sources and with different disease histories.

- PRE: Pre-loading mixing of animals from different sources should be avoided.

Condition inside the vehicle: Poor ventilation results in increased humidity and levels of dust and noxious gases such as NH_3 and these are known risk factors for respiratory disorders (Boulton et al., 2018).

- PRE: Adequate ventilation should be ensured inside the truck.

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Loading of animals with reduced fitness: Transporting cattle with previous signs of respiratory disorders may spread the pathogens among other individuals.

- PRE: The absence of pre-loading clinical signs of respiratory disorders should be confirmed.

Time with limited access to feed and/or water: These are risk factors for respiratory disorders, reducing the resistance to pathogens to infect the respiratory tract and allowing the infection to spread (Earley et al., 2010, 2012, 2017) as transport may impair immune function (Blecha et al., 1984; Murata, 1989; Murata and Hirose, 1991).

- PRE: Time off feed and water should be kept to a minimum.

Corrective/mitigating measure of respiratory disorders

An animal that has become sick during a journey should be appropriately treated or euthanised. When necessary, veterinary advice should be sought in the care and treatment of these animals.

vi) Resting problems

Resting problems are regarded as highly relevant 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 cattle 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 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.

Vehicle motion: The moving vehicle is not only stressful for cattle in itself, but can also lead to resting problems as transported cattle lie down less than non-transported cattle. See the section on motion stress above for a review of the consequences of vehicle motion.

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

Insufficient horizontal space (space allowance): When the stocking density is too high, the animals do not have the space to lie down and get up again, increasing the risk of resting problems during journeys.

- PRE: Cattle should be provided with enough space to allow them to rest, as described in detailed in quantitative examination of the space requirements (Section 3.5.3.2).

Unsuitable floor and inadequate bedding: Poorly maintained or poorly designed flooring with corrugated patterns does not allow animals to comfortably maintain balance during the journey, predisposing them to slipping, falling and constant changes of position during the journey. Anti-slip materials should be used. In addition, without adequate bedding (type and/or quality and/or quantity) animals will be less motivated to rest lying down during journeys and may be exposed to slips, falls and weakness.

- PRE: Non-slippery floors are essential, and they should be properly maintained to avoid edges and cavities that can lead to falls and injuries. Sufficient bedding should be provided for the transit stage, made of adequate materials such as straw for young cattle, straw for adult cattle in winter and straw or sawdust for adult cattle in summer. The use of long straw helps to minimise dust.

Insufficient vertical space: Low decks are problematic because animals will be forced to adopt abnormal positions during the journey, and this can be exacerbated if the floor also does not provide the necessary conditions for a comfortable resting position.

- PRE: 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 cattle during the transit stage.

Corrective/mitigating measure of resting problems.

If resting problems are severe, the journey should stop, the animals be offloaded and provided sufficient time and conditions to rest.

vii Restriction of movement

Restriction of movement is to some extent inherent to animal transport as the animals cannot move freely, especially during the transit stage. Thus, restriction of movement is regarded as highly relevant during this stage. The prevalence is high, as movement restriction will affect all animals in the transit stage. Therefore, the duration of restriction of movement depends on the journey duration, and severity is expected to increase with increasing duration, as the lack of possibility to move freely is expected to become more problematic for the animals. Restriction of movement may lead to frustration, discomfort and potentially distress. The main hazard leading to the restriction of movement of cattle during the transit stage is the provision of insufficient space for the animals. 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 of cattle.

Corrective/mitigating measure of restriction of movement.

If inappropriate restriction of movement is suspected during the transit stage, the journey should be terminated as soon as possible. Upon arrival, the animals should be unloaded with no delays and they should be provided with sufficient space and conditions to rest.

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

3.5.3.1. Threshold of microclimatic conditions

A) Introduction

For cattle, the relative importance of sweating and increased respiratory rate as a means to lose heat can be significantly different between different breeds, and also between *Bos indicus* and *Bos taurus* breeds, and can occur at different temperatures and after different intervals (Pereira et al., 2014).

Dairy breeds are typically more sensitive to heat stress than beef breeds, and higher-producing animals are more susceptible because they generate more metabolic heat (Kadzere et al., 2002; Bernabucci et al., 2010). Aggarwal and Upadhyay (2013) described upper critical temperatures (UCTs) in dairy cattle ranging from 20°C to 24°C in Holstein cows, more than 24°C in Jersey crossbreeds and more than 32°C in indigenous cattle.

B) Background

Thermoregulation is the physiological process allowing the balance between heat production and heat loss mechanisms. The approach taken in this Scientific Opinion to recommend microclimatic conditions during cattle transport is based on the thermoregulatory concepts and model as described by EFSA AHAW Panel (2004) (Figure 3), and originally formulated by Mount (1974). The figure covers a range of environmental temperatures from cold to very hot. This assessment of thresholds for microclimatic conditions focusses on temperatures higher than B as indicated on Figure 3.

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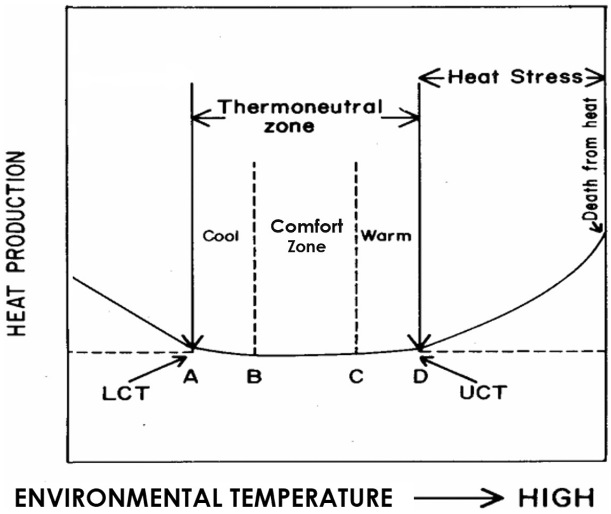

LCT/A: Lower critical temperature (LCT), UCT/D: Upper critical temperature; B: Lower limit of thermal comfort zone; C: Upper limit of thermal comfort zone.

Figure 3: Schematic representation of thermal zones as a function of the environmental temperature (adapted from EFSA AHAW Panel, 2004)

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

Thermoneutral zone (TNZ): As reviewed by Bracke et al. (2020), the TNZ covers the range of environmental temperatures within which metabolic rate and heat production are constant and independent of the ambient temperature. The zone is limited by the lower critical temperature (LCT) (marked as A in Figure 3) and the UCT (marked as D in 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 (TCZ): 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) 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, and may start to display thermoregulatory behaviour. The TCZ is sometimes called the safe zone as it is referred in EFSA Scientific Opinion on the welfare of animals in containers during transport (EFSA AHAW Panel, 2022b).

Upper critical Temperature: 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).

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According to the definition of WCs published by 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, focusing on lack of ability to cope or on performance loss (as discussed by Wang et al. (2020) in a review on heat stress in calves and heifers).

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, cattle 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 cattle 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 animal WC heat stress, addressing a situation where an animal experiences stress and/or negative affective states such as discomfort and/or distress.

C) Heat stress and animal welfare during transport of cattle

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

The water vapour content of the air is important because it impacts the rate of evaporative heat loss through the skin and respiratory tract (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.

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. 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.

In the context of animal transport, ventilation functions to replace the metabolic heat and moisture produced by the animals inside the vehicle with air of a certain humidity and temperature from outside the vehicle. Ventilation also serves to mix and redistribute internal air to attempt to make the internal thermal microenvironment more homogeneous. In addition, concentrations of different gases (O_2, CO_2, NH_3) can be modulated. The effect on individual animals depends on the rate of air change and the flow around the bodies of the animals. In this way, the temperature and humidity (and all other

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microclimatic conditions) in the vehicle can, in theory, be maintained only slightly elevated compared to the level of those outside the vehicle, but only if ventilation is very efficient.

However, in a passively ventilated vehicle, air flow over the surface of the moving 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. The red area in the front indicates where air heated by the animals accumulates and the blue area show the colder rear spots. Adapted from picture taken by (Adapted photo taken by Magnus and Morten Bach Sørensen, Simested Vognmandsforretning)

For all passively ventilated vehicles, when the vehicle is stationary (for example during mandatory driver breaks) there is no driving force for ventilation other than buoyancy or free convection or external factors such as cross winds (as illustrated by Kettlewell and Mitchell, 2005). 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, and air will tend to enter towards the rear of the vehicle and exit towards the front side of the load. The presence of the sides 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 as indicated in Figure 4.

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 the transport vehicle for cattle.

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 cattle transport, as far as we know, as the relevant studies have not been found.

When trucks are stationary, for example for loading and unloading, the risk for heat stress increases. Naturally ventilated vehicles must, therefore, either have sufficiently large or otherwise

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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 AHAW Panel, 2004).

However, very little information exists on how effectively different transport ventilation designs are able to keep cattle within the required temperature ranges when using mechanical versus 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, depending on the local climatic conditions through which the vehicle will travel.

D) Thermal heat indices

Several indices have been developed to predict stressful microclimatic conditions that take into consideration multiple weather-related factors and allow execution of abatement strategies. The majority of these have been based on ambient temperature and relative humidity. One, 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 thresholds is mainly focused on avoiding production losses, such as in-transit mortality, means that it is not necessarily aligned with animal welfare, as defined by affective states. Recent studies have 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 cattle and other livestock (Herbut et al., 2018; Wang et al., 2018).

To avoid the limitations presented by the different available THI's 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 an 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 welfare indicators during animal transport and 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 on the temperature immediately surrounding the animals 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). However, none of these indices have been validated for cattle during transport.

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 hot as well as colder spots (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 (Sammad et al., 2020).

E) Identification of microclimatic conditions to protect cattle from heat stress during transport

Respiratory rate is a sensitive indicator of heat stress in cattle (Figures 5 and 6), with increases occurring prior to changes in core body temperature. Studies have shown that respiratory rate increases gradually at temperatures below 25°C, with the rate of sweating demonstrating a significant increase at ~ 25°C and the rectal temperature starting to increase very shortly thereafter, at ~ 26°C. McLean (1963) and McDowell et al. (1954) also found that sweat production starts to increase at ambient temperatures of ~ 25°C. Various studies have also reported that cattle increasingly seek shade starting at 25°C (Kendall et al., 2006; Fisher et al., 2008; Rovira and Velazco, 2010; Becker et al., 2020).

While Lefcourt and Adams (1996) reported that respiration rate nearly doubled when the ambient temperature was above 24°C, and Li et al. (2020) reported a steep increase in respiratory rate at a

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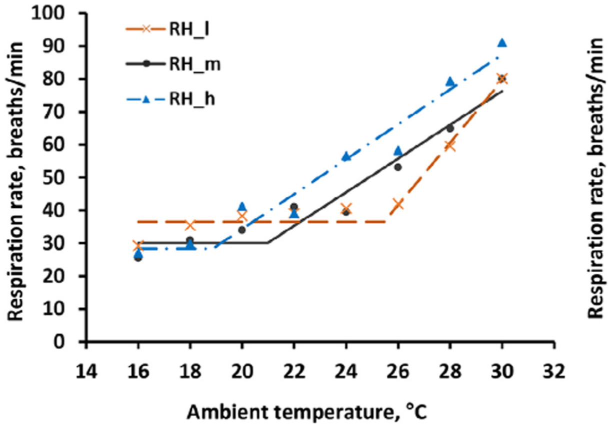
temperature of ~ 25°C, Norris et al. (2003) found that the respiratory rate of cattle increased by 5.7 breaths per minute for every 1°C ambient temperature greater than 25°C. These studies are aligned with EFSA AHAW Panel (2020) quoting Silanikove (2000), and Aggarwal and Upadhyay (2013) for the UCT for cattle being ~ 24–26°C.

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 sweating and panting, increase even further in an effort to stop the rise in core body temperature above normal. During transport of cattle, the WC heat stress, may start when the animals are no longer in their TCZ, and the risk and the severity of heat stress, is likely high when the thermal conditions reach the UCT (Figure 5).



RH_l (low relative humidity = 30% day and 50% night); RH_m (medium relative humidity = 45% day and 70% night) and RH_h (high relative humidity = 60% day and 90% night).

Figure 5: Relationship of ambient temperature and respiratory rate expressed as breaths per min, in dairy cows, from Zhou et al. (2022)

The changes in respiratory rates reported by Scharf et al. (2008) (Figure 6) are in agreement with the findings of other studies. Gaughan et al. (2000) reported that over a range of ambient temperatures from 24°C to 39°C, the increase in respiratory rate varied from 2.8 breaths/min to 3.3 breaths/min for each 1°C increase in ambient temperature.

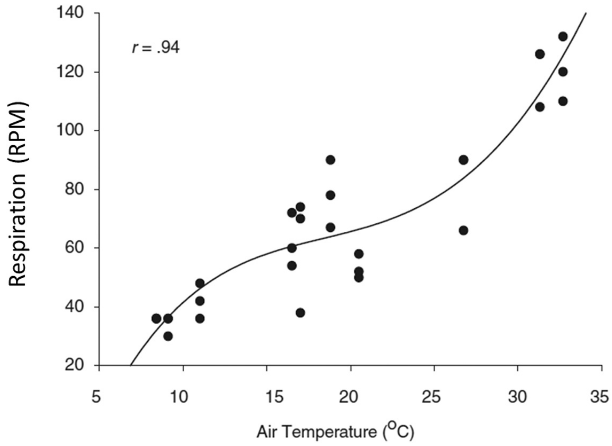


Figure 6: Changes in respiratory rate expressed as respirations per minute (RPM) with respect to changes in air temperature in six crossbred Angus steers (Scharf et al., 2008)

F) Summary of microclimatic conditions

Respiratory rate is a sensitive indicator of heat stress in cattle, with increases occurring prior to changes in core body temperature. In cattle, the relative importance of sweating and increased respiratory rate as a means to lose heat can, though, be significantly different between *Bos indicus* and *Bos taurus*, and also between different cattle breeds, and can occur at different temperatures and

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after different intervals of exposure. In addition, dairy breeds are typically more sensitive to heat stress than beef breeds, and higher-producing animals are more susceptible because they generate more metabolic heat than lower-producing individuals.

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

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

The available information has not allowed for proper estimates of thresholds for thermal comfort zone, but only for the UCT for cattle. Based on the available information, an estimate of 25°C can be proposed as the UCT, corresponding to the upper threshold of the thermal comfort zone, for cattle. For variations of dry temperature and relative humidity, the higher the levels of relative humidity, the lower the upper thresholds of thermal comfort zone and 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 taking account of humidity effects in addition to temperature as already stated in the Council Regulation (EC) No 1/2005[1].

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 cattle within a specified area of floor space (or occasionally the number of cattle of a specified live weight range per unit area).

The space allowance can be quantified as the floor area per animal. In this Scientific Opinion, space allowances are given as m^2 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 cattle 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 cattle respond to space during transport, the variability in types of cattle and in journey conditions, it is considered preferable to provide minimum rather than target or recommended space allowances for different types of cattle. The evidence of WCs when inadequate space is available is stronger than that available for determining optimal conditions. The minimum space requirement will vary with the breed of cattle, what the space is needed for, for how long, and 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 cattle during transport, the following approach will be used:

During transport, cattle 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 cattle to undertake one of the above biological 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 1/2005[1] 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 cattle in a standing posture depends on the size of the cattle, 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 all cattle have a similar shape. Therefore, allometric equations provide estimates of space requirements rather than definitive calculations of areas. 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 occupied by pigs, rabbits and birds when in different positions (i.e. when standing and lying). However, limited research was identified for cattle.

Specific factors can affect how much space some animals require. For example, cattle with horns may require more space as do heavily pregnant cows versus non-pregnant cows. 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 cattle. Previously, additional space corresponding to 5–10% was recommended for horned cattle (SCAHAW, 2002; EFSA AHAW Panel, 2011). However, no specific research was found for that recommendation.

A k-value of at least 0.02 has been proposed to provide sufficient space for a standing posture (Petherick and Phillips, 2009) and reduce the risk of cattle experiencing stress, injury and fatigue. However, the UK Farm Animal Welfare Council (FAWC, 2019) proposed a minimum k-value of 0.021 for an acceptable floor space for cattle, which translates into 1.16 m^2/animal for a 400 kg animal.

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

As reviewed by Tarrant (1990), maintenance of balance on moving vehicles is a major consideration in cattle transport in view of the hazards associated with large animals falling down in vehicles and the attendant distress and risk of injury or suffocation. Thus, space allowance is a major factor influencing cattle welfare during transport.

During transport, cattle need extra 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, cornering, stopping and changes in direction). If insufficient space is provided, cattle may experience the WC of restriction of movement, leading to reduced stability and bruising, and further potentially leading to stress and distress. If vehicles are driven well on good quality roads, cattle benefit from plenty of space.

In the SCAHAW (2002) report, it was discussed whether cattle, in situations of poor driving or emergency responses, could benefit from a stocking density that provided mutual support. However, based on the available studies, cattle seem to be at greater risk of stress and injuries at low space allowances than at high (Tarrant et al., 1988; Tarrant et al., 1992).

Tarrant et al. (1988) compared 4 h of transport of dehorned Friesian steers (weight 500–735 kg; mean 603 kg) at stocking densities of 196, 312 or 591 kg/m^2 (equivalent to 0.05, 0.03 and 0.02 k-values, respectively). In their design, half of the 6 experimental journeys changed stocking density by manipulating group size (9, 5 or 3) but kept space the same (9.8 m^2) (called G) and vice versa (always 5 steers; 4.9 m^2, 9.8 m^2 or 14.7 m^2) (called A). Table 17 lists their findings.

Table 17: Loss of balance event frequencies and indicators of carcass bruising at different stocking densities of steers (Tarrant et al., 1988). The condition 'G' describes journeys where space allowance was manipulated by changing group size, whereas 'A' describes journeys where compartment size was changed but group size kept the same

	Stocking density: 591 kg/m^2 (k = 0.017)	**Stocking density: 312 kg/m^2 (k = 0.034)**	**Stocking density: 196 kg/m^2 (k = 0.051)**
Plasma cortisol concentration mmol/L	121	84	43
Plasma creatine kinase activity units/L (indicator of muscle damage and fatigue)	200	32	34

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	Stocking density: 591 kg/m^2 (k = 0.017)	Stocking density: 312 kg/m^2 (k = 0.034)	Stocking density: 196 kg/m^2 (k = 0.051)
Bruising score G**	5.4	3.2	1.6
Bruising score A**	11.9	3.6	3.1
Position shifts	16	*	109
Loss of balance G events/group	11	12	10
Loss of balance A events/group	30	3	1
Struggle to get on foot G events/group	4	2	2
Struggle to get on foot A events/group	20	0	0
Down but up again G events	5	1	1
Down but up again A events	8	1	0
Down and stay G events	0	0	0
Down and stay A events	2	0	0

*: Not reported in original paper.
**: Carcass bruising was scored from 0 (no bruising) to 7 (maximum score of bruising) following the Australian bruise score system (Anderson and Horder, 1979).

As can be seen from Table 17, irrespective of whether the space allowance was changed by group size or compartment size, several of the ABMs associated with posture adjustment were affected by space allowance. Between k-values of 0.017 and 0.051, the greater the space allocation the lower the rate of loss of balance events and the lower the indicators of bruising. However, for most of the variables assessed, there was not marked difference between a k-value of 0.034 and a k-value of 0.051.

In a later study, Tarrant et al. (1992) transported 4 groups of 24 dehorned Friesian steers (539–900 kg; averaging ~ 618 kg) for 24 h and compared space allowances of 1.33–1.41 m^2/animal (corresponding to a k-value of 0.015) versus 1.19–1.24 m^2/animal (k-value of 0.017) and 1.03–1.08 m^2/animal (k-value 0.019). The behaviour of the animals was observed by video during the first 10 min of each half hour when the vehicle was in motion and blood samples were taken within 1 h after arrival at a lairage. Table 18 summarises the findings, and again suggests that the lowest space allowance was the most negative for animal welfare.

