STUDY

Requested by the ANIT Committee

# Particular welfare needs in animal transport: unweaned animals and pregnant females

Workshop on Animal Welfare during Transport of 25 May 2021


Policy Department for Structural and Cohesion Policies
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RESEARCH FOR ANIT COMMITTEE

# Particular welfare needs in animal transport: unweaned animals and pregnant females

Workshop on Animal Welfare during Transport of 25 May 2021


**Abstract**

This study provides a technical overview and policy recommendations of the welfare need during transport of unweaned animals and pregnant females. During long journeys unweaned calves may experience negative welfare consequences such as prolonged hunger and thirst, resting problems, thermal stress and diseases. Further research is needed to develop appropriate methods to determine the gestational age during late pregnancy when the date of insemination or matting is unavailable, as well as to establish the gestational age at which females are at particular risk of suffering poor welfare during transport.

This document was requested by the European Parliament's Committee of Inquiry on the Protection of Animals during Transport (ANIT).


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# LIST OF ABBREVIATIONS

| | |
|---|---|
| **ABM** | Animal-based measure |
| **AHA** | Animal Health Australia |
| **ANIT** | European Parliament's Committee of Inquiry on the Protection of Animals during Transport |
| **BRD** | Bovine respiratory disease |
| **BW** | Body weight |
| **Comext** | Eurostat's database for statistics in international trade in goods |
| **CK** | Creatinine kinase |
| **EC** | European Commission |
| **EU** | European Union |
| **EUROSTAT** | Statistical Office of the European Union |
| **FAO** | Food and Agriculture Organisation of the United Nations |
| **OIE - WAHIS** | World Organisation for Animal Health |
| **MJ** | Megajoules |
| **MS** | Member States |
| **TRACES** | Trace Control and Expert System |

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# EXECUTIVE SUMMARY


KEY FINDINGS

- Unweaned calves between 2 and 4 weeks of age experience an immunological gap due to the shift between passive and active immunity, and therefore they are more susceptible to long journeys than weaned calves.
- To meet the basic physiological and behavioural needs, unweaned calves need between 10 and 20% of BW as temperate milk or milk replacer daily with 16-22 MJ and 160-240 g crude protein.
- On the basis of farm practices, 12 h may be recommended as maximum interval between milk meals, but this needs validation under transport conditions.
- Confirmation of the date of insemination or mating should be obtained before the transport of pregnant females to ensure that the stage of gestation can be ascertained.
- Alternatively, determination of gestational age could be possible with ultrasonography. However, the available data do not currently allow reliable benchmarks to be derived during late pregnancy.
- Further research is needed to establish the gestational age at which females are at particular risk of suffering poor welfare during transport.


## Background

The European Implementation Assessment of October 2018 on the Regulation (EC) 1/2005 highlighted the long-distance transport of unweaned animals and the ascertaining of the state of pregnancy of live animals as two of the most important issues that remain unsolved.

This study aims to analyse the welfare needs in transport of unweaned animals (focused on calves) and pregnant females (focused on cattle, sheep, goats and sows). The study examines the available academic literature, analyses the data related to the transport of these animals and provides an overview of the main current protocols/guidelines implemented in the EU Member States and third countries. It also highlights the main issues remaining unsolved and describes the best animal welfare practices in the transport of unweaned animals and pregnant females and identifies the main conditions for its proper enforcement. Finally, the research provides policy recommendations to improve the EU animal welfare standards in this area.

## Particular welfare needs in the transport of unweaned calves

The term 'unweaned animals' refers to those young animals that are still on a milk diet. The focus of the study is on unweaned calves of 2-4 weeks of age, transported over long journeys (> 8h), regardless of the means of transport (road and sea transport). In the dairy industry, calves not kept for replacement are considered by-products. It can happen that the amount and timing of colostrum feeding is not adequate if provided. Furthermore, calves could be sold and placed into collection centres before long distance transportation and sometimes without receiving an appropriate quantity and quality of feed (milk replacer) and water during that time. At this age calves experience an immunological gap due to the shift between passive and active immunity that compromise animal health and welfare during and after transport. During the journey, unweaned calves may experience negative welfare consequences such as