Table 18: Frequency of loss of balance events and other indicators of carcass bruising in steers at different space allowances (Tarrant et al., 1992)

	1 m^2/animal k = 0.015	1.2 m^2/animal k = 0.017	1.4 m^2/animal k = 0.019
Changes in position (event/pen/10 min)	4	6	9
Favoured orientation	43%	66%	79%
Shift to maintain footing total events	26	142	153
Struggle to maintain footing total events	10	4	5
Fall	8	1	1
Hair loss top tail (Number of animals)	15	13	8
Plasma cortisol concentration nmol/L, difference between pre- and post-transport	3.03	1.38	0.28
Plasma CK activity units/L, difference between pre- and post-transport	367	234	132
Bruise score*	8.5	5.0	3.7

*: Carcass bruising was scored from 0 (no bruising) to 7 (maximum score of bruising) in each of the seven areas of the carcass following the Australian bruise score system (Anderson and Horder, 1979), and then sum up.

Several more recent publications have presented data from surveys in America, based on reports from transporters or slaughterhouses, and reported effects of space allowance on, for example, bruising score. Typically, these studies involved not only much less controlled conditions than intervention studies, but also much larger data sets. As examples, Garcia et al. (2019) and Mendonça

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et al. (2019) reported that bruising scores increased with lower space allowance (stocking densities above 401 kg/m^2, equivalent to k-value of 0.019, in both studies).

In a comparable North American study based on survey data, González et al. (2012b) reported an increased risk of mortality in fat and cull cattle in some locations on the trailer when k-values were lower than 0.015 and higher than 0.035, and an increased risk of becoming non-ambulatory when k-value was less than 0.015. However, the authors explained that within these extreme categories the conclusions were based on very limited data.

iii) Space required to rest in lying posture

In housing conditions, dairy cattle spend an average of 8–13 h per day lying down, while finishing beef cattle may spend 13 to 15 h (Wechsler, 2011; Tucker et al., 2021). Lying is considered a behavioural need of cattle, the deprivation of which leads to stress responses and development of abnormal behaviour over time (Munksgaard and Simonsen, 1996).

During journeys, cattle may lie down, especially if the conditions in terms of driving and bedding are appropriate. Whether cattle need to lie down and rest during transport is dependent on the age of the animals, their health and physical condition, as well as the transport conditions. Journey duration, space allowance, driving quality, road conditions and suspension characteristics of the vehicle influence lying behaviour. At some point, however, it may be necessary for cattle to rest in order to avoid states of fatigue. Currently, it is unknown whether all cattle categories have the same need to lie down during transport.

Whether all animals in a compartment will lie down simultaneously during transport has not been investigated. In non-transport conditions, cattle may demonstrate shared vigilance, and some members may remain standing while others rest lying down (Phillips, 2002).

If all cattle within a pen are in a sternal position simultaneously, a k-value of at least 0.027 has been proposed (Petherick and Phillips, 2009) in a housing environment. However, for all animals to lie down, the space provided should also include room to perform lying-down and getting-up movements (as described by Niss et al., 2009). A limited number of studies have focused on this extra space needed for cattle to change position from standing to lying and vice versa. In an indoor housing study using continental beef steers (mean liveweight of 590 kg at the start of the study), Keane et al. (2018) investigated the effect of three fixed (2.0 m^2, 2.5 m^2 and 3.0 m^2 per animal) and two allometric (dynamic) space allowances (Equation 1 (E1) $y = 0.033w^{0.667}$ and Equation 2 (E2) $y = 0.048w^{0.667}$) on animal welfare and performance of steers over a 105-day period. The mean lying time and mean number of animals lying simultaneously was significantly lower for steers accommodated at 2.0 m^2 (equivalent to *k*-value = 0.027) than any of the other treatments (equivalent to *k*-value = 0.033 and 0.048) (Keane et al., 2018).

As discussed by Petherick (2007) and Petherick and Phillips (2009), if all cattle within a pen lie down in a sternal position simultaneously, a k-value of at least 0.027 has been proposed but in order for cattle to move between lying and standing, and vice versa cattle require an area that was best described as an area (m^2) where k = 0.047. However, the k = 0.047 has not been investigated during transport, or in indoor housing until the work by Keane et al. (2018). Based on the findings of Keane et al. (2018); however, obtained under housing conditions, a k-value of 0.033 is suggested to be enough for all cattle in a compartment to lie down and (get up again) simultaneously.

iv) Space required to thermoregulate

The minimal space allowance that can protect the welfare of cattle during transport, will be influenced by the environmental conditions, i.e. temperature and humidity inside the vehicle, the effectiveness of the ventilation system (while the vehicle is in motion and is stationary) and by the ability of the animals to thermoregulate effectively. Decreasing space allowance increases the number of cattle in a given compartment or vehicle, and thus the amount of metabolic heat and moisture that they produce (Wikner, 2003). Unless this extra metabolic heat and moisture can be effectively removed by ventilation, it can be detrimental at warmer temperatures and high humidity, and predisposes the animals to WCs such as heat stress, involving discomfort and potentially leading to distress.

On hot and humid days, increasing space allowance reduces the risk of heat stress. Increasing space allowance by 20% when ambient conditions increase the risk of heat stress has been suggested (Council Regulation (EC) No 1/2005)[1]. It has, however, not been possible to find scientific validation of this value for cattle. Estimating the actual influence of the increase in space allowance on the microclimatic conditions inside vehicles transporting animals requires detailed modelling and precise

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data, not only on the heat and water vapour produced by the animals, but also on the heat loss from the vehicle, the ventilation and the dynamic nature of these interactions. No experimental studies have been found on the effect of changes in space allowance on measures of heat stress in cattle during transport.

In cold conditions, cattle require sufficient space to be able to move away from cold areas, such as air movement at ventilation inlets. Otherwise, they may experience the WC cold stress, involving discomfort and potentially distress, causing for example frostbite.

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

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

Cattle need to eat and drink. If feeding and/or watering needs to take place onboard a vehicle, extra space is required to site the drinkers and troughs, and to provide access to these. As social animals, cattle are motivated to feed simultaneously with other members of their group. Moreover, if cattle are allowed access to feed and water after a period of fasting, all cattle will most likely attempt to drink and eat simultaneously. If there is insufficient space for all animals to feed simultaneously and/or the availability of feed or water is restricted, there is likely to be competition between the animals, and acts or threats of physical aggression will be shown (DeVries, 2019). If access to the feed and water is restricted, subordinate individuals will have reduced access to the feeders and drinkers. Thus, if feed and water are provided after periods of fasting, all animals in a compartment must be able to access it at the same time, if they are to be protected from the WCs of prolonged hunger, prolonged thirst, and group stress, and thus not be at risk of the development of frustration, distress and/or fatigue.

The appropriate linear length of a trough is likely to be affected by the breed, sex and age (size) of the cattle, the type of feed offered, the amount of feed available, the feeder/trough design, the social composition of the group and how hungry or thirsty the cattle are (Petherick, 2007; DeVries, 2019). Petherick (2007) suggested that the linear space required for cattle to eat and drink from a trough could be calculated from allometric relationships using the equation length (m) = $0.064 \times W^{0.33}$ where W = body weight (kg) of the animals. However, this recommendation was not based on in-transit conditions and has not been validated under such.

A k-value of 0.0315 has previously been proposed if cattle are to be offered feed and drink as well as space to rest on a vehicle (SCAHAW, 2002). This value has not been validated by research and should be regarded as a guiding framework since physical condition of animals, animal categories, ambient climatic conditions and likely journey duration may require adaptations.

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. The need for vertical space depends on several factors such as the type of ventilation, size of the animals, ambient climatic conditions, type of vehicle and ventilation openings.

Earlier, most cattle trucks had only one deck, but in recent years, the use of two-deck cattle trucks has increased (Figure 7).

Figure 7: Two-deck cattle truck used to transport dairy heifers on long distance journeys inside and outside EU. Photo by Lars Kloster, VikingDenmark

Cattle trucks may have varying types and sizes of ventilation openings. The height of compartments within which cattle are transported influences the ability of the animals to adopt a comfortable unimpeded posture and may lead to injuries (especially on the back and root of the tail) if the height is too low. In addition, it is necessary for adequate temperature regulation and removal of noxious gases that the height of the compartment is sufficient for effective ventilation to occur (SCAHAW, 2002). Finally, the compartment height may also affect the manoeuvrability of the animals as well as the ability to locate resources such as preferred orientation, feed and water, but at the same time, unrestricted compartment height may facilitate unwanted (and potentially dangerous) behaviours such as mounting.

Earlier recommendations stated that the compartment height must be well above the head of the tallest animals when standing with their head in a natural position (SCAHAW, 2002; TRAW, 2009). However, the natural position was not specified (see Figure 8 for an example).

Only limited research has, however, investigated different compartment heights, often taking the wither height as reference. Steinkamp and Marahrens (2012) studied the ventilation capacity and risk of injuries for heifers during long distance transport with compartment heights of 10 or 20 cm above the withers of the tallest animal, in combination with different space allowances. In their study, no evidence of injuries, swellings or hairless patches were found for any of the heights after a journey of over 1,000 km. Continuous behavioural recordings revealed that the heifers did touch the ceiling, but this was interpreted as exploring, not as butting.

Based on data from a study involving rather short journeys of less than 2 h, Lambooij et al. (2012) concluded that cattle did not have freedom of movement when provided with 20 cm over the withers, whereas the situation had improved at 40 cm. Lambooij et al. (2012) suggested to 'have a clearance of more than 20 cm above the withers during transport'.

Figure 8: Heifers during transport with their heads significantly above the height of the withers – illustrating the need for sufficient vertical space during transport. Photo by Lars Kloster, VikingDenmark, taken inside a two-deck cattle truck

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Based on a Danish pilot study, it was recommended to use 20 cm plus the height of the withers times 1.17 to allow cattle the possibility to perform natural movements and to ensure ventilation during transport. This was recommended when outdoor temperatures were ≤ 20°C and the vehicle was mechanically ventilated (Elmholt, 2008). This recommendation was based on measurements of height of the head and the withers of dairy cattle (Holstein-Friesian (500–700 kg) and Jersey (up to 500 kg)) when standing calmly in a cubicle. Based on data from 14 to 15 animals per breed, the average height at withers was reported to be 146 cm and 126 cm, respectively. In more than 90% of the observations, the animals kept their head higher than the withers (see Figures 8 and 9, in two-deck and one-deck cattle trucks, respectively) and the head height was up to 17% more than the height of the withers. Thus, in order to allow cattle room for the highest part of their body plus space for ventilation, the above formula was recommended. For higher temperatures, no specific recommendations were given, but it is emphasised that more space is then needed (Figure 9).

Figure 9: Cull dairy cows transported to slaughter in Denmark in a one-deck cattle truck. Photo: Kirstin Dahl-Pedersen, University of Copenhagen

For a Holstein cow with a wither height of 146 cm, the 17% above the withers equals 24.8 cm. Adding this to the recommended 20 cm of free space results in a height above the withers of approximately 45 cm. For a Jersey cow (wither height 126 cm) the corresponding number is approximately 41.5 cm.

D) Summary on space requirements

The spatial dimensions of compartments holding cattle during transport are of major importance 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 – 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.

The ability to maintain stability on moving vehicles was the second biological function of space and is a major consideration in cattle transport in view of the hazards associated with large animals falling down in vehicles and the attendant distress and risk of injury or suffocation. Earlier it has been discussed whether cattle, in situations of, for example poor driving could benefit from a space allowance that provided mutual support. However, based on the available studies, cattle seem to be at greater risk of stress and injuries at low space allowance compared to high.

A space allowance higher than k-value of 0.034 would benefit cattle by providing them with space to maintain stability. However, only a few studies have included space allowances larger than the current Council Regulation (EC) No 1/2005[1], and where space allowances were compared, the largest space allowance led to the lowest occurrence of several ABMs indicative of restricted movements. Thus, a cut-off point has yet to be determined and even further space allowance may be beneficial. At present, such data are not available.

The third biological function of space was lying, which is a behavioural need for cattle. Deprivation of lying behaviour leads to stress responses. During transport, the space required for lying behaviour

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of cattle has been subject to limited scientific focus, but if all cattle within a compartment need to be able to lie down simultaneously, a k-value of at least 0.033 is currently considered the best estimate.

For the fourth and fifth biological functions of space, taking into consideration, room to thermoregulate, and to eat and drink, no research was identified to provide specific quantitative information on appropriate space allowances. However, earlier a k-value of 0.0315 has been recommended to allow animals to eat and drink.

Regarding deck height, the available research is limited. Allowing cattle at least 20 cm of free space between withers and compartment ceiling seems to be enough to avoid injuries and head butts against the ceiling, but whether other functions of compartment height are fulfilled at this allowance is not known. A pilot study recommended to use [wither height (cm) $\times$ 1.17 + 20 cm] to allow natural movements and ventilation in trucks with mechanical ventilation when outdoor temperatures were $\leq$ 20°C. This recommendation corresponds to at least 40 cm above the withers for adult cattle.

3.5.3.3. Thresholds for journey time

As previously mentioned in this Scientific Opinion and noted by Nielsen et al. (2011) and Cockram (2022), 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, including 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 microclimatic 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 transit stage, the available research has been examined to identify what factors associated with transport have the potential to either increase or decrease the risk of WCs as a journey continues.

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

a) there are aspects of welfare that are negatively affected after a specific journey duration, and thus stopping a journey before this occurs would help to minimise these effects;
b) transported animals are exposed to continuous or periodic 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 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 cattle, 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 cattle. As the quality of these journey conditions are likely to be high, and only fit and healthy animals were used, the results of many of these studies may not have identified major WCs associated with commercial journeys (as discussed by Cockram, 2007, 2019).

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 dead-on-arrival (DoA)) and not on the

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protection of animals from WCs. In North America, an analysis of a survey of long-distance cattle transport showed that, as the duration of time on the vehicle (3–45 h) increased, the risk of the cattle dying, becoming non-ambulatory or lame increased, with a marked increase when the duration was > 30 h (González et al., 2012a,c). In this Scientific Opinion, signs of activation of coping mechanisms are taken as an indication of the presence of a hazard potentially leading to the corresponding WC.

Among the highly relevant WCs during the transit stage, the following are considered relevant for this work, and dealt with in further detail below: 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. Due to the lack of knowledge about relations between respiratory disorders and journey time, this highly relevant WC is not included in the assessment of journey time. Below, the relation between journey duration and these WCs is examined, ordered to align with the three different categories suggested by Cockram (2007): continuous or semi-continuous WCs, progressively developing WCs, and the more sporadic health conditions.

The scenarios considered in this section refer to the transport of animals within the EU and take into account the recommendation made on microclimatic conditions (Section 3.5.3.1) and space allowance (Section 3.5.3.2). In addition, it is taken as a prerequisite that the animals do not have effective access to food and water during the journey.

A) Motion stress and sensory overstimulation

Several studies involving transport of cattle have found increased plasma concentrations of cortisol (Tarrant et al., 1992; Fazio et al., 2005; Theurer et al., 2013), which is an indicator of stress. The studies typically examined plasma concentration of cortisol before and after journeys, thereby potentially involving many different WCs.

Increased plasma concentration of cortisol has been reported after journeys as short as 0.75 h (Odore et al., 2011). When Holstein steers (~ 5 months of age, weight approximately 230 kg) were transported for 10 h (no access to feed or water, no less than 2.1 m^2 space per animal, mean speed 80 km/h), in comparison with non-transported steers (NTC) (n = 6–7 for both treatments), the cumulative mean plasma concentration of ACTH (not shown) and cortisol (Figure 10) was greater across the entire sampling period (Knights and Smith, 2007).

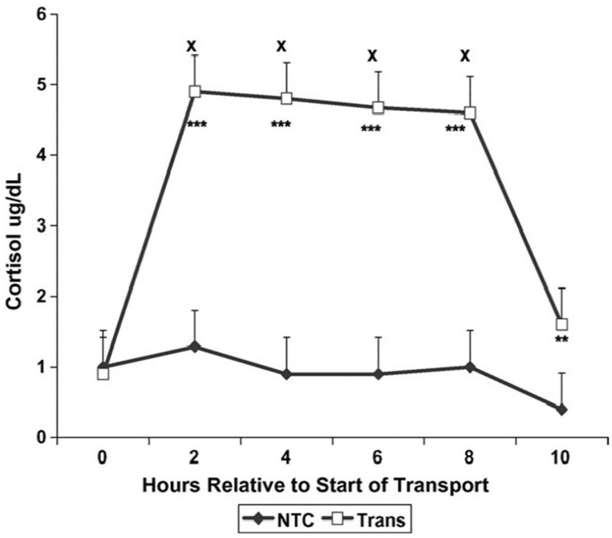

Figure 10: Plasma cortisol concentration in Holstein steers (approximately 5 months of age, weight ~ 230 kg) transported for 10 h (no access to feed or water, no less than 2.1 m^2 space per animal, mean speed 80 km/h), in comparison with non-transported steers (NTC) (n = 6–7 for both treatments) (Knights and Smith, 2007)

Post-transport, when the anterior pituitary gland was stimulated by the administration of corticotropin-releasing factor (CRF), that would normally have stimulated the release of ACTH, transport reduced the magnitude and duration of the ACTH secretion (Figure 11).

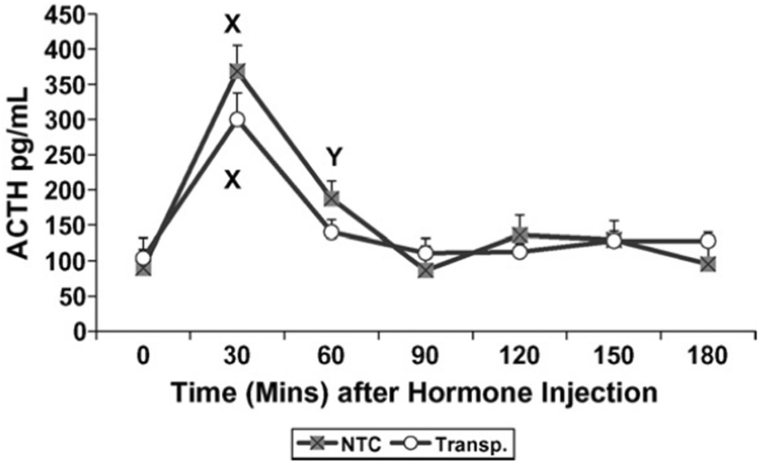

Figure 11: Plasma ACTH concentration in Holstein steers (approximately 5 months of age, weight ~ 230 kg), when stimulated with corticotropin-releasing factor (CRF) after 10 h of transport (no access to feed or water, no less than 2.1 m^2 space per animal, mean speed 80 km/h), versus non-transported steers (NTC) (n = 6–7 for both treatments) (Knights and Smith, 2007)

This suggests that at least some of the reduction in the cortisol response that occurs during a long journey is due to reduced responsiveness of the HPA axis to sustained stimulation by a stressor rather than the cattle becoming habituated to the stress of transport (Knights and Smith, 2007). Thus, even though it is not always reflected in the peripheral plasma cortisol concentration, cattle likely continue to perceive transport as an aversive stimulus.

Importantly, although the assessment of circulating cortisol levels is the most predominant measure of stress studied in cattle, there are limitations to relying solely on this measure as an indicator of the extent of stress that an animal experiences. First, it has been shown that the blood sampling necessary to obtain plasma or serum for the assay of cortisol can itself induce HPA activation (Mostl and Palme, 2002). Blood glucocorticoid concentrations are also influenced by factors such as diet, time of day and glucocorticoid receptor sensitivity (Sapolsky et al., 2000; Nader et al., 2010). A study using intensive sampling illustrated the circadian rhythm of plasma cortisol in the absence of stress. The study sampled sexually mature bulls at 30 min intervals for 24 h and demonstrated rapid and frequent cortisol fluctuations throughout the day with lowest concentrations in the evening and highest concentrations in the morning (Thun et al., 1981). Interestingly, a blind bull included in the same study did not exhibit a circadian rhythm, implying that the light–dark cycle of each day is in part responsible for this rhythm. Protocols for assaying glucocorticoids in faeces or saliva have been developed in order to avoid confounding results due to stress incurred by handling and blood sampling (Loerch and Fluharty, 1999; Mostl and Palme, 2002).

Some studies can be used to provide information on specific aspects of motion stress. As reviewed by Tarrant (1990), maintenance of balance on moving vehicles is a major consideration in cattle transport in view of the hazards associated with large animals falling down in vehicles and the attendant distress and risk of injury or suffocation. Kenny and Tarrant (1987) compared confinement of steers for 1 h on a stationary or a moving truck and found that cortisol increased in response to confinement on the moving truck. In addition, after a 2-h journey, maximum faecal cortisol metabolite concentrations in cows occurred 10 h post-transport, and these were greater than in animals that had been loaded and not transported (Palme et al., 2000).

Aspects of novelty also seem to affect cattle responses to transport. The plasma cortisol response to transport of 14 Angus steers was greater during their first journey compared to when they had experienced repeated journeys (nine 1.5 h journeys over 15 days), indicating that the steers might have become habituated to transport (Stockman et al., 2011).

B) Resting problems

If non-transported cattle are deprived of lying, their motivation to lie down will increase (Metz, 1985; Jensen et al., 2004). During transport, cattle lay down less than non-transported controls (Earley et al., 2013). Cattle have been shown to lay down more during the first 3 h after completing a

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3-h journey than they did before the start of the journey (Booth-McLean et al., 2007). Knowles et al. (1999b) also noted that cattle showed increased resting behaviour for several hours after a 31-h journey. In contrast, in young bulls, Earley et al. (2013) reported lower post-transport lying time compared to non-transported controls. It is possible that this different result is related to increased post-transport eating behaviour, but eating behaviour was not recorded in the study.

Although some cattle will lie down during a journey, e.g. 27% of a 9-h journey (Earley et al., 2013), many cattle stand during journeys (Cockram and Spence, 2012). Towards the end of journeys lasting 24–31 h, some cattle lay down after about 18 h (steers and heifers at a stocking density of 369 kg/m^2) (Knowles et al., 1999b) and after 16–20 h (steers at a stocking density of 444–599 kg/m^2) (Tarrant et al., 1992). Whether an increase in lying behaviour represents an adaptation to the environmental conditions and 'rest', or a deterioration in the ability of the cattle to stand and 'exhaustion' is, however, not clear (Cockram and Mitchell, 1999).

Fatigue is the endpoint of unsolved resting problems. The behavioural responses that could be quantified to identify fatigue include qualitative characterisation of posture and facial features, reduced responses to external stimuli and increased resting, i.e. lying down (as recently reviewed by Gallo et al., 2022). Blood and tissue measurements indicative of exhaustion of body energy reserves (e.g. hypoglycaemia and reduced liver and muscle glycogen concentration) and the accumulation of metabolites, e.g. blood and muscle lactate concentration (Frese et al., 2016) in response to prolonged and anaerobic exercise might also be associated with fatigue. The plasma CK-activity can increase in response to exercise (Frese et al., 2016). If cattle, without obvious signs of injury, become reluctant or show reduced ability to walk at unloading (Frese et al., 2016) or become non-ambulatory during a journey, this might be a consequence of fatigue (Thomson et al., 2015).

Gebresenbet et al. (2012) found increased plasma concentration of lactate and CK-activity when calves, bulls and cows were transported on journeys of 10–11 h compared with shorter journeys (< 2 h or 4–6 h). In bulls (367 kg) transported for 12 or 24 h, plasma CK-activity was increased 12 h post-transport compared with pre-transport (Earley et al., 2010). Heifers transported at low space allowance and high air temperature and humidity for 17 h had reduced muscle glycogen concentration compared with animals transported for 9 h (Abubakar et al., 2021). Knowles et al. (1999b) found reduced muscle glycogen concentration after steers and heifers had been transported for 21 h.

C) 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 in the digestive systems of ruminant 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,b).

The WC prolonged hunger describes a situation where an animal experiences a 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 (Table 3). Thus, when feeding motivation is thwarted, hunger involves a negative subjective perception that differs from short-term appetitive regulation.

Despite the existence of motivational tools, including operant techniques (e.g. Franchi et al., 2019) to quantify affective states such as hunger in cattle, these approaches have not been applied to transport conditions. In studies of the welfare implications of transport, 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. Fasting results in increases in NEFA (due to mobilisation of fat reserves) and increased plasma BHB concentration (from hepatic ketogenesis) (Ginane et al., 2015). In ruminants, plasma glucose concentration is influenced more by

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endogenous glucose production in the liver (hepatic gluconeogenesis) than from direct dietary intake (Nafikov and Beitz, 2007). In cattle with a functional rumen, hypoglycaemia only occurs in extreme negative-energy situations. Although changes in peripheral blood concentrations of metabolites can indicate mobilisation of energy reserves in response to fasting, these changes are not reflected by corresponding changes in the cerebrospinal fluid concentration of these metabolites, and therefore they probably do not act as direct signals for hunger (Laeger et al., 2012). Some studies have included post-transport measurements of feed intake, which are easier to interpret in terms of motivation, but more difficult to associate with negative affective states.

In cattle, 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 during 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. Heat production falls during fasting (Kim et al., 2013), and ruminal activity (production of methane) is reduced, but cattle (50–400 kg) can maintain about 80% of their non-fasted heat production after 4 days of fasting (Blaxter and Wainman, 1966). Increased ruminal pH, reduced numbers of rumen bacteria, and reduced ruminal volatile fatty acid concentration indicate that reduced rumen microbial activity occurs after 24–32 h without access to feed and water (Cole and Hutcheson, 1981; Galyean et al., 1981).

Under non-transport conditions, fasting for 12 h led steers to show increased NEFA concentration (Ortolani et al., 2020), and after 24 h, steers and calves show further evidence of mobilisation of body energy reserves, with increased serum NEFA concentration (Marques et al., 2012), increased plasma BHB concentration (Bravo et al., 2018), increased serum urea concentration (Ortolani et al., 2020) and reduced liver glycogen concentration (Carr et al., 1973).

Only a few studies have quantified potential indicators of hunger in a transport setting. A reduction in ruminal volatile fatty acid concentrations has been reported in both non-transported and transported steers that had been fasted (and transported) for 13 and 46 h (Cole et al., 1986). Earley and O'Riordan (2006) found that bulls (250 kg) had a greater concentration of non-esterified fatty acids (NEFA) after 12 h of transport compared to controls. Deng et al. (2017) reported that after bulls had been transported for 14 h without access to feed and water, the rumen pH was reduced, rumen acetic acid concentration was increased and there were changes in the rumen microbial population that may have affected rumen digestion. When the pH decreases below 5.6, *Lactobacillus* spp., more acid-resistant than other microbes, become dominant in the rumen, which can cause metabolic disorders (Nagaraja and Titgemeyer, 2007). Therefore, in a state of lactic acidosis, the host ruminant may experience intake depression, reduced fibre digestion, milk fat depression, diarrhoea, rumenitis, lameness, liver abscesses, inflammation, pneumonia and even death (Lean et al., 2000).

Thus, although the rumen can provide a source of energy for cattle that can last several days, it is possible that the mobilisation of body energy reserves seen in some cattle within 12 h without feed might be associated with the initiation of hunger.

D) Prolonged thirst

If cattle 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. 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. From a physiological point of view, 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 cattle to drink (McKinley and Johnson, 2004).

The WC prolonged thirst describes a situation where an animal experiences a 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 (Table 3). If access to water is limited, or prevented, then a significant and prolonged increase in the motivation to drink may result, the thwarting of which may be associated with a negative subjective experience of thirst (Jensen and Vestergaard, 2021).

During long journeys, the amount of water lost in the faeces and urine can be greater than that in untransported cattle fasted without feed and water for the equivalent period (Cole et al. (1986), studying 46-h journeys in steers). In response to 48 h without feed and water during transport, steers lost 8–9% of their initial body weight. Loss of faeces and urine can contribute up to 65% of this total loss in body weight (Phillips et al., 1991). The remaining weight is lost by respiratory and cutaneous

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water loss and by the mobilisation of body energy reserves. The rate at which cattle lose weight during transport increases with both ambient temperature and journey duration (González et al., 2012b). Animals need to drink water to rehydrate tissues, to restore electrolytes and enzymes in the liver and muscle, as well as kidney function (Hogan et al., 2007). Shrink is defined as the loss of bodyweight following periods of food and water deprivation due to the loss of urine and faeces and body tissue, which can take from a few hours up to 30 days to recover. The primary factor affecting shrink is the duration of feed and water withdrawal. The shrink rate averages at 1%/hour during the initial 3–4 h and decreases to as low as 0.1% after 9 h or more on the vehicle (Coffey et al., 2001). A study by Meléndez et al. (2020) using 7- to 8-month-old beef calves (258 ± 24 kg BW) found a transport effect for mean BW and shrink, where calves transported for 12 h had greater mean body weight than a group transported for 36 h, and the 36-h transported group had greater mean shrink than the 12-h transported group after the initial 12 and 36 h of transport. Marques et al. (2012), reported feed and water deprivation in transport as the major factor that accounts for lost performance. They transported steers and heifers for 24 h and allowed one period of rest at 12 h with no access to feed or water, and their study had a separate group of non-transported animals that was restricted from ration and water. These treatments had statistically significant decreases in average daily gain, compared with control animals that were rested and received rations.

Plasma total protein concentration increases in transported as well as NTC after 10–11 h without feed and water (Cole et al., 1986; Phillips et al., 1991). Cole et al. (1986) carried out two transport trials, each involving 16 crossbred steers (261 kg), transported for 13 h (trial 1) and 46 h (trial 2), respectively. In each trial, 16 animals were assigned to 4 groups in a 2 × 2 factorial arrangement of treatments. Treatments consisted of either transported or non-transported groups and two pre-transport dietary regimens (alfalfa hay or a 50% concentrate diet fed for 3 days before fasting). Steers in the transport group were transported for 13 h in trial 1 and 46 h in trial 2, while the remaining steers were not transported. Transport increased urinary and total N excretion and non-evaporative water losses compared with fasting alone. The rate of water excretion was 54% greater (0.37 vs. 0.24 L/h, respectively) in transported steers than in NTC at 11 h, but at 33 h the values were similar (0.08 L/h). Concentrations of total protein, plasma glucose, triglycerides and cholesterol were not affected by transport stress in trials 1 or 2.

Warriss et al. (1995) transported steers (mean initial weight 341 kg, age 12–18 months, stocking density 1.m^2/animal) by road for either 5 (286 km), 10 (536 km) or 15 h (838 km). During transit, the temperature increased steadily from 7°C at the start of the journey at 07:00 h, stabilised at 17°C between 5 and 10 h after the start and then fell with the onset of evening to approximately 10°C. There were no differences in temperature between the pens, or between the internal and external air temperature. Animals that were transported for 5, 10 and 15 h lost 4.6, 6.5 and 7.0% of their BW, respectively; and recovery to pre-transport BW generally took 5 days. Plasma cortisol concentrations were increased by loading, but not by journey duration. The activity of plasma CK increased in proportion to the journey length; the percentage change from baseline for the 5 h, 10 h and 15 h transport journeys were + 268% (raised over 3-fold), + 855% (raised over 10-fold) and + 1,698% (raised over 18-fold), respectively. The CK activity remained high for two days for the 10 h and 11 h journeys, but returned to control values after 5 days. Plasma urea and albumin concentrations and osmolality recovered more slowly for the longer journeys compared to the 5 h journey. In the 6 days pre-transport the animals consumed 5.24 kg of hay and 22.7 L of water each, per day. In the 7 days post-transport the corresponding consumption averages were 5.55 kg hay and 21.8 L of water, and the consumption did not differ between treatments. The authors concluded that a 15-h transport period under good conditions was acceptable from the viewpoint of the animal's welfare (Warriss et al., 1995).

Earley et al. (2006) investigated the effect of fasting Holstein-Friesian bulls (230 kg) for 8 h prior to an 8-h road journey. The treatments were: (1) fasted and then transported (n = 20); (2) non-fasted and transported (n = 18); (3) non-fasted at pasture (n = 18); (4) fasted then fasted (n = 18), and (5) non-fasted then fasted (n = 18). There was no significant difference in rectal temperature, pre- or post-transport, or live weight among treatments on days 0 (pre-transport), 1, 4 and 10 (post-transport). The ambient relative humidity and temperature of the outside environment ranged from 82.8% to 99.8% and 9.9°C to 14.5°C, respectively. The bulls undergoing an 8-h journey at a stocking density of 0.82 m^2/animal showed physiological and haematological responses that were within normal referenced ranges (Schalm, 1984; Kaneko, 1997; Radostits et al., 2000). Animals that were fasted for 8-h and transported lost 9.4% of live weight while non-fasted transported animals lost 7.2%. The control non-fasted animals remaining on pasture gained 2% of live weight. Animals that were fasted

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continuously but not transported and the initially non-fasted control animals that were subsequently fasted for 9 h lost 6.1% and 6.2% of live weight, respectively. Following transport, plasma protein concentration was statistically greater in the fasted and transported animals (8.13 mg/100 mL) than in the non-fasted animals at pasture (7.68 mg/100 mL), which was not different from the other treatments.

Earley et al. (2010) transported bulls (367 kg) at a space allowance of 1.02 m^2/animal on journeys of 6, 9, 12, 18 and 24 h. During the journeys, the bulls had access to hay and drinking water via nipple drinkers on the vehicle, similar to the type that the bulls were accustomed to in their home pens. During the 12-h journey, the water consumption was zero, but during the other journeys (6, 9, 19 and 24-h duration), they consumed between 1.8 and 5.0 L/animal during the journey compared with 4.1 L/animal consumed by non-transported controls with access to feed. During the first 4 h post-transport, the bulls transported for 6, 9, 12, 18 and 24 h consumed 8.0, 11.5, 8.5, 5.5, and 9.0 L/animal, respectively, compared with 20 L/animal consumed by non-transported controls. The live weight of the animals decreased after journeys lasting 18 and 24 h, but there was no significant difference after journeys of 6, 9 or 12 h. The plasma total protein concentration was increased after all journey durations.

Earley et al. (2013) transported bulls (510 kg) that had been fasted for 11 h, on two successive 9-h journeys separated by a 12-h rest period where they were offloaded and offered hay, concentrates and water. During the journeys at 12°C and a space allowance of 1.3 m^2/animal, the bulls had access to water, but they only drank 0.09 and 0.20 mean L/animal, respectively, during each of the two journey stages and drank 2.91 mean L/animal during the mid-journey period. The plasma total protein concentration in the transported bulls was increased, although the increase was small, after each journey stage compared with pre-transport.

Total protein and albumin concentrations are markers of protein homeostasis, which increase with dehydration (Earley and O'Riordan, 2006; Earley et al., 2013). An alteration in protein metabolism is evidenced by changes in circulating total protein, albumin, and urea, which are usually increased (Crookshank et al., 1979; Tarrant et al., 1992; Knowles et al., 1999b; Earley and O'Riordan, 2006).

Taken together, the results of the studies by Earley et al. (2010) and Earley et al. (2013) suggest that, if during a journey cattle are provided with access to drinking water on a moving vehicle, they may not drink, and if they do, their consumption may be reduced compared with non-transported cattle. The provision of feed and water on the vehicle during the transit stage does not necessarily prevent live weight loss and biochemical changes indicative of dehydration and mobilisation of body energy reserves. Although the rumen can provide a source of water for cattle that can last several days, it is possible that the increase in total plasma protein concentration (indicative of a reduction in plasma volume) seen in some cattle within 9 h without water might be associated with the initiation of thirst.

E) Summary on journey duration

Regardless of how optimal the conditions of the journey provided are, cattle can potentially be exposed to several hazards during transport that might, either on their own or in combination, result in impaired animal welfare. The amount of time the animals are exposed to these hazards is dependent on the journey duration. Any aversive effects of resting problems, or reduced availability of, or restricted access to water and feed, are likely to increase with journey duration 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 19) has been created to show the estimated journey duration after which the WCs are expected to be present. The assessment of 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 cattle 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. For cattle, maintenance of balance on moving vehicles is a major consideration in view of the hazards

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associated with large animals falling down in vehicles and the attendant distress and risk of injury or suffocation. Motion stress will vary over time depending on the journey conditions, but the severity of its effects will most likely increase over time and may eventually lead to fatigue. Based on the constant presence of motion stress, 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 cattle during journeys. Depending on factors such as time off feed before journey start, cattle 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 frustration, exhaustion and a weakened condition. The available data do not allow a detailed determination of the interval between journey start and initiation of prolonged hunger. Based on the available knowledge from studies of transport of cattle, mobilisation of body energy reserves can occur after about 12 h and these changes might be associated with the initiation of hunger.

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 studies documenting proper intake of water, even in journeys on vehicles fitted with drinkers, are available. Depending on factors such as time off water before journey start and/or microclimatic conditions before and during the journey, cattle may not be thirsty during the initial phase of the transit stage, but thirst will develop over time if water is not accessed. The duration of prolonged thirst depends on accessibility of water and journey duration, and severity is expected to increase with increasing duration, as the need for water becomes more problematic for the animals. Prolonged thirst may lead to dehydration, discomfort and suffering. The available data do 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 the available knowledge from studies of transport of cattle, it is possible that the increase in total plasma protein concentration (indicative of a reduction in plasma volume) seen in some cattle within 9 h without water might be associated with the initiation of thirst.

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. Duration depends on journey duration, and severity is expected to increase with increasing duration, as the lack of resting becomes 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 cattle, potentially leading to fatigue. Although the initiation of lying behaviour after 16–20 h of a journey might indicate that the cattle have started to adapt to the transport conditions, the initiation of lying behaviour is likely to be associated with fatigue. This, together with some evidence that the risk of cattle dying during transport increases when the journey duration exceeds 30 h, suggests that some cattle are likely to experience severe WCs at some point during journeys lasting 16–30 h. Based on 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.

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 cattle while they are on-farm, that could subsequently affect their ability to respond to transport (Dahl-Pedersen, 2022).

In addition, animals which did not show a health condition before the journey may get injured during the journey due to, for example falls or aggressive behaviour, and the pain and discomfort from such conditions will continue, and likely worsen, until the animal can be unloaded. In this weakened state, cattle may be less able to cope with the challenges associated with transport, and their condition may deteriorate with time and journey duration (Dahl-Pedersen et al., 2018a; Cockram, 2019).

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

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

Table 19: 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 9 h of transport without effectively accessing water, 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, 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, cattle will need feed, water and rest in order to avoid WCs. This can be done in two ways: 1) by providing the animals with feed, water and rest while the truck is stationary; and 2) by unloading the animals and providing them with feed, water and rest there (at a 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

When animals are offered opportunities to feed, drink and rest on a stationary vehicle, the conditions on the vehicle and the time provided may not result in beneficial consequences for the animals. Some studies have investigated the effects of stopping a long journey to provide cattle with a mid-journey feed, water and rest period on the vehicle, reporting limited success. After an 18-h journey, at temperatures of up to 13°C and a stocking density of 300–350 kg/m^2, pregnant heifers (476–533 kg) offered hay and straw, and water from buckets during a 3-h rest period on the vehicle, ate and drank between 1 and 6.3 L/animal and then laid down (Lambooy and Hulsegge, 1988). After steers and heifers had been transported for 24 h at 1.55 m^2/animal and then offered the opportunity to drink from troughs on the vehicle during a 1-h stationary period, 58% of the cattle drank (Knowles et al., 1999b).

Without direct comparison, these data suggest that journey breaks on a stationary truck of 1 h cannot be used to provide cattle with enough feed, water and rest to an extent that protects their welfare.

3.6.2. Unloading of the cattle into a pen where water and/or feed is provided for a period of time before reloading to continue the journey

Theoretically, unloading cattle into a pen allows the animals to have access to resting, watering and feeding areas in order to mitigate the WCs of transport. However, stopping a journey to unload the animals to provide a period of rest, feed, and water involves a number of hazards relevant for animal

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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, the extra unloading and reloading, and the likely mixing of unfamiliar animals as well as the inherent biosecurity risk, cannot be avoided. Thus, whether there are always beneficial effects for the welfare of cattle for a long journey to be interrupted to offer feed and water while unloaded is equivocal.

Studies have reported the behavioural and physiological responses of cattle after they have been unloaded from a vehicle into a pen and offered opportunities to eat, drink and rest. Some studies report on the effect of a mid-journey lairage period before a subsequent journey (Earley et al., 2013; Marti et al., 2017; Meléndez et al., 2020) and others report on post-transport lairage (Booth-McLean et al., 2007; Earley et al., 2013; Ross et al., 2016). If cattle are offered feed in a lairage/rest area, the latency to feed might be increased and the amount of feed consumed might be reduced 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. Consumption of forage is associated with the secretion of large volumes of saliva and a transient increase in rumen volume. After feeding, the rumen can become hypertonic relative to plasma, and this can cause movement of water from the blood to the rumen, and a dehydration of extracellular fluid occurs (Silanikove, 1994). When cattle have access to water, drinking is stimulated by eating. When cattle do not have access to forage, water intake is reduced. When cattle do not have access to water, feed intake is reduced (Bond et al., 1976; Silanikove and Tadmor, 1989; Silanikove, 1992). Therefore, if feed is provided, it is important that cattle are also provided with sufficient water and allowed time to drink it before reloading.

Compared with steers and heifers that had been transported for 1,290 km without any rest periods, cattle that had been unloaded twice during the journey into a pen where they were given the opportunity to eat and drink for 2 h on each occasion had, at the end of the journey, less live weight loss and lower concentrations of plasma cortisol and serum NEFA (Cooke et al., 2013).

Earley et al. (2013) transported bulls (510 kg) that had been fasted for 11 h on two successive 9-h journeys separated by a 12-h period where they were unloaded and offered hay, concentrates and water. During the journeys at 12°C and a space allowance of 1.3 m^2/animal, the bulls had access to water. The corresponding average water intake per animal for non-transported controls during the periods corresponding to the 9–12–9 h transport phase was 1.47 L, 0.78 L and 2.98 L, respectively. At the end of the 24-h post-transport rest period, the mean water consumption/animal, for transported (T) and non-transported animals (NT) during the 24-h post-transport period, which was sub-divided into three time blocks of 1–4 h, 5–12 h and 13–24 h, was T 1.40 versus NT 0.84, T 0.16 versus NT 0.69 and T 1.71 versus NT 2.13 L, respectively. During the 12-h mid-journey period, the bulls ate less than non-transported controls and consumed similar amounts of silage during the 24-h post-transport period. The plasma NEFA concentration in the transported bulls was increased after each 9-h transit stage compared with pre-transport, but there was no significant difference after the 12-h mid-journey period.

After 15-h journeys, Marti et al. (2017) compared the effects of providing calves (260 kg) with 0, 5, 10 and 15 h rest periods, where they were unloaded and offered hay and water. All calves then continued the journey for another 5 h. Based on recordings of behaviour and physiology during 5 h post-transport, the authors suggested that 5- or 10-h rest stops were possibly not long enough to ensure all calves fed until satiation.

Meléndez et al. (2020) transported calves (258 kg; N = 12 per experimental treatment) for 12 or 36 h, then unloaded them and offered hay, silage, grain and water for 0, 4, 8 and 12 h before a final 4-h journey. No significant beneficial or detrimental effects of providing the rest periods were reported on lying and feeding behaviour, feed intake, subsequent weight gain, or serum concentrations of cortisol, NEFA and serum CK-activity.

After unloading into a novel pen, not all cattle will drink from a water trough or bowl within 3 h of arrival (e.g. median latency of 1.15 h), whereas they are likely to start eating within 0.11 h (Jarvis et al. (1996), observing 378 animals upon arrival at a slaughterhouse).

After arrival in a novel environment, cattle 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). During this state, their rest can easily be disturbed by activity. How quickly cattle start to lie down and for how long is likely to depend on factors such as the availability of feed, the novelty of the new environment, their degree of fatigue, disturbance from other animals, noise and human activity, the

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space provided, time spent drinking and the presence of bedding as well as the type of floor surface. Some animals lie down immediately after arrival in lairage, but many take several hours to lie down, with the amount of lying increasing with increased duration in the lairage.

3.6.3. Control posts

CPs are specialised livestock facilities, usually private, where animals 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).

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

Porcelluzzi (2013) stated that a resting period in a CP is the most appropriate solution to the challenges of long journeys in terms of animal welfare, because the animals are supposed to be getting adequate rest, feed and water, according to their needs, and to access comfortable bedding areas, as well as feeding and watering resources. Based on this, the authors of the report concluded that the use of CPs is an efficient means to improve animal welfare. However, as mentioned by Padalino et al. (2018), this conclusion does not seem to be supported by scientific data, as currently very limited studies on cattle welfare at CPs in the EU are available.

3.6.3.1. Current practice

In January 2022, there were CPs for cattle on the EU approved list in 16 MS (https://ec.europa.eu/food/system/files/2022-01/aw_list_of_approved_control_posts_0.pdf). Except for the UK, no list of approved CPs outside the EU exists, which has been described as a concern for competent authorities, as they may have difficulty verifying whether the place outside the EU, where animals are planned to stop to be unloaded, has suitable conditions for this.

CPs are used during the transport of cattle within the EU and during export of cattle from EU MS to third countries. CPs are mainly used for calves transported to other EU MS for fattening and for the very long-distance transport of cattle, for example from the EU to the countries bordering Europe/central Asia (e.g. Kazakhstan) to the Middle East or North Africa, either for slaughter or breeding. The text of this section focuses on adult bovines sent for slaughter or breeding, as these are the main cattle category experiencing long journeys with rest stops. To a lesser extent, other categories are mentioned when relevant. Unweaned calves are addressed in Section 3.7.

3.6.3.2. Highly relevant welfare consequences

The highly relevant WCs are: group stress, handling stress, prolonged hunger, prolonged thirst, resting problems and sensory overstimulation. The WCs and 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, milking equipment) and training of the staff at the CP. In addition to the WCs listed above, two major welfare hazards related to the use of CP are animals developing health conditions or becoming unfit for further transport, and the presence of lactating females at CPs. Below, these hazards are listed together with

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other welfare hazards associated with the different WCs. In addition, preventive and corrective/mitigating measures are listed.

i) Group stress

Group stress commonly appears in the CP as a consequence of mixing animals from different origins, or transported in different sections of the vehicle, or due to limited resources at the CP.

Mixing animals: Due to the biology of cattle, mixing animals will unavoidably lead to aggression and agonistic behaviours (e.g. cows trying to displace other cows by pushing with head or body, or cows being displaced) which may impair animal welfare and lead to injuries.

- PRE: It is recommended that CP pens are divided so that animals can be kept in the same groups as in the vehicle, and thus avoid mixing unfamiliar cattle in a CP.

Limited resources: The consequences of aggression/agonistic behaviour in terms of animal welfare are increased when resources are limited. After many hours in a vehicle, cattle arriving in a CP are hungry and thirsty. The presence of aggressive pen-mates may limit the access of the aggressive individuals as well as other cattle to the resources. In addition, limited resources, e.g. fewer eating places than cattle and too few drinkers may increase the likelihood of aggression.

- PRE: Feed, water and space should be provided in enough quantity in the CP, to avoid competition and allow animals to recuperate from the journey.

Corrective/mitigating measures of group stress

If signs of group stress as aggressions are observed in the CP, animals from different groups should be separated as much as possible, to allow a proper rest, feeding and drinking.

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 and loading/unloading phase (Sections 3.3 and 3.4).

iii) Prolonged hunger.

See Sections 3.5.2iii and 3.5.3.3C for examination of the WC prolonged hunger during the transit stage. The hazards for this WC in a CP are:

Time off feed: After a journey, if no feed has been provided or only little eaten, cattle will typically be hungry when they arrive at a CP. In cold weather conditions, which can be exacerbated by wind chill in a moving vehicle, animals rapidly mobilise body energy reserves to try to maintain body temperature (cumulative effect of feed deprivation and too low effective temperature), in which case feed intake needs will be higher (Fisher et al., 2009). The severity of the WC will increase if cattle are not able to eat quickly after unloading and at least according to their body maintenance requirements during the stay at the CP. If not all feed-motivated cattle can eat simultaneously, it may lead to aggression and frustration. If the animals, for some reason (such as fear of other animals, fatigue or fear of novelty) are not able to ingest enough feed, hunger may persist and/or develop.

- 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. If animals are not fed for *ad libitum* intake, one feeder per animal has been recommended (Porcelluzzi, 2013). No studies, however, have been found to document these recommendations.

Novelty of the situation in the CP: If cattle 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,b). Although most animals readily consume feed when it is offered after a journey, a novel environment can decrease feed intake.

- 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 cattle.

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Competition for access to feed: Feeding during journey breaks may cause competition between animals, and the stronger individuals may exclude the weaker ones.

- 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, feed should be provided, allowing sufficient time for the animals to eat.

iv) Resting problems

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

Insufficient space allowance: As previously discussed during the transit stage, cattle require a minimum space to allow them to rest and lie down comfortably.

- PRE: To reduce the risk of resting problems, enough space should be provided so that animals can rest comfortably lying down in the CP. In addition, group pens should provide enough space for the behavioural needs of the cattle to be met in terms of space to rest, move away from others and express natural species-specific behaviour.

Bedding: Floors of CPs should be non-slippery, cleanable and sufficiently drained. In addition, the quality, type and quantity of bedding influence the resting of the animals. Cattle spend more time lying down on well-bedded surfaces. For each additional kilogram of bedding, cows spent 3 and 12 min/day more lying down for wood shavings and straw, respectively (Tucker et al., 2009). Therefore, additional bedding, at least above a certain level, improves the comfort of lying surfaces. Manninen et al. (2002) showed no differences in lying time of cows when comparing concrete versus rubber floored stalls, both bedded with 6.5 kg of straw. With sawdust-bedded mattresses, lying time decreased 12 min/day for every 1-kg reduction in sawdust use (Tucker and Weary, 2004). The softness or compressibility of the lying surface may underlie the behavioural response to the amount of bedding. Body size, measured by body weight, may influence the response to the bedding levels. For example, calves (4–21 weeks old), unlike cows, showed no difference in lying behaviour when housed on concrete compared with rubber mats (Hanninen et al., 2005).

- PRE: Suitable bedding should be provided in the CP. Porcelluzzi (2013) recommended 8–12 kg straw per adult cattle and 2–3 kg per calf, possibly mixed with wood shavings. Short straw (> 10 cm) bedding and/or wood shavings or special mats has been recommended as being preferable over sawdust.

Corrective/mitigating measures of resting problems

If resting problems are suspected in the CP, a thorough inspection of the facilities and conditions should be performed ensuring they allow cattle to rest.

v) Prolonged thirst.

See Sections 3.5.2iv and 3.5.3.3D for examination of the WC prolonged thirst during the transit stage. The hazards for this WC in a CP are:

Time off water: After transport, at the arrival of the CP, cattle will be thirsty if water has not been provided during the transit stage, or if the animals for some reason (such as lack of familiarity, neophobia or fear of other animals) have not been able to drink enough water. Adult and growing cattle require a large volume of water to maintain homeostasis. For example, growing beef cattle drink approximately 27 to 66 L/day (Von Keyserlingk et al., 2016. Dairy cows producing over 30 kg of milk per day require a significant supply of fresh water as they can consume from 80 to > 100 L/day of water (Von Keyserlingk et al., 2016). Therefore, water requirements of cattle are high (around 0.09 L/kg live weight per day).

- PRE: In the CP, access to drinkers should be easy at all times. Porcelluzzi (2013) recommended that CP pens for cattle should be equipped with drinking bowls with a flow of minimum 12 L/min. The drinking height is specified per age group, starting with 0.5 m height for smaller calves (50 kg) to 0.75 m for cattle above 650 kg. At least one drinker per 10 cattle is recommended, with a minimum of 2 drinkers per pen, with at least 60 cm separation to

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allow free access. However, whether these recommendations are enough to avoid WCs is not known, as no specific research has been found to validate it.

Corrective/mitigating measures of prolonged thirst

If prolonged thirst is suspected, additional drinking sources should be provided, allowing sufficient time for the animals to drink.

vi) Sensory overstimulation

Sensory overstimulation is one of the consequences for the welfare of cattle 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 the animals may face novel auditory, olfactory, visual and tactile stimuli that can provoke fear or anxiety (Wemelsfelder and Farish, 2004). This is especially important if animals of various origins are brought together under the same housing, and consequently of different physiological conditions (i.e. males vs. females, or adults vs. juveniles) (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, no odours that frighten the animals (e.g. aggressive smelling cleaning products or disinfectants), well ventilated and visually separated 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 highly relevant during the loading/unloading stage and the transit stage.

Corrective/mitigating measures of sensory overstimulation

If sensory overstimulation is suspected in the CP, affected animals can be moved to a separate pen in calm conditions allowing time to rest.

In addition to the highly relevant WCs reviewed above, animals may experience pain and/or discomfort in CPs, as a consequence of changes in their clinical condition. Hazards, preventive measures and corrective/mitigating measures for this is listed below.

Animals developing health conditions or becoming unfit: During a journey, changes in the clinical condition of animals are a significant risk to their welfare. To date, only one study has been identified reporting data on the occurrence of mortality or morbidity (including animals declared unfit for further transport) at a CP. Padalino et al. (2018) reported the official recordings of mortality and morbidity in an Italian CP during 2010–2015, a period where 111,536 animals of different species passed through. Of these, 80.2% (corresponding to 1,116 of the 1,391 trucks) were cattle, the majority of which were heavy cattle (between 550 and 700 kg). According to the data retrieved by Padalino et al. (2018), the mortality rate for cattle was 0.01%, the morbidity rate 0.006% and 3 cattle were recorded as 'unfit for further transport'. It was noted that morbidity only included severe injuries and diseases as described in the Annex 1 of the Council Regulation (EC) No 1/2005[1], and that no minor injuries or pathologies were counted in the data set. However, no scientifically published data are available that have documented changes in the clinical condition of cattle during and/or after journeys involving journey breaks. Hence, it is not possible to assess this risk. To the best of our knowledge, whether the physical conditions at CPs allow checks of fitness for transport or not, is not known.

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 becoming unfit for further transport. The use of mid-journey rest periods where cattle are unloaded to obtain rest, feed and water have the potential to be counterproductive if inadequate attention is given to biosecurity (Greger, 2007; Canadian Food Inspection Agency, 2018). There are numerous infectious diseases to which cattle are susceptible that require biosecurity practices to prevent transmission. When cattle are unloaded during a journey, there is an increased risk of contact with infected animals or facilities, e.g. inadequately cleaned and disinfected areas. Viruses can be transmitted to susceptible cattle by direct contact with infected animals, by mechanical transfer on people, non-susceptible animals, birds, vehicles and fomites, and by aerosols (Sellers and Parker, 1969; Sattar et al., 1987; Sanson, 1994). Bovine respiratory syncytial virus (BRSV), bovine coronavirus (BCoV), bovine herpesvirus-1 (BoHV-1), and foot-and-mouth disease virus are examples of pathogens that could be spread by unloading cattle to offer them feed, water and rest (Greger, 2007; Frössling et al., 2012).

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- PRE: Staff should be trained on the inspection of animals at the CP to detect signs of weakness or illness in fatigued or injured animals that may reduce their fitness for further transport (see Section 3.3.3). To prevent the spread of infectious diseases in CPs, adequate cleaning and disinfection should be performed between lots and animals from different farms and vehicles should not be mixed at the CP.

Lactating females: Lactating cows need to be transported with special care. Lactating cows that are not regularly milked can experience discomfort and likely pain because of increasing intramammary pressure (Vilar and Rajala-Schultz, 2020). They are also at much higher risk of developing mastitis or other illnesses. Lactating cows should be milked every 12 h as stated in the Council Regulation (EC) No 1/2005[1] and recommended in the Consortium of the Animal Transport Guides Project, 2018. However, many CPs do not have a milking parlour and some do not specify it on the EU approved CP listing.

- PRE: The CP should be specially equipped with a milking machine and additional equipment to collect and store the milk. Staff should also be trained on the correct procedures and cleaning and disinfection around milking of the cows. Special attention should be given to checking all lactating cows for mastitis.

Corrective/mitigating measures for animals developing health conditions or becoming unfit (including lactating cows developing mastitis)

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

3.6.4. Journey break – summarising considerations

At some stage during a journey, cattle will need feed, water and rest in order to avoid being exposed to hazards leading to WCs such as prolonged hunger, prolonged thirst and resting problems. If cattle are provided with water on a moving vehicle, they may not drink, and if they do, their consumption is reported to be reduced compared with non-transported cattle (Earley et al., 2010). When animals are offered opportunities to feed, drink and rest on a stationary vehicle, the conditions on the vehicle and the time provided may not result in beneficial consequences for the animals, as some studies have investigated the effects of stopping a long journey to provide cattle with a mid-journey feed, water and rest period on the vehicle, reporting limited success (Lambooy and Hulsegge, 1988; Knowles et al., 1999a). Without direct comparison, these results suggest that journey breaks on a stationary truck of 1 h cannot be used to provide cattle enough feed, water and rest to an extent that protects their welfare.

Theoretically, unloading cattle into a pen allows the animals to have access to resting, watering and feeding areas in order to mitigate the WCs of transport. However, stopping a journey to unload the animals to provide a period of rest, feed and water involves a number of hazards relevant for animal welfare such as the risk of stress, injury and infectious diseases. Some of these 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, the extra unloading and reloading, and the likely mixing of unfamiliar animals as well as the inherent biosecurity risk, cannot be avoided. Thus, whether there are always beneficial effects for the welfare of cattle for a long journey to be interrupted to offer feed and water is equivocal.

If a stay in a CP or similar should be beneficial for the welfare of cattle during transport, any journey break needs to be long enough for each animal to eat and then drink and rest. At present, the available studies are not conclusive as regards the potential benefits from a stay and the required duration of a stay. However, the available evidence suggests that a break of 5–10 h is not enough to allow all animals to eat, as it may take hours until all animals have even started eating and drinking upon arrival. In addition, no beneficial effects were found of journey breaks of up to 12 h.

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

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3.7. The transport of cattle by air

A) Current practice

The transport of livestock by air happens at a low level when compared to road transport but it can still be part of industry practice, involving especially breeding animals. According to TRACES, on average 3,700 cattle (range: 1,200–7,431) were transported annually by air from 2019 to 2021 between MS and exported to third countries.

The transport of cattle by air starts with a journey by road from a farm or assembly centre to an airport, where the cattle 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 cattle are removed from the crates and loaded into road transport vehicles to continue their journey.

B) Concerns specific to cattle travelling by air

The WCs, hazards, preventive and corrective/mitigating measures explained in the transport by road (Sections 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 cattle 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 cattle 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. Other factors that may cause animal discomfort are motion stress and loud noises.

3.8. The transport of cattle by rail

A) Current practice

Rail is the means of transport used the least for cattle. According to TRACES, less than 600 bovines per year were transported by rail between MS since 2018. The transport of cattle by rail starts with a journey by road from a farm or assembly centre to a train station where the cattle are unloaded from the road transport vehicle and loaded onto the train. Upon arrival at the train station of destination, the cattle are unloaded from the train, and loaded into road transport vehicles to continue their journey.

No scientific literature pertaining to the transport of cattle by train were found. The WCs and hazards identified in Sections 3.3, 3.4, 3.5 and 3.6 are all applicable to the transport of cattle by rail. The unloading of cattle from a vehicle at a railway station with the subsequent loading onto a railway carriage is a procedure requiring care. Similarly, the unloading from a railway carriage and the loading onto 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 cattle 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 cattle are high density confinement in railway carriages, lengthy waiting times, extended periods of water and feed deprivation, variation in microclimatic conditions and potential exposure to noxious gases.

3.9. Specific scenario: Welfare of unweaned calves during long journeys by road

A) Current practice

According to TRACES, around 370,000 unweaned calves were transported across all means of transport between MS per year in 2019–2021. Road transport constituted the vast majority of this. Unweaned calves constituted almost 10% of the approximately 4 million cattle transported per year.

Unweaned calves are for the most part a surplus product of the dairy industry where the focus on milk production means that male calves are surplus to requirements. In addition, some female calves are also surplus to requirements as 60% of the milking herd can produce a sufficient number of replacement females (De Vries et al., 2008).

As reviewed by Goetz et al. (2021), the calves are typically separated from their dams immediately, or within hours, after birth, and provided with colostrum by bottle or tube. The calves will often be kept in individual hutches (or similar) and fed milk or milk replacer twice daily. The amount of milk (or milk replacer) given to the calves is variable depending on the country of origin, but often calves are

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fed less than their nutritional needs (< 20% of their BW) (Khan et al., 2011**;** Palczynski et al., 2020). The calves will be kept on the farm of origin until collected and transported to an auction market or assembly centre, where they are grouped to constitute a full truck load.

After grouping at the market or assembly centre, the calves are transported, typically on long journeys and often between MS, to be raised for veal or beef (Velarde et al., 2021). Thus, during the journey from the farm of origin to the destination, unweaned calves can be loaded and unloaded multiple times. Transiting through auction markets or assembly centres increases the risk of impaired welfare due to handling (associated with slips or falls, for example during hurried loading and unloading), and deprivation of feed and water.

In October 2021, 46 CPs on the EU approved list (EC-DG SANTE, 2021) mentioned unweaned calves. However, the remarks for unweaned calves provide a list of options that in some cases are not very specific. There are, for example 17 CPs where 'feeding unweaned calves possible' is mentioned; however, only 5 specify that the calves will be provided with milk replacer, one mentions that calves will be provided with electrolytes and 11 do not specify. No list of approved CPs outside the EU exists, but it is not common to export unweaned calves outside of the EU. No studies on unweaned calves at CPs are available documenting the costs and benefits of a stay in a CP (including physical layout and/ or the animal management) in terms of calf welfare, and no data are available to assess the required duration of such a stay, either.

Many of the WCs, ABMs, hazards, corrective and preventive measures detailed in Sections 3.3, 3.4, 3.5 and 3.6 above are applicable to transport of unweaned calves. The following is a list of additional concerns (defined as an area or a topic to which special attention should be given in order to potentially avoid WCs) that are particular to the transport of this cattle category.

B) Concerns specific to unweaned calves being transported by road

i) Immunity/ provision of colostrum

During transport, calves from different farms of origin are mixed and exposed to new environmental conditions and management practices. These hazards occur at an age at which the calves are immature and several physiological systems are still developing. For example, young calves are still developing their gastrointestinal tract, and their thermoregulatory and acquired immune systems are not completely functional (Figure 12) (Morein et al., 2002; Chase et al., 2008; Hulbert and Moisá, 2016; Chase, 2018, 2022; Osorio, 2020; Roadknight et al., 2021a).

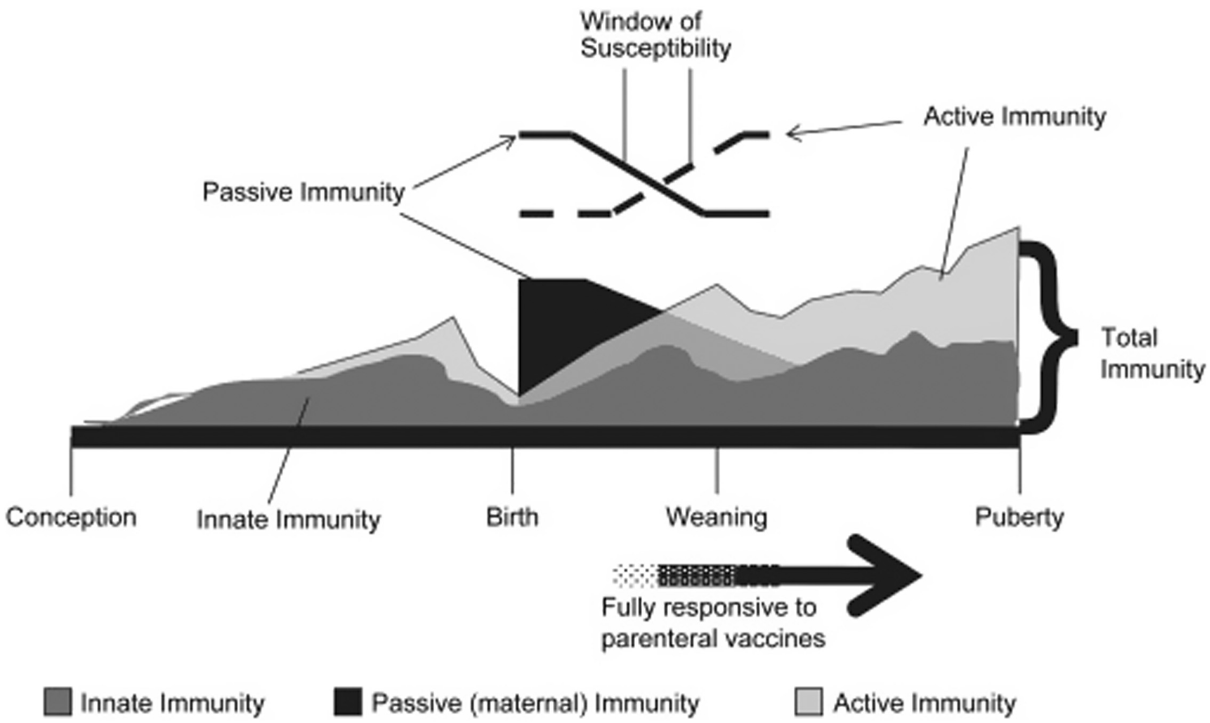

Figure 12: Development of the immune response in the calf: from conception to puberty. Adapted from: Morein et al., 2002 and Chase, 2018)

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The bovine placenta does not allow passive transfer of antibodies to the fetus, which means that calves are born agammaglobulinaemic with a high susceptibility to environmental pathogens (McGee and Earley, 2019; Hammon et al., 2020). In terms of colostrum management, one of the obvious primary differences between dairy and beef calves is that the beef suckler calf usually remains with and suckles the dam, whereas typically, the dairy calf is removed from its dam soon after birth and generally receives colostrum through artificial means.

With the ingestion of colostrum, the calf immune system is enhanced. Colostrum immunoglobulin mass ingested relative to birth weight is the most important factor determining calf passive immunity (McGee and Earley, 2019; Hammon et al., 2020). Ideally, a newborn calf that is separated from the dam should receive 3–4 L of colostrum within the first 6 h after birth (Cortese, 2009). The colostrum contains antibodies, immune cells (neutrophils, macrophages, T cells and B cells), complements, lactoferrin, insulin-like growth factor-1, transforming growth factor, interferon and other soluble factors as well as nutrients (sugars and fat-soluble vitamins) (Nagy, 2009). Colostral components, including antibodies and bioactives, influence the neonatal gastrointestinal microbiome, morphological and functional development, and digestion and absorption, and also have systemic effects on calf metabolism and development (Hammon et al., 2020). When the amount, quality or timing of colostrum provision is not adequate, calves may experience failure of passive transfer (FPT) of immunity, and their immune system is compromised (Renaud et al., 2018).

Passive immunity test results are generally categorised for FPT using test-specific cut-off values (Todd et al., 2018). For dairy calves, cut-off points applied for FPT can vary from 3.5 to 18 mg/mL blood serum/plasma IgG (Raboisson et al., 2016), but the most commonly used cut-off is 10 mg/mL IgG (e.g. Hogan et al., 2015; Raboisson et al., 2016), although the basis of such widespread adoption is not clearly apparent. Moreover, cut-offs for tests that indirectly estimate IgG concentration are most often established by simply identifying the test equivalent to 10 mg/mL serum IgG (Hogan et al., 2015). Similarly, multiple IgG cut-off values, ranging between 8 and 24 mg/mL, have been applied to classify failure of FPT in beef calves (Raboisson et al., 2016). Clearly, the 'prevalence' of FPT can fluctuate depending on what cut-off value is assumed or how it is classified (McGee and Earley, 2019).

In Canada, Italy, Australia, and New Zealand, the prevalence of FPT immunity was recently reported to be 37–43% (Trotz-Williams et al., 2008; Elsohaby et al., 2019), 41% (Lora et al., 2017), 36–42% (Vogels et al., 2013; Abuelo et al., 2019) and 25% (Lawrence et al., 2017), respectively, and it may have a negative impact on the susceptibility of calves that are transported early in life.

As calves are born agammaglobulinaemic, ingestion of colostrum containing maternal antibodies is required for the provision of maternal passive immunity to the neonatal calf (Barrington, 2001; Chase et al., 2008; Cortese, 2009; Chase, 2018, 2022). Maternal immunoglobulins have a half-life of ~ 16–32 days in calf serum, and decline during the first month of the calf's life (Husband et al., 1972). Maternal IgM and IgA disappear from the calf's circulation as early as at 2–5 days of age (Husband et al., 1972). Endogenous production of IgG and IgM by the calf's own immune system occurs approximately 8–16 days after birth while production of IgA does not begin until 64 days post birth (Husband et al., 1972).

i) Feeding

The minimum milk allowance to meet requirements for maintenance (National Research Council, 2001) and growth and to avoid signs of hunger has been estimated as 15–20% of bodyweight/day (Thomas et al., 2001; Jensen and Holm, 2003; de Paula Vieira et al., 2008; Herskin et al., 2010). The exact requirement for feeding during transport is unknown, but might be higher than on farm (Miller-Cushon et al., 2013), due to the energy demands from being on a moving vehicle (Velarde et al., 2021).

Unweaned calves are suction drinkers and therefore, for the physiological act of sucking, they need to take a teat into their mouth. After birth the dam spends a significant amount of time licking the newborn calf (Edwards and Broom, 1982; Lidfors and Jensen, 1988; Lidfors et al., 1994). One of the functions of this licking is to stimulate calf activity and thereby facilitate the first sucking attempt. During journey breaks or at assembly centres, calves might encounter feeding systems that impede them to drink the liquid feed properly. The teats need to be flexible and of rubber to enable sucking with a negative and positive pressure phase triggering the abomasal groove reflex (Khan et al., 2011).

Liquid feeding of unweaned calves requires observation, and often handling, of each individual animal. In addition, feeding milk requires attention to hygienic presentation of the feed which must be provided at the correct temperature and concentration in order to avoid digestive problems.

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When the preparation of calves for transport starts in an assembly centre (calves are typically not prepared for transport while on the farm of origin), calves are often fed electrolyte solution as their last meal before initiation of a journey. Feeding electrolytes (or a mixture of glucose and electrolytes) as a pre-transport diet does, however, not fulfil the nutritional requirement of calves, and has been associated with increased post-journey activity of creatinine kinase and lactate in the blood compared with calves fed milk replacer (Marcato et al., 2020a,b), potentially indicating increased tissue damage or fatigue during transport in calves fed this way (Chacon et al., 2005; Averós et al., 2008).

The provision of milk replacer before transport is a better measure to prevent or mitigate energy depletion or hypoglycaemia (Schaefer et al., 1997; Marcato et al., 2020a,b). The higher content of nutrients and energy of milk, as compared to electrolytes, seems to protect calves against nutrient mobilisation and loss of body weight associated with transport (Marcato et al., 2020a,b). However, caution should also be taken with milk replacer, as sudden changes in the quantity and quality of milk replacer may cause intestinal problems. Reduced body weight and increased occurrence of liquid faeces have been observed during the first 3 weeks post-transport in calves fed milk replacer at the assembly centre, probably associated with exposure to stressors either during (Marcato et al., 2020a, b) and/or after transport (Devant and Marti, 2020). Commercially, high osmolality calf milk replacers are being promoted as a means to reduce calf dehydration if fed to calves prior to transport – yet there are insufficient published scientific studies to support such benefits (Wilms et al., 2019).

Unweaned calves should not be loaded immediately after being fed, as the stress of loading prevents the formation of a casein clot in their abomasum. This will increase the likelihood of diarrhoea during the journey and the risk of longer-term digestive disorders. Operators have been recommended to allow calves to rest for 1–3 h after feeding before loading (DAFM, 2020).

ii) Development of hunger

At present, no studies have documented that it is possible to feed calves on trucks. This means that the interval between milk meals during transport may be considerable. In a recent report summarising the current knowledge on the effect of milk feeding management on milk intake, behaviour and health of young calves in relation to transport, Jensen et al. (2020) were not able to find studies establishing minimum or maximum intervals between successive liquid feeding. However, the authors identified that intervals lower than 6 h may reduce milk intake due to the presence of curd in the abomasum from previous liquid feeding (Miyazaki et al., 2019).

When calves are deprived of feed, metabolic changes have been identified after 12 h. Todd et al. (2000) reported increased occurrence of behavioural indicators of hunger in newborn calves fed at 12-h intervals compared to animals fed at 4-h intervals. Low glucose concentration after transport may indicate that energy has run out. Roadknight et al. (2021b) observed that for calves transported more than 500 km, each additional kilometre was associated with a greater decline of glucose concentration compared to calves that were transported shorter distances. Mormede et al. (1982) studied calves less than 32 days of age, and observed that journey duration, especially a long stay in an assembly centre, caused hypoglycaemia. Marcato et al. (2020a,b) observed that 18-day-old calves of 45 kg had lower glucose concentration at arrival after being transported for 18 h compared with calves transported for 6 h, and found that 12% of the calves showed signs of hypoglycaemia (plasma glucose < 2.8 mmol/L) after 6–12 h of transport. Similar results were observed by Pisoni et al. (2022) when feed restriction of 8 or 19 h was performed in 14-day-old calves, suggesting that their energy balance was less affected after 8 h compared to 19 h of feed restriction. For this study, 20 unweaned Angus-Holstein bull calves [44.1 ± 2.04 kg of body weight (BW) and 14.7 ± 0.63 d of age ($\pm$ standard error)] were used to evaluate the effects of feed restriction and fasting on performance, energy status [serum concentration of glucose, BHB and NEFA], and gastrointestinal permeability [serum concentration of citrulline, chromium (Cr)-EDTA, lactulose and d-mannitol]. Calves were randomly assigned to treatments that simulated the feed restrictions of an assembly centre situation on one hand, and the fasting hours during transportation on the other (Pisoni et al., 2022).

Fisher et al. (2014) did not observe differences in glucose concentration between calves transported for 6 or 12 h (both groups were fasted for 30 h). The authors observed that glucose concentration was relatively stable for 18 h and then started to decrease. However, the finding that the plasma concentration of glucose did not start to decrease until after 14–18 h in one of the studies, does not mean that calves were not experiencing a sensation of hunger, as hunger is a motivating sensation that has evolved to maintain metabolic homeostasis (Jongman et al., 2020). Negative energy balance described as an increase of NEFA and BHB have also been observed (Todd et al., 2000; Fisher et al., 2014; Marcato et al., 2020a,b) due to transport and fasting. Marcato et al. (2020a,b)) observed

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greater concentration of NEFA and BHB in calves transported for 18 h as compared with calves transported for 6 h, indicating that longer transport duration increases fat mobilisation. However, the authors observed alteration of the parameters related to fat mobilisation and energy even before the calves were transported, indicating that the animals were already challenged before transport. Similar results were reported by Roadknight et al. (2021b), and the lack of effect of journey length on BHB may indicate that the animals were loaded on to the truck with low body fat reserves.

Recently, Devant and Marti (2020) suggested that feed restriction during 19 h of transport may cause alterations in the intestinal permeability causing endotoxin infiltration, thereby activating an inflammatory response (as described by Bischoff et al. (2014)).

Thus, feed restriction during time spent at an auction market or assembly centre, together with the fasting period during a journey, infers that prolonged hunger is one of the highly relevant WCs during transport of unweaned calves (as also discussed by Velarde et al., 2021).

The hazard of time off-feed cannot be prevented by feeding calves one large liquid meal or by applying long intervals between two daily milk feedings, as this will limit the intake of milk and increase stress due to hunger (Stanley et al., 2002; Kehoe et al., 2007; Hulbert et al., 2011; Saldana et al., 2019; Jongman et al., 2020).

In order to prevent prolonged hunger, maximum transport duration should take into account the time from the last feeding. The available evidence suggests that around 12 h can be suggested as the interval between milk meals, as plasma glucose concentration starts to decrease after 14–18 h of transport. Milk-fed calves need at least 3 h of rest for a proper digestion after a milk meal (Marahrens and Schrader, 2020). Improper digestion increases the risk of diarrhoea. During digestion, calves must therefore have enough space to lie down and rest (Marahrens and Schrader, 2020). Based on this knowledge, feed (milk replacer) should thus be provided to the calves around 4 h before loading, according to calf needs (minimum 20% of their BW), to allow calves at least 3 h of rest for proper digestion. Based on the above synthesis, maximum journey duration cannot exceed 8 h unless new technology to allow on-truck feeding is developed. This constitutes a gap in knowledge.

iii) Provision of water

Even though unweaned calves are fed liquid (milk or milk replacer), they still need water (Jensen and Vestergaard, 2021). At a CP, the drinking height for unweaned calves should be 0.5 m (50 kg) and at least one drinker per 10 calves has been recommended (Porcelluzzi (2013)).

The water intake of calves on farm (and therefore likely also in a CP) depends on the ambient temperature, as compared to a 0°C condition, the water intake at 30°C increases more than twice (Quigley et al., 2001; Broucek et al., 2019). Severely sick calves under heat stress may require up to 19 L to replace fluid that has been lost.

Some authors have observed dehydration after transport of calves (Atkinson, 1992; Knowles et al., 1997; Todd et al., 2000; Fisher et al., 2014; Pempek et al., 2017). When severe, thirst may cause lethargy, weakness (Kells et al., 2020), hypovolemic shock and death (George and Zabolotzky, 2011). Renaud et al. (2018) found a relationship between dehydration and post-transport mortality in veal calves.

iv) Inflamed navel

In the EU, according to the Council Regulation (EC) No 1/2005[1], calves cannot be transported if their navel 'has not completely healed' (Annex I, chapter I). In a recent Italian study by Roccaro et al. (2022), it was reported that 'navel healing' should be defined as the scarring of the umbilical wound, which occurs no earlier than 3–4 weeks of life. Transporting calves with a completely healed navel should be considered best practice because it ensures that too young calves are not transported.

Navel inflammation is one of the most common health problems reported upon arrival at white veal facilities in the US, with a prevalence ranging from 20% to 32% (Wilson et al., 2000; Pempek et al., 2017; Scott et al., 2020). It has been associated with an increased risk of mortality in the first 3 weeks after transport to a veal calf rearing facility in Canada (Renaud et al., 2018). In Canadian auction markets 43% of male dairy calves had at least one health abnormality and 8% of the calves had a generally unhealthy appearance before transport, while 6.8% had a wet umbilical stalk or navel (Marquou et al., 2019). No similar studies have been reported in the EU to ascertain the fitness for transport of unweaned calves.

v) Gastro-enteric disorders

Diarrhoea has been reported to affect 6–14% of transported calves (Pempek et al., 2017; Renaud et al., 2018; Marquou et al., 2019). In a report from National Animal Health Monitoring System for U.S. Dairy (2007), half of the deaths among unweaned calves were attributed to diarrhoea. Multiple pathogens are known or postulated to cause or contribute to the development of calf diarrhoea. Other factors, including the environment and management practices, influence disease severity or outcomes, but co-infection is frequently observed in diarrheic calves (Cho and Yoon, 2014) and if sick animals are transported, it may increase the dispersion of the pathogen(s) when calves are confined in a means of transport.

The calf's digestive system undergoes three distinct developmental stages; the pre-ruminant phase, the transitional phase and the ruminant phase (Drackley, 2008) (Table 20). Different enzymes are present in the calf's digestive system during the different developmental stages and so dietary requirements need to be satisfied in a different way at each stage (Longenbach and Heinrichs, 1998).

Table 20: Enzyme activity in the pre-ruminant (to 30 days) and ruminant (after 30–60 days) calf

Pre-ruminant			**Ruminant**		
Enzyme	**Age (days)**	**Action**	**Enzyme**	**Age (days)**	**Action**
Intestinal lactose	1	Absorption of lactose in intestine	**Rumen bacteria and protozoa**	30	Functional in the digestion of nutrients
Chymosin	2	Binds casein and fat into a clot	**Isomaltose, maltose sucrose**	60	Aid in the digestion of carbohydrates
Pre-gastric esterase **Pancreatic lipase**	Birth	Hydrolysis and digestion of nutrients in milk	**Intestinal amylase**	60	Digestion of carbohydrates
Somatostain	Birth	Regulates gastric motility from abomasum to duodenum	**Somatostatin**	No change	Regulates gastric motility
Pepsin	Birth	Aids in digestion	**Pepsin**	No change	Aids in digestion
Isomaltose, maltase and, intestinal amylase and protozoa are absent during the first 30 days of life. Rumen bacteria and protozoa – limited or not present for first 30 days			**Chymosin not present after 30 days of age. Intestinal lactose is not present after 60 days of age.**		

Source: Longenbach and Heinrichs (1998).

In the pre-ruminant phase, calves have a behavioural and physiological need to ingest their milk by sucking (from a natural teat or rubber teat) and not to drink from a surface (De Passillé, 2001), as the position of the head during milk drinking is essential to prevent liquid from flowing into the developing rumen (Heinrichs, 2003; Brammertz, 2014) and reduce the associated risk of gastro-enteric disorders. Dairy calves have 8–12 suckling bouts per day and the frequency reduces with age (Lidfors and Jensen, 1988). Suckling behaviour is highly motivated, if calves are unable to express sucking, the motivation can be redirected as cross-sucking behaviour. Limited milk allowance (e.g. < 6 L/day) is associated with cross-sucking (Cantor et al., 2019).

vi) Handling unweaned calves

Unweaned calves are more difficult to move than older cattle because they do not show a natural herding behaviour (Broom and Fraser, 2007), thereby increasing the risk of poor handling (Roadknight et al., 2021a) and injury, discomfort and pain during handling. Gregory et al. (2013) suggested that slips and falls were the main problems during loading, and according to Bravo et al. (2020) slips and vocalisations were most frequently observed during unloading, whereas slips and turning around happened most frequently during loading.

In general, group-housed calves are more difficult to load than individually kept calves (Trunkfield, 1990; Albright et al., 1991; Lensink et al., 2001). Albright et al. (1991) suggested that

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group-housed calves seem more explorative when loaded onto a vehicle, and Trunkfield (1990) reported more balking and turning in group-housed calves than in individually housed calves. Lensink et al. (2001) observed that group-housed calves stopped more often during loading, as indicated by the number of pushes performed by the handlers.

Mixing of unfamiliar conspecifics is inevitable in most of the farms of origin of unweaned calves, as often they are kept in individual hutches. In addition, full loads are not common. The lack of aggressive behaviour at this age would make the mixing easier, but mixing animals from different origins may increase the risk of spread of diseases among them.

vii) Facilities for loading and unloading of unweaned calves

Similar to older cattle (Lapworth, 1999), slippery surfaces, the lack of appropriate floor type, inadequate slopes of the loading/unloading ramp and inappropriate side protections may result in increased intervention by handlers, thereby increasing the handling stress of the calves. A maximum ramp angle of 20° is appropriate for cattle providing it has non-slip floors and appropriate cleats at 30 cm intervals (Consortium of the Animal Transport Guides Project (2018). Calves, especially those which have had little pre-transport exercise, require lower gradient loading ramps (Figure 13) (Consortium of the Animal Transport Guides Project (2018).

Figure 13: Unloading of unweaned calves from a truck with more than one deck. Photo: Sonia Marti, IRTA Institute of Agrifood Research and Technology

Depending on equipment design, calves can have a high risk of falls using ramps during unloading. For example, 7.2% of calves fell with a ramp incline of 4.2°, whereas 80% fell with a ramp angle of 18.8° (Bremner et al., 1992). Falls are likely to have a negative effect on calf welfare due to potential pain and injury on impact. The fear of falling when descending steep ramps may also contribute to poor welfare.

viii) Space allowance.

Given sufficient space, young calves prefer to lie down during transport (Jongman and Butler, 2014). Therefore, even on short journeys, space requirements have been recommended to be able to account for those preferences (Eicher, 2001). During non-transport conditions, a k-value of at least 0.027 has been proposed to estimate the minimum space that would allow all calves in a pen to lie down simultaneously (Petherick and Phillips, 2009). 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, this equation was not made for unweaned calves, and has, thus, not been validated scientifically, and not under transport conditions. A k-value of 0.027 equates to a space allowance of 0.37 m^2/animal for a 50-kg calf.

Some studies are available, providing information about space allowance and the occurrence of lying behaviour in unweaned calves during transport (Figure 14), such as Knowles et al. (1997, 1999a, b), Kent and Ewbank (1986a,b) and Fisher et al. (2014). Due to the way the behavioural data were collected, none of the studies can be used to give precise information on the space required for all calves in a compartment to lie down during transport. However, based on the available knowledge, as well as the tendency for young calves to perform social lying combined with their limited body weight and thus limited risk of damaging each other when moving up and down, a k-value of 0.027 is suggested to be able to accommodate this.

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Figure 14: Unweaned calves inside truck. The calves are provided with bedding and many calves are lying down. Photo: Sonia Marti, IRTA Institute of Agrifood Research and Technology

Not only space allowance in the horizontal plane, but also in the vertical plane, influences welfare of calves during transport. Trucks transporting unweaned calves typically have more than one deck (Figure 14). The deck height in trucks transporting calves influences the ability of the animals to adopt a comfortable unimpeded posture, and may lead to injuries (especially at the back and root of the tail), if the height is low. In addition, it is necessary for adequate temperature regulation and removal of noxious gases that the height of the compartment is adequate for effective ventilation to occur (SCAHAW, 2002). Finally, the compartment height may also affect the manoeuvrability and ability of the calves to locate resources such as preferred orientation, feed and water.

Earlier recommendations stated that the deck height must be well above the head of the tallest animals when standing with their head in a natural position (SCAHAW, 2002). However, the natural position was not specified. No research was found to document the need for deck height in unweaned calves. In earlier Scientific Opinions, it is stated that the minimum space between the head of an animal (when standing in a natural position) and the compartment ceiling should be 20 cm because of ventilation (SCAHAW, 2002) or because of unhindered movements and risk of injuries (EFSA, 2011). In addition, the Standard Operating Procedures of the Irish Department of Agriculture, Food and the Marine Staff working at Export Assembly Centres (DAFM, 2020) recommended that all animals should be able to hold their heads upright while standing in a natural position, in all cases requiring a minimum of 10 cm above the withers of the tallest calf on that deck.

ix) Microclimatic conditions

Calves are homeothermic animals, which regulate body temperature by controlling the balance between the heat they produce through their basic metabolism and the loss of heat from their body to the environment. The heat production is the result of the metabolic rate, physical activity and the feeding heat increment (Vermorel et al. 1989). However, it should be taken into account that during transport unweaned calves already have low body fat reserves and possible FPT that make them more susceptible to thermoregulatory failure (Hulbert and Moisá, 2016).

The TNZ of young calves varies with age, weight, environmental conditions and other stressors, and ranges from 15°C to 25°C (Bianca and Hales, 1970; Spain and Spiers, 1996; Davis and Drackley, 1998) observed an increase of respiratory rate when air temperature was higher than 25°C in 56 kg Holstein and Guernsey calves. Gebremedhin et al. (1981) observed that in air temperatures higher than 24°C, unweaned Holstein calves (from 1 to 8 weeks of age) showed increased evaporative heat loss through sweating and raised respiratory rate. Exposure of unweaned calves to raised temperature and humidity can be stressful and potentially fatal (Neuwirth et al., 1979).

Cold has negligible effects on growth and feed conversion efficiency in very young calves until air temperature falls below 5°C, as long as the calves are dry, out of draughts and eating normally. Below 5°C, calves must increase heat production by shivering or other means in order to maintain body temperature. Intermittent exposure to temperatures around 0°C would seldom increase heat loss and thus feed energy requirements more than about 25%. Sick calves, or animals that are prevented from getting a normal energy intake have a lower metabolic heat production, and are thus less cold tolerant, so would benefit from some form of supplementary heating.

Bedding is a useful measure to mitigate cold stress by reducing heat loss. If the bedding is sufficiently deep, a calf can nest and trap a boundary layer of warm air around itself, which reduces the LCT of the calf (Nordlund, 2008). A minimum of 15 cm of bedding has been recommended, and a

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variety of bedding materials can be used. In addition, adequate bedding absorbs moisture, which will help to keep the haircoat dry, and thereby maintaining the insulating function (Davis and Drackley, 1998). While straw is a gold standard for bedding in winter time for the nesting ability, sand or sawdust will not retain as much heat, making them suitable options for summer. Regardless of bedding choice, a clean and dry resting space is required.

As previously discussed (Section 3.5.3.2), space allowance influences heat production and humidity inside a vehicle. In addition, in cold conditions, calves require sufficient space to be able to move away from cold zones of vehicles, such as ventilation inlets. Otherwise, they might experience discomfort and frostbite. The ability of the calves to adopt a compact posture could be affected by a high stocking density. Therefore, in most cold situations avoiding high stocking density is likely to be preferable. No research was found to provide recommendations for the minimum space allowance to avoid heat or cold stress in unweaned calves.

x) Mortality

Mortality and distance or duration of journeys have been reported to be related (Cave et al., 2005; Boulton et al., 2020). Roadknight et al. (2021a) suggested that reducing transport distances and journey duration may reduce mortality and improve calves' welfare during and after transport.

Cave et al. (2005) followed 220,519 calves older than 4 days in 1376 consignments destined for slaughter, and observed an increase in mortality with increasing transport distance, specifically when distance was greater than 400–500 km. In the study, distance was used as an estimate of journey time, considering journey time from the last on-farm feeding and until arrival at destination. Boulton et al. (2020) estimated that the odds ratio for young calf mortality increased by a factor of 1.45 for each additional hour of transport. In the study, calves were a minimum of 4 days old, and maximum journey times were less than 8 h. As suggested by Roadknight et al. (2021a), reducing transport distances and duration or increasing calf age may thus reduce mortality and improve calves' welfare during and after transport.

xi) Age and weight at transport

Currently, the majority of unweaned dairy calves are transported to a rearing facility at an age of 2–4 weeks. However, as mentioned previously, transporting calves at this age coincides with a vulnerable time in the calf's life in terms of the development of their immune system (Hulbert and Moisá, 2016). The immune components of calves are not completely functional at 2 weeks of age and they are in the so-called 'immune gap period' due to the combination of decreasing passive immunity and the absence of a mature adaptive immune system (Chase, 2022). A recent study by Marcato et al. (2022b) found that calves transported to a veal farm at 4-weeks of age showed a more advanced development of their adaptive immunity than calves transported at 2 weeks of age. The calf's immune system does not reach full maturity until they are ~ 6 months of age (Morein et al., 2002; Chase et al., 2008, Chase, 2018, 2022; Figure 12).

Calves younger than 4 weeks of age do not have a typical HPA response to transport stress so it is difficult to evaluate how sensitive the animals are to transport (Mormede et al., 1982; Swanson and Morrow-Tesch, 2001). It was reported that for calves (n = 6,649) transported at some time during the interval from birth to 3 weeks of age, increased mortality rates within 4 weeks of purchase were found (Staples and Haugse, 1974), with pneumonia as the most frequent cause of death, followed by diarrhoea. If calves experience FPT due to poor colostral management at the dairy farm, it is doubtful whether they have a good immune status, and if they should be transported, when younger than 5 weeks of age. From 6 to 8 weeks of age, the active immune system may be developing sufficiently for calves to withstand the transport challenge (Velarde et al., 2021).

The influence of the duration of a journey on the response of calves of different ages to transport has been extensively studied. For example, Kelley et al. (1981) transported calves ranging in age from 1 to 20 days, for up to 10 h. Transport did not reduce plasma IgG_1 or IgM concentrations, which were presumably reflective of colostrally derived immunity, but mitogen-stimulated blastogenic response of peripheral blood mononuclear cells was suppressed. Mormede et al. (1982) transported Friesian calves that were 1 month of age or less. One treatment group was transported by road for approximately 3 h, whereas another group was held without feed or water overnight and then transported for 8 h. There was no consistent cortisol response to transport, however, calves on the longer journey duration treatment were clinically dehydrated by the end of the journey, and hypoglycaemic until 1 week after transport. There was also a greater incidence of respiratory disease during the 3 weeks after transport among the calves from the long journey treatment. Kent and Ewbank (1986a) transported calves

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(age 1–3 weeks) by road for either 6 or 18 h. In comparison with control calves that were not transported, but feed deprived for 18 h, transported calves showed increased neutrophil and decreased lymphocyte counts. In contrast to the results from Mormede et al. (1982), Kent and Ewbank (1986a) did not record evidence of dehydration in calves transported for up to 18 h, as measured by plasma protein concentration and packed cell volume. Calves transported for 6 h became hypoglycaemic when they were not fed for a further 12 h, but there were few biochemical differences and no difference in proportion of time spent lying between calves transported for either 6 or 18 h (Kent and Ewbank, 1986a). In a subsequent study (Kent and Ewbank, 1986b), calves aged 3 months were transported for either 6 or 18 h, or feed deprived for 18 h without being transported. Plasma glucose concentrations were elevated in transported calves compared with feed deprived controls, as were plasma cortisol, NEFA and total white blood cell count. Plasma NEFA concentrations were elevated for longer post-transport in calves transported for 18 h compared with 6 h, but there were no other differences in biochemical measurements related to journey duration. Calves transported for 18 h spent a greater proportion of the journey lying and ruminating. Kent and Ewbank (1986a,b) concluded that transport of calves aged 1–3 weeks or 3 months, for either 6 or 18 h did not cause significant problems for the animals.

There are also studies focusing on body weight (and not age) of calves to evaluate the risk of transport, and it is reported that calf weight has a high impact on morbidity and mortality (Marcato et al., 2018). In a cross-sectional study involving 174 veal meat farms in Europe, calves with body weight lower than 51 kg at arrival presented a higher risk of hampered respiration when examined 3 weeks after arrival (Brscic et al., 2012). No calf age was given. Similarly, the risk of early mortality (before 21 days after arrival) of 4,825 calves was higher in calves with lower body weight at arrival (average weight at arrival 47 kg (SD: 5 kg) with a range from 28 to 71 kg) (Renaud et al., 2018). The likelihood of diarrhoea, respiratory disease and mortality was also higher in calves arriving at a very young age and light weight (Wilson et al., 2020). However, the latter study included very young animals (median of 5 days of age (range 1–54 days) with very low weights (estimated mean 44 kg, ranging from 27 to 77 kg).

Establishing thresholds for calf body weight at transport is not straightforward. Masmeijer et al. (2019) observed that low body weight calves (< 46 kg; 2–4 months of age) after 2 h of transport had leucocytosis and more pro-inflammatory states compared with heavier calves (> 46 kg). In a recent review on the preparation of male dairy calves for transport, 50 kg (independent of age) was proposed as a weight threshold (Renaud and Pardon, 2022). However, such thresholds should not be considered alone, as older calves with low body weight for their age (even if heavier than 50 kg) could arrive at the rearing facility with severe health issues and reduced growth performance for their age. For example, Cuevas-Gómez et al. (2020) reported that weaned suckler beef calves (306 kg (S.D. 26.3) at arrival) diagnosed with lung lesions, detected using TUS, had a reduction in live weight gain of 28% (0.23 vs. 0.32 kg/day) during the first 65 days after housing compared to those without lung lesions. The ADG of pre-weaned artificially reared dairy calves (mean weight 57 kg (S.D. 7.1) at arrival) diagnosed with severe lung lesions (lung lobe completely consolidated or pulmonary emphysema) was 0.09 kg/day less than calves without lung lesions during the first 53 days after arrival (Cuevas-Gómez et al., 2021). Scott et al. (2019) reported that body mass index on arrival at a veal facility was negatively associated with morbidity and mortality. They developed a body mass index as a composite measure of weight, height, and length so that the entire calf could be evaluated, rather than focusing solely on weight. Previous disease has also been shown to reduce ADG (Virtala et al., 1996; Windeyer et al., 2014), affect body weight (Stanton et al., 2010), and may reduce body mass index.

Based on a synthesis of the available literature, the main welfare concerns relating to the transport of unweaned calves are: reduced adaptive immunity, handling difficulties, transport stress and health issues. Therefore, it is recommended that unweaned calves should not be transported until 5 weeks old and at a minimum body weight of 50 kg.

Postponing the minimum age of transport from the present 2 weeks to 5 weeks of age, would mean that calves remain longer at the farm of origin. This Scientific Opinion addresses only the protection of calves during transport, but in order to obtain a welfare benefit from advancing the minimum age at transport from 2 to 5 weeks, it is important that calves are properly cared for during the prolonged stay on-farm or in collection farms or similar. For further information on the topic, EFSA will publish a Scientific Opinion on the protection of calves discussing the welfare conditions of the animals in different housing systems.

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3.10. Specific scenario: Transport of cull dairy cows to slaughterhouses

A) Current practice

In Europe, the current dairy cow population has been estimated to be approximately 23 million animals (Augère-Granier, 2018). Overall, the annual culling rate is 25–30% (Nor et al., 2014). This means, that annually at least 5 million dairy cows are transported to slaughter by road (Figure 15).

Figure 15: Cull Holstein dairy cows inside a truck on the way to a slaughterhouse in Denmark. Photo: Kirstin Dahl-Pedersen, University of Copenhagen

Health issues such as lameness, injury and disease are major causes for culling dairy cows (Hadley et al., 2006), and severe WCs may occur when cull dairy cows, that are not fit for the intended journey, are transported to slaughter (Cockram, 2021).

The WCs, ABMs, hazards, corrective/mitigating and preventive measures detailed in Sections 3.3, 3.4, 3.5 and 3.6 are all applicable to transport of cull dairy cows to slaughterhouse. The following is a list of additional concerns that are specific to the transport of cull dairy cows.

B) Concerns specific to cull dairy cows being transported to slaughterhouses

i) Fitness for transport

The text in Section 3.3.3 also apply to cull dairy cows, but since the key animal welfare issue affecting the transport of cull dairy cows to slaughter is the fitness of the cows for transport, this concern is given extra attention here. Slaughterhouse surveys show that the risk of transport of dairy cows to slaughter with gross pathological lesions (greater prevalence of condemnations) is greater than in other types of cattle (Dupuy et al., 2014). The risk of cull cows experiencing severe WCs during transport to slaughter due to lack of fitness can vary considerably, though, depending on the reasons for culling and on the type of journey.

As most cull dairy cows are not in prime condition, there are additional challenges faced by cull dairy cows that are more severe than those experienced by many beef cattle, and the animals will often be less able to cope with the stressors associated with transport (Cockram, 2021) than the average animal. In a data set including cull dairy cows from 20 Danish dairy herds, Dahl-Pedersen et al. (2018c) identified that 75% of the animals had one or more signs of a clinical condition before transport.

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Major health reasons for culling include those that affect reproductive performance and milk production and are potentially painful, including mastitis, injury to the udder, and lameness (Beaudeau et al., 2000; Armengol and Fraile, 2018; Sánchez-Hidalgo et al., 2019). Sickness, including specific conditions such as milk fever, pneumonia and displaced abomasum, are important reasons for culling as well. Cull cows sent for slaughter with pre-existing conditions are more likely to die in transit, become non-ambulatory or be euthanised on arrival than those that are healthy (Cockram, 2019). Cull cows that die during long journeys can show extensive pathology, including subcutaneous and muscle bruising, lung and kidney damage, reduced liver glycogen concentration and signs of dehydration (Burns et al., 2014). In the Czech Republic, Malena et al. (2007) recorded a mortality rate of cull dairy cows during transport or shortly after arrival at the slaughterhouse of 0.04%. This was greater than that in fattened cattle (0.007%), and the mortality rate was greater in dairy cows from farms that were more than 100 km from the slaughterhouse, than in those that had been transported on a shorter journey.

In some cases, cull cows may arrive unfit at the slaughterhouse because their health deteriorated during the journey. In Denmark, Dahl-Pedersen et al. (2018a) examined cull dairy cows on-farm before loading, and again at unloading at the slaughterhouse, and showed that the severity of lameness increased, and that the cows had significantly more wounds than when examined before loading (mean journey duration 3 h, range 0.5–8.5 h). Thus, depending on how animal fitness for transport is defined (e.g. if fitness means that animals should arrive at their destination in a similar condition to that assessed before loading with minimal deterioration during their journey), it can be discussed whether cull dairy cows in general are fit for the presently used transport conditions at all. There has, however, not been extensive research on how cows with different health issues respond to transport.

There are, though, also cows culled for reasons not related to health. Culling can be considered in two categories: voluntary, cows culled purely based on productivity (milk production) and involuntary, cows culled as the result of an underlying health issue including but not limited to infertility, lameness, mastitis or injury (Grohn et al., 2003). Thus, for some cows culled voluntarily, issues of fitness for transport may not be present.

Lameness can result in decreased milk production (Warnick et al., 2001) and lower reproductive performance (Hernandez et al., 2001) and is a common reason for culling. As most lameness reflects underlying pain (Flower et al., 2008), severe lameness in cull dairy cows transported to slaughter is a serious welfare issue. Animals with painful foot lesions are more reluctant to bear weight on their feet than healthy animals (Flower and Weary, 2009), and pressure on a lesion causes additional pain (Dyer et al., 2007; Flower et al., 2008). Therefore, walking during loading, unloading, and handling, and foot placements in response to vehicle movements during transport, will likely be painful. As examined in Section 3.5.3.2ii, cattle have to adjust their footing at regular intervals to maintain balance in response to vehicular movements, and are at risk of falling over (Bulitta et al., 2015). In accordance with the results from Dahl-Pedersen et al. (2018a), the condition of lame animals during a journey is likely to deteriorate as exercise associated with prolonged standing and responses to other animals, as well as vehicular movement, have the potential to aggravate the lameness. A lame cow is more likely to adopt a lying posture (Ito et al., 2010) while the other cattle on a vehicle are likely to remain standing; if she falls down, the cow will be susceptible to injury from trampling (Tarrant et al., 1988) and thereby subjected to severe WCs such as pain and distress.

Although one study showed that producers, veterinarians and livestock drivers were able to identify lame dairy cows to a similar extent, there were differences in the assessment of whether the cows were fit for transport based on the severity of the lameness (Dahl-Pedersen et al., 2018b).

Many cull dairy cows sent for slaughter are thin with a low body condition score. A high producing dairy cow uses her body fat reserves as well as the energy in the feed to meet the energy demands of lactation. If a cow does not fully regain the lost body fat, there is a cumulative decrease in body fat reserves during subsequent lactations. The net effect is that by the time a dairy cow is culled from the herd, she can have a low body condition score that reflects the low body fat reserves. A low body condition score may also occur following health issues (Bewley and Schutz, 2008). The evidence that a low body condition score is a hazard to animal welfare during transport is not strong, but thinness may increase the risk of discomfort in cold environments (Roche et al., 2009). Thin animals are also more likely to be injured or bruised during transport and have an increased risk of becoming non-ambulatory (Grandin, 2001; Strappini et al., 2010).

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ii) Lactation

Many dairy cows are culled while lactating. As examined in Section 3.6.3.2, lactating cows need to be transported with special care. If a lactating cull dairy cow is not milked at regular intervals after leaving the farm, milk will accumulate in the udder, causing increased intramammary pressure, and this can result in tissue damage, discomfort and potentially pain (Bertulat et al., 2013; Vilar and Rajala-Schultz, 2020). Dahl-Pedersen et al. (2018a) observed more cull cows with milk leakage after transport to slaughter than on-farm before loading. The risk of milk leakage increased with journey distances greater than 100 km and in cows in early lactation. A lactating cow that has not been milked regularly can be identified by milk leaking from the teats and by udder oedema (that likely occurs due to inflammation and reduced venous drainage) (Balmer et al., 2019). A lactating dairy cow that is transported to slaughter should be milked to reduce potential udder discomfort, and milked every 12 h until slaughter. The best preventive measure is to dry-off the cow about 3 weeks before transport so that the udder is involuted at the time of transport (Vilar and Rajala-Schultz, 2020; Larsen et al., 2021).

iii) Cold stress

Cull dairy cows are susceptible to cold stress if they are exposed to environments that are colder than those to which they have been acclimatised during housing. Exposed udders are also susceptible to frostbite (Fisher and Rothwell, 1999).

iv) Bruising

Cull dairy cows are at greater risk of bruising than fattened cattle (Strappini et al., 2010). Some of this bruising has been attributed to handling on-farm and during the pre-slaughter period (Strappin et al., 2013) and from unsuitable vehicle design and operation. Bruising in the hip region and on the back is thought to occur when Holstein cows (that are taller than beef cattle) hit their hips or back when loaded into cattle trailers with too low deck height (Rezac et al., 2014). See Section 3.5.3.2C for a discussion on deck height.

v) Marketing route

An important aspect of the culling decision is the marketing route chosen for the cows. Cull cows may be sent for slaughter via auction markets. Sending cull animals to an auction market often will involve consecutive journeys, increase the overall journey duration and expose the animals to extra handling (Sánchez-Hidalgo et al., 2020), novel environments, restricted availability of feed and water, cold or hot environments; provide reduced opportunities for rest; and more opportunity for any existing health condition to deteriorate. If the marketing process is prolonged, it can result in a loss of body weight (Arp et al., 2011), body condition (Stojkov et al., 2020) and signs of dehydration (Vogels et al., 2011). Reduced feed intake and fasting can cause hunger, weakness and increased susceptibility to cold conditions. If a cull cow is already in negative energy balance because of, for example early lactation or reduced feed intake due to a health issue, such as metritis, ketosis, mastitis, lameness and oral pathology, her condition is likely to further deteriorate during prolonged marketing (Herdt, 2000; Ingham, 2001; Bareille et al., 2003; Esposito et al., 2014; Norring et al., 2014).

3.11. Specific scenario: The export of cattle by road

A) Current practice

The export of cattle is defined as the transport of cattle from an EU MS to a country that is not a member of the EU. In the last 4 years, an average of 414,000 cattle were exported by road, with a decreasing trend observed with a reduction of almost 40% of cattle exported between 2018 and 2021. However, these journeys are only recorded in TRACES when the animals need to pass through another MS. This means, that animals leaving the EU directly are not included in these numbers. The main countries of destination for cattle were Russia, Lebanon and Israel, but animals have been transported by road to countries as far away as Qatar, Kazakhstan, Iran and Eritrea. These very long journeys involve repeated unloading and reloading to facilitate rest periods in CPs or CP-like facilities.

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 export of cattle by road. The following is a list of additional concerns that are particular to the export during the various transport stages.

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B) Concerns specific to the export of cattle by road

i) Border crossing when leaving the EU

Cattle exported by road typically leave the EU via a few and busy border crossings. In the previous years, most of the cattle left EU through two exit points: Bulgaria–Turkey border and Poland–Belarus border, with Turkey being the main destination for the exported cattle. There, problems may arise due to: the large number of vehicles crossing, high temperatures in the summer months (i.e. on most summer days the temperature reaches or exceeds 30°C in the shade), and administrative challenges such as restricted opening hours and complicated administrative procedures for processing consignments, as well as the absence of animal facilities and shaded areas (DG SANTE, 2019). Vehicles with cattle may, therefore, be forced to wait for a long time – often without the possibility for provision of feed and/or water to the animals, and with high ambient temperatures.

ii) Very long journeys and 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 and there are long waiting times for animals at the EU borders. Cattle being transported to distant third countries will need to cope with journeys that can take many 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 fast (UNEP, 2022). Mean summer temperatures for cattle at their destination are likely to be in the region of 35–40°C (daily maximum), i.e. well above the UCT for cattle (as examined in Section 3.5.3.1). The competent authority at the place of departure, where the animals are loaded, does not usually take weather conditions into account (DG SANTE, 2019).

iv) Health risks

As previously studied by several authors (e.g. Chirase et al., 2004; Earley et al., 2017; Pratelli et al., 2021), it is likely that cattle can be infected by pathogens during and after export. Based on knowledge of bovine medicine, relevant examples are pneumonia, other respiratory diseases including coronavirus and salmonellosis. The role of bovine coronavirus in the development of BRD is unclear (Panciera and Confer, 2010). While bovine coronavirus may cause high morbidity (O'Neill et al., 2014) it is not a significant cause of mortality in cattle and is present also in healthy cattle.

v) Handling upon arrival

EU countries and many destination countries are bound to comply with the World Animal Health Organisation (WOAH) guidelines for the export of livestock by road (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.

The handling facilities at 3rd country destinations are not subject to any routine inspection of quality control. In addition, animals may be slaughtered without prior stunning and thus experience severe pain, fear and distress, as described by EFSA AHAW Panel (2020), a procedure that EFSA recently concluded should not be practised (EFSA AHAW Panel, 2020).

3.12. Specific scenario: Transport of cattle in livestock vessels

A) Current practice

Cattle to be exported are loaded in the farms of origin and driven to a port, where they are unloaded and loaded in the vessels, on which a journey can last from 5 to 8 days and up to several weeks. These journeys are only recorded in TRACES when the animals need to pass through another MS (i.e. animals exported from Croatia or Slovenia). However, the majority of cattle leaves the EU from Spain, France, Ireland, Portugal and Romania, where they come from, so all these movements are not recorded in TRACES database (DG SANTE, 2020).

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A livestock vessel is a large 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 (see Section 3.5.3.1 for examination of microclimatic requirements of cattle. 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 the competent authority of a MS or by a body designated by a MS. The approval is valid for a maximum of 5 years, and it becomes 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 capacity of the livestock vessels approved in the EU is variable. The biggest livestock vessel can transport approximately 18,000 cattle.

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 cattle 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 cattle 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 in the vehicles to be unloaded and loaded in the vessel 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) 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. If a negative impact on animal welfare from the microclimatic conditions during journeys is to be fully prevented, animals should be transported in their thermal comfort zone, 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).

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.

iii) Noxious gases

The accumulation of manure during the journey, especially in poorly ventilated pens, leads to increased levels of noxious gases, mostly NH_3, CO_2 and H_2S. High levels of NH_3 irritate the throat, nose and eyes, which can be identified by coughing, sneezing, nasal secretion and lacrimation (Pines and Phillips (2013) in sheep, and Earley et al. (2011) in bulls). Philipps et al. (2010) used a simulated ship journey of 12 days to study the effect of different concentrations of gaseous NH_3 on the welfare of steers. The authors concluded that high NH_3 concentrations (23 and 34 mg/m^3) induced inflammatory responses and could adversely affect animal welfare.

iv) Space requirements

As previously discussed in the road transport Section 3.5.3.2, if cattle 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.

v) 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. However, no studies on the consequences of motion stress on the welfare of cattle during journeys in livestock vessels were found.

vi) Handling upon arrival

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

3.13. Specific scenario: Transport of cattle on roll-on-roll-off ferries

A) Current practice

Roll-on-roll-off (RO-RO) 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 (Figure 16).

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 cattle by RO-RO ferries. The following is a list of additional concerns that are particular to the RO-RO transport.

Figure 16: A cattle truck inside a roll-on-roll-off ferry, during a 1.5 h crossing in Denmark. In this picture, no other vehicles were standing next to the cattle truck. This may not always be the case. Photo by Mette S. Herskin, Aarhus University

B) Concerns specific to roll-on-roll-off ferries

No studies have been found focusing on the welfare of cattle 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 cattle 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. When the maximum journey time is reached, animals have to be unloaded to rest, eat and drink. However, in reality, this is not always practical as there are not always dedicated CPs in the vicinity of the port. Consideration of the journey to the ferry, time taken while waiting to board the ferry and onward to the destination or CP, must be taken into account.

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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 thermal comfort zone. Ventilation is also crucial. Airflow around and through the vehicle's animal compartments, 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.

Special attention needs to be given to 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.

3.14. Specific scenario: 'Special health status animals'

A) Current practice

In some cases, cattle 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 cattle does not want them exposed to the general cattle population during the course of any given journey. Therefore, journeys taking place without unloading the cattle during the journey may be advantageous. The focus here is on the transport of cattle on long journeys by road without unloading them before their final destination.

The WCs, ABMs, hazards, corrective/mitigative and preventive measures detailed in Sections 3.3, 3.4, 3.5 and 3.6 are all applicable to the transport of special health status cattle. The following is a list of additional concerns that are particular to transport of special health status cattle.

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

The WCs selected as highly relevant for cattle during the transit stage (heat stress, motion stress and sensory overstimulation, prolonged hunger, prolonged thirst, respiratory disorders, resting problems and restriction of movement) have been dealt with in Section 3.5.2, including recommendations that attempt to prevent hazards and/or mitigate WCs. However, not unloading cattle 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, cattle 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. No scientific studies have been found that have demonstrated effective feeding and watering of cattle during transport. Knowledge about the quality of resting on a vehicle (moving or stationary) is also lacking. Air and bedding quality are other important issues that have not been studied in such a context.

The maximum duration of long journeys without unloading the cattle has not been studied. 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.

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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 – measured in a qualitative or quantitative way – and hazards causing these WCs. Based on this identification and listing of WCs and ABMs, conclusions and recommendations are formulated allowing for different mitigation and preventing measures for the identified WCs (resource and management-based measures). As the identification and listing of the highly relevant WCs and ABMs was mainly based on expert opinion (integrating the severity, duration and prevalence 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. Table 21 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 most relevant WCs and ABMs

Source of uncertainty	Nature or cause of the uncertainty	Impact of the uncertainty on the assessment
Literature search – Language	The search was performed exclusively in English. More studies could have been identified by including references 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	The studies considered included primary research studies identified through the extensive literature search and grey literature (fact sheets, guidelines, conference papers, EU reports, book chapters, etc.) known to the EFSA Experts, but an extensive search of the grey literature was not conducted. Therefore, there may be reports and other guidance documents on animal welfare of which the EFSA Experts were not aware off.	Underestimation of the published relevant studies.
Literature search – Search strings	Although the search criteria were thoroughly discussed, some synonyms may have not been used in the search strings, and thus less hits might have been retrieved. In addition, literature from non-transport conditions, that may still have been relevant for the assessment, may also not have been found.	The understanding of the relation between hazards and ABMs may not be complete due to having missed data.
Literature search – data sources	The search was limited to Web of Science. Although the search was complemented by internet searches and manual searches of the publicly available literature, no data were retrieved from other sources (e.g. industry, NGO or authority data). More information could have been retrieved by applying a different methodology (e.g. public call for data).	The understanding of the relation between hazards and ABMs may not be complete due to having missed data.
Literature search – inclusion and exclusion criteria	The screening phase might have led to the exclusion of certain studies that could have included relevant information.	Underestimation of the published relevant papers.

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

4. Conclusions

The following section lists the conclusions of this Scientific Opinion.

4.1. General conclusions on transport of cattle

- There are published protocols to assess animal welfare on farm and at slaughter, but no validated protocols are available to assess the welfare of cattle during transport.

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- 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, have 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 access to animals particularly during the transit stage, but may be more feasible during other transport stages (e.g. loading/unloading).
- Technological development, such as artificial intelligence-based camera systems 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.
- Many hazards have been identified (see examples below for each transport stage) during the transport of cattle and the consequences of the exposure to them often go across transport stages (See Section 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 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 have been studied much less than for example animal housing, especially under European conditions; (b) lack of ABMs that are documented to be useful during transport in terms of feasibility, sensitivity or specificity; (c) lack of available studies done under the recommended conditions; (d) lack of time; and (e) 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 cattle 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 is one of the highly relevant WCs during the preparation of cattle for transport. For this WC, education and training of handlers are among the most important preventive measures.
- Group stress is the other highly relevant WC during this stage of transport. The primary hazard for group stress is mixing of unfamiliar animals.
- 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 initiation of the journey and 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 rendering animals unfit for transport are mainly related to health impairment, but not always as, for example certain age groups or certain physiological stages lead to animals being unfit for transport. Even though some guidance for assessing fitness for transport is available, variation between assessments is documented. A list of conditions has been provided in this Opinion, of which some still require scientific validation and require further study to identify the severity of the potential WCs that can arise when they are present.
- At present, thresholds for ABMs as indicators of animals being unfit for transport have most often not been established or validated. Thus, knowledge about risk associated with transport

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of animals with conditions potentially leading to negative affective states (e.g. lameness), as well as the establishment of ABMs useful to identify these and their thresholds (suitable for use across professional groups), are needed. This knowledge may lead to additional conditions that will need to be added to the list provided in this Opinion.

- Successful assessment of fitness for transport requires well-educated staff (including professional groups such as veterinarians, farmers, herdsmen 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 cattle should not be transported in the last 10% of their pregnancy. However, scientific evidence to support this threshold is lacking, and the risk of reduced animal welfare may be present earlier.
- A special case of cattle transport is the export of early lactation dairy cows. All existing guidelines for the fitness of transport of cattle mention that females should not be transported shortly after giving birth, and different thresholds are mentioned (e.g. 48 h or 7 days). However, at least until 3 weeks post-calving, dairy cows are in the so-called transition period, where they are known to be vulnerable due to physiological, metabolic and nutritional changes. No studies relating the characteristics of transition dairy cows to fitness for transport have been found, which constitutes a gap in knowledge, as these animals are likely to be more prone to WCs, especially during long and complicated journeys, as compared to the average animal.
- Among the potential measures mitigating the WCs of transport of animals with reduced fitness are reduced journey duration, increased bedding, loading last and unloading first and providing space to lie down. However, the effectiveness of such mitigation measures to avoid the additional suffering is questionable. This constitutes a gap in knowledge.

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

- The highly relevant WCs during loading/unloading of cattle are: handling stress, heat stress, restriction of movement, injuries, and sensory overstimulation.
- Across the highly relevant WCs, the major hazards are inappropriate handling, unsuitable facilities, delays, high temperatures and noise, sights and smells.
- The main preventive measures are establishment and maintenance of proper facilities, avoiding loading during hot hours and education and training of handlers.

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

- The highly relevant WCs for cattle during the transit stage are heat stress, motion stress and sensory overstimulation, prolonged hunger, prolonged thirst, respiratory disorders, resting problems and restriction of movement.
- Among the major hazards for animal welfare during the transit stage are high effective temperatures, insufficient space allowance, time off feed and water, ineffective access to water, vehicle movements and lack of possibility to rest.
- 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, it seems that cattle 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 cattle 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

- Respiratory rate is a sensitive ABM of heat stress in cattle, with increases occurring prior to changes in core body temperature.

- If the temperature in the transport vehicle remains below the upper limit of the thermal comfort zone, cattle will most likely not experience stress or negative affective states associated with heat stress during transport.
- The WC, heat stress, may start when cattle are no longer in their thermal comfort zone, 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 cattle during transport, such as humidity, thermal radiation, temperature of surrounding surfaces and wind speed. These will all influence the microclimatic conditions experienced by cattle and should in theory, all be taken into account when microclimatic conditions of cattle during transport are evaluated. In addition to dry temperature, humidity is considered the most important of these to take into consideration.
- It has not been possible to determine the upper limit of the thermal comfort zone with a reasonable degree of certainty.
- The UCT of cattle is estimated to be 25°C. For variations of dry temperature and relative humidity, the higher the levels of relative humidity, the lower the upper thresholds of thermal comfort zone and UCT will be, when measured as a dry bulb temperature only.
- Although sensors recording dry temperature have commonly been used in transport of livestock so far, it would be a significant refinement to use improved sensors taking account of other environmental conditions influencing heat load placed on cattle during transport (preferably a combination of temperature and humidity). For variations of dry temperature and relative humidity, the higher the levels of relative humidity, the lower the upper thresholds of thermal comfort zone and 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 m^2 per animal and W is liveweight in kg) can be used to calculate the physical space requirements of any category of cattle.
- The minimum space allowance is set by the first limiting factor reducing the ability of cattle to undertake relevant biological functions during transport. The available evidence suggests that a k-value of at least 0.034 is required. Providing cattle 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 sternal position, including room to perform the lying-down and getting-up movements. The suggestion with respect to space to lie down has, however, not been validated during transport.
- Table 22 gives estimations of the minimum space allowance suggested for cattle during transport.

Table 22: Estimates of minimum space allowance suggested for cattle of different weights

Approximate weight	Area (m^2/animal)
200 kg	1.16
400 kg	1.84
500 kg	2.13
700 kg	2.67

- 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 may result in WCs such as heat stress and restriction of movement.
- No studies have established a proper deck height for cattle during transport. Based on the available evidence, 20 cm free space above the withers of the tallest animal seems to be enough to avoid injuries and head butts against the ceiling, whereas wither height × 1.17 + 20 cm has been suggested to allow natural movements and ventilation, however specifying that on warmer days and in naturally ventilated trucks this is likely not enough. 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

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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 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 23.

Table 23: Summary of conclusions on the development of welfare consequences over journey time

Type of welfare consequence	Welfare consequence	Number of hours until the estimated start of the welfare consequence	Expected development over time
Continuous or semi-continuous	Motion stress	Motion stress continuous throughout the transit stage	Severity will increase over time leading to fatigue
	Sensory overstimulation	Sensory overstimulation repeated intermittent	Can lead to fear and distress
	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 9 h of transport without effectively accessing water, 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 effectively accessing feed, physiological changes indicative of hunger can be present.	Severity will increase with time leading to weakening 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 continues while the vehicle is moving, affecting all animals in the moving vehicle. Animals experience stress potentially leading to fatigue and negative affective states such as fear and distress;
- The 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 may affect a large proportion of animals in a moving vehicle. The severity is expected to increase with increasing duration, as the lack of resting becomes more problematic for the animals and may lead to fatigue;
- The prevalence of prolonged thirst may be high, even when a transport vehicle is fitted with water drinkers. Prolonged thirst may lead to dehydration and associated negative affective states. Physiological changes that are likely to be associated with thirst have been identified after 9 h 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. Physiological changes indicative of hunger can be present after 12 h of transport.

To conclude, during transport cattle 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.

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4.5. Conclusions for journey breaks and control posts

- Per definition, breaks in journeys function to remove the animals from the hazards that they are exposed to during transit and to allow them to recover from the associated WCs.
- No studies have documented successful feeding of cattle 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 cattle to drink and rest according to their needs, in a journey break on a stationary vehicle. This constitutes a gap in knowledge.
- If cattle 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 cattle during the CP stage are group stress, handling stress, prolonged hunger, prolonged thirst, resting problems 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 handling stress, heat stress, injuries, and sensory overstimulation (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.
- When lactating cows are transported via CPs, prolonged interval between milking is a hazard, the exposure to which may lead to pain and discomfort.
- Across the categories of cattle 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.
- If a stay in a CP or similar should be beneficial for the welfare of cattle during transport, any journey break needs to be long enough for each animal to eat and then drink and rest. The available evidence suggests that a break of up to 12 h is not enough to allow all animals to eat, as it may take hours until all animals have even started eating and drinking upon arrival. The duration of a rest at a CP, allowing WCs from the transit stage to be mitigated, constitutes a gap in knowledge.

4.6. Conclusions on transport of cattle by air and rail

- For cattle, air transport involves only a small fraction of transported animals, and mainly breeding animals. Rail is the mode of transport used the least for cattle.
- The WCs, hazards, preventive and corrective/mitigating measures explained in the transport by road (Sections 3.3–3.6) also apply here. However, there are additional concerns for cattle welfare during air or rail transport expressed by the EFSA experts. They 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. Other factors that may lead to WCs are motion and loud noises.
- The available evidence to evaluate the welfare of cattle when transported by air or rail is very scarce. This constitutes a gap in knowledge.

4.7. Conclusions on specific scenarios

A) Unweaned calves during long journeys by road

- When only few weeks old, unweaned calves are transported, often over long distances, for further fattening. Journeys might be directly from the farm of origin to a rearing farm, but may also involve different combinations of markets, assembly centres and stays at CPs. Unweaned calves constitute approximately 10% of transported cattle.
- The majority of the WCs, hazards, preventive and corrective/mitigating measures referred to previously for cattle transported by road (Sections 3.3, 3.4 and 3.5) also apply to unweaned calves, but certain additional concerns for their welfare during transport exist.
- Unweaned calves are difficult to handle because they do not show natural herding or group behaviour.

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- When the preparation of calves for transport starts in an assembly centre calves may be fed electrolyte solution (or a mixture of glucose and electrolytes) as their last meal before initiation of a journey. This does not fulfil the nutritional requirements of calves, and is no substitute for a meal of milk or milk replacer.
- In order to prevent prolonged hunger, the available evidence suggests that calves can be fed a milk meal with an interval of around 12 h. Milk fed calves need at least 3 h of rest for proper digestion of a milk meal. This infers that a journey break of 1 h does not meet the needs of calves for feeding and resting.
- In addition to the milk or milk replacer, calves benefit from having access to water to drink.
- For unweaned calves, the TNZ is estimated to be within 15–25°C.
- Calves prefer to lie down during the journey. In order to do so, at least k = 0.027 is required. Even though this value only takes into account the area of the floor occupied by the animals, but not the extra space required by an animal to change position from standing to lying or vice versa, it is considered appropriate due to the tendency for young calves to lie socially and their limited body weight. Provision of bedding (of various materials) limits the risk of cold stress and the risk of resting problems during the journey stage.
- As for other cattle, deck height influences welfare of calves during transport with similar hazards and potential WCs as for the other cattle categories. For this cattle category, no studies have been done. Establishment of evidence-based thresholds constitutes a gap in knowledge.
- Based on a synthesis of the available literature, the welfare concerns during transport of unweaned calves are; reduced immunity, handling difficulties, transport stress and health issues. In the first 4 weeks of life unweaned calves are at a greater risk of WCs during and after transport than later in their life.

B) Cull dairy cows transported to slaughterhouses

- Cull dairy cows are transported to slaughter by road. Across the EU, at least 5 million cull dairy cows are transported to slaughter annually.
- The WCs, hazards, preventive and corrective/mitigative measures explained for transport of cattle by road (Sections 3.3, 3.4 and 3.5) also apply here, but when exposed to the hazards, the severity of several of the resultant WCs will most likely be higher for cull cows.
- In addition to the above, the most important animal welfare issue affecting the transport of cull dairy cows to slaughter is the fitness for transport, as a major reason for culling is impaired health.
- No studies allowing a quantitative assessment of journey time for cull dairy cows are available. Due to the general health impairment of a large proportion of cull dairy cows, they are most likely experiencing a higher risk of worsening of pre-existing health conditions (e.g. lameness), as well as a higher risk of new health conditions occurring during transport, than average cattle.
- Cull dairy cows would, thus, benefit from a restriction on journey duration, which is shorter than for other categories of cattle in order to take into account all WCs, continuous, progressively developing, and sporadic, thereby reducing the duration that the dairy cows are exposed to hazards. The current knowledge does not allow estimates of such a journey duration restriction, which constitutes a gap in knowledge.

C) Export by road

- 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.
- These exports usually involve long journeys to long distance destination(s) with all the implications stated before in Section 3.5.3.3 [Thresholds for journey time].
- Export of cattle 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 the EU. In addition, the legal protection of these animals after leaving the EU is unknown. Thus, export by road likely involves more hazards and higher exposure to hazards than intra-EU transport.
- Other hazards can be predicted, such as temperature and lack of plans made to mitigate extreme external temperatures.

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D) Export by livestock vessels

- Very little is documented about the possible welfare implications of transport of cattle in livestock vessels.
- Typically, the WCs, hazards, preventive and corrective/mitigative measures explained in the sections on transport by road (Sections 3.3, 3.4 and 3.5) also apply here.
- Transport of cattle in livestock vessels increases risks for the welfare of the animals, as they are exposed to additional hazards, as compared to road transport. Among the additional hazards and concerns for animal welfare are microclimatic conditions during the waiting time in ports and during the journey, motion arising from sea conditions and post-journey handling.

E) Roll-on-roll-off ferries

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

F) Special health status

- 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 cattle during journeys where animals cannot be unloaded is very scarce. This constitutes a gap in knowledge.

5. Recommendations

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

5.1. General recommendations on transport of cattle by road

- Protocols for the assessment of welfare of cattle during transport should be developed and validated, preferably involving aspects of pre-and post-transport housing and 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 outcomes more than input.

5.2. Recommendations for preparation of cattle 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, ingrown horns, hernias, rectal and vaginal prolapse, late pregnancy, bone fractures, body condition score, eyesight deficiency.
- Risk associated with transport of animals with a number of conditions, potentially involving negative affective states during transport, such as pregnancy, should be examined.
- 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.
- The effectiveness of measures to mitigate risk of transport of animals with reduced fitness should be examined.

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

- In order to minimise handling stress and other WCs during loading and unloading, handlers should be properly educated and trained.
- Loading and unloading facilities should be fit for purpose in order to avoid WCs such as injuries.
- Delays in loading and unloading increase exposure to hazards such as high effective temperatures, may lead to WCs such as heat stress, and should be avoided.

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

A) Microclimatic conditions

- Among the environmental factors affecting the heat load placed on cattle during transport, it is recommended to take temperature and at least humidity into account when cattle 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.
- In order to reduce the risk of WCs due to exposure to high effective temperatures, the temperature inside vehicles transporting cattle should not exceed the UCT estimated to be 25°C.
- 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 comfort zone levels. Technical issues (e.g. accuracy, maintenance, placement, reliability and calibration) relating to this improved approach should be clarified.
- Future research should be carried within:
  - Development of systems to maintain the microclimatic conditions in stationary as well as moving vehicles across different compartments and deck heights by, e.g. air conditioning.

B) Space allowance

- In order to protect the welfare of cattle during transport, it is recommended to use the allometric equation $A = kW^{2/3}$ to calculate the minimum space allowance.
- It is recommended that the minimum space allowance is based on a k-value of at least 0.034, thereby allowing the cattle to be able to adjust to acceleration events, and lie down during journeys, including the performance of the lying-down and getting-up movements.
- The minimum vertical height during transport should be at least 40 cm above the withers of the tallest animal in a compartment (corresponding to [wither height (cm) × 1.17 + 20 cm]). Research is required to establish evidence-based thresholds for deck height, taking the microclimatic conditions and the motivation to move around inside the means of transport into account.

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- Further research is recommended in the following areas in order to support informed decision making on the protection of the welfare of cattle during transport:
  - Determine the effect of different space allowances, as based on the biological functions of cattle during transport and the minimum space requirement to perform these functions as determined by k-values, on the behaviour and clinical condition of cattle during transport. In particular, research should explore the ability of cattle to lie, to stabilise themselves and to thermoregulate during transport of long and short duration.
  - Determine the space required and the behaviour of cattle to consume water during transport, including the location of drinkers, the drinker type and the cattle to drinker ratio.
  - Determine vehicle design components, including the deck height and ventilation capacities to protect the welfare of cattle.

C) Journey times

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 WCs, continuous, progressively developing and sporadic, it is recommended to consider that:

- Animals experience motion stress and sensory overstimulation all along the journey potentially leading to fatigue, fear and distress;
- The pain and/or discomfort might be relatively rare but if happened, the consequences might be severe, and will worsen over time;
- Resting problems 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 9 h of transport;
- Physiological changes indicative of hunger can be present after 12 h 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 cattle 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 cattle and the current practice, the following is recommended to protect the welfare of cattle during this transport stage. New scientific knowledge may lead to adjustments of these.

- Cattle should be unloaded from the vehicle to effectively provide food, water and rest.
- Cattle from different vehicle compartments should not be mixed in a CP.
- The microclimatic conditions in the CP should allow for cattle to be kept in their thermal comfort zone.
- Lactating cows should be milked every 12 h.
- Feed should be provided for ad libitum intake and all cattle 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 cattle stay in a CP should be as low as possible.
- Until evidence-based thresholds are established for the duration of rest periods, compliance with the current 24 h period is recommended.

- Future research in the following areas is recommended:
  - Examine whether CPs fulfil their function, and how they should be designed and managed to protect cattle welfare.
  - Scientifically define an appropriate duration of stays at CPs.

5.6. Recommendations for the transport of cattle by air and rail

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

5.7. Recommendations for specific scenarios

A) Unweaned calves

- While still on-farm, adequate provision of colostrum as early as possible post-birth, should be part of the preparation for transport.
- Handlers of unweaned calves should be trained specifically in this animal category, as they differ from other cattle categories, e.g. in their tendency to follow each other and in their feed requirements. Enough time should be allocated to handle and feed the calves.
- When fed, calves should be provided milk or milk replacer of appropriate temperature and hygiene level, and not be fed electrolytes. The milk or milk replacer should be fed from teats allowing the calves to feed by sucking from an appropriate height.
- During transport, intervals between milk meals should not exceed 12 h, and not be shorter than 6 h. After a milk meal, calves should be allowed to rest (lying) in a calm place for 3 h to digest their meal.
- Maximum journey duration should take into account the time from last feeding. In order to allow calves to be loaded/unloaded and a 3-h post-meal rest, journeys should not exceed 8 h.
- In order for all calves to be able to lie down during journeys, a k-value of at least 0.027 is required.
- In order to reduce the risk of the WC heat stress, the temperature inside vehicles transporting unweaned calves should not exceed 25°C.
- Until evidence-based thresholds for deck height are established for this category of cattle, the minimum vertical height during transport should be at least [wither height (cm) × 1.17 + 20 cm], as recommended for other cattle categories.
- Based on the available knowledge, calves should be at least 5 weeks of age and of 50 kg weight when transported.

B) Cull dairy cows

- Due to the similarity in the WCs and hazards, alignment with the recommendations for road transport for preparation, loading/unloading and transit microclimatic conditions and space allowance (see Sections 3.3, 3.4, 3.5.3.1, 3.5.3.2 and 3.6) is recommended.
- 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.
- 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 questions on responsibility between the groups should be clarified.
- If these animals are fit for transport, the journey to a slaughterhouse should be kept to a minimum, be direct and not involve any unloading and reloading at any interim premises.
- If these animals are not fit for transport and are without the prospect of recovery in a reasonable period of time, they should be killed on farm as soon as is possible.

C) 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.

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D) 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 cattle 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 cattle when transported in livestock vessels is recommended.

E) 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 stabilised.
- The driver or animal attendant must be able to have access to the animals at regular intervals during the voyage in order to check and care for them.
- Lactating cows should not be transported on a Roll-on-roll-off if the total journey will be longer than 12 h, as milking is impossible.

F) 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 vehicles and procedures, including stationary resting periods, to ensure that the welfare of cattle during this type of transport is protected.

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Abbreviations

ABM	animal-based measure
ACTH	adrenocorticotropic hormone
AET	apparent equivalent temperature
AHAW	EFSA Animal Health and Welfare Panel
BHB	β-hydroxybutyrate
BPM	breaths per minute
BRD	bovine respiratory disease
CK	creatinine kinase
CRF	corticotropin-releasing factor
DoA	dead-on-arrival
ECI	Enthalpy Comfort Index
EKE	Expert Knowledge Elicitation
FAWC	UK Farm Animal Welfare Council
FPT	failure of passive transfer
HPA	hypothalamic–pituitary–adrenal
HR	heart rate
Ig	immunoglobulin
LCT	lower critical temperature
NEFA	non-esterified fatty acid
NTC	non-transported steers
PRE	preventive measures
RH	relative humidity
RO-RO	roll-on-roll-off
RR	respiration rate
THI	temperature–humidity index
TNZ	thermoneutral zone
TRACES	Trade Control and Expert System
TUS	thoracic ultrasonography
Twb	wet-bulb temperature
WC	welfare consequence
UCT	upper critical temperature
WOAH	World Animal Health Organisation

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

Name	Example			
Animal species	Example			
Animal type	Example			
Husbandery system	Example			
Code	**1**	Clearly relevant		
	32	Clearly not relevant		
	33	Not applicable		
	2 to 31	**Please rank the remaining WCs**	VVV	
ID	**Abbr**	Welfare Consequence	**Ranking**	Binding
B03	**SCS**	Group stress	0	0
H03	**HEA**	Heat stress	1	0
H04	**CLD**	Cold stress	1	0
B02	**RSP**	Resting problems	1	0
B06	**GFS**	Motion Stress	1	0
H01	**HNG**	Prolonged hunger	1	0
B01	**MOV**	Restriction of movement	1	0
B04	**VAS**	Sensory Overstimulation	1	0
B05	**HNL**	Handling stress		0
B09	**CMF**	Inability to perform comfort behaviour		0
B12	**EXP**	Inability to perform exploratory or foraging behaviour		0
H02	**H2O**	Prolonged thirst	1	0
H05	**LOC**	Locomotory disorders (including lameness)		0
H06	**SKL**	Skin lesions and wounds		0
H10	**RES**	Respiratory disorders		0
H12	**GED**	Gastro-enteric disorders		0
H15	**MTB**	Metabolic disorders		0
H16	**MUS**	Muscle disorders		0
H17	**UMB**	Umbilical disorders		0
B08	**SEP**	Separation stress	32	0
B16	**PLY**	Inability to perform play behaviour	32	0
H07	**MAN**	Pain resulting from management procedures	32	0
H08	**BNL**	Bone lesions (incl. fractures and dislocations)	32	0
H09	**SKD**	Skin disorders (other than pododermatitis or skin lesions)	32	0
H11	**EYE**	Eye disorders	32	0
B07	**ISO**	Isolation stress	33	0
B10	**WSX**	Inability to perform sexual behaviour	33	0
B11	**USX**	Inability to avoid unwanted sexual behaviour	33	0
B13	**MAT**	Inability to express maternal behaviour	33	0
B14	**SUC**	Inability to perform sucking behaviour	33	0
B15	**CHW**	Inability to chew and ruminate	33	0
H13	**RPD**	Reproductive disorders	33	0
H14	**MAS**	Mastitis	33	0
Please sort this table by the "Ranking" and complete the "Binding" (No. of cells with same rank)				

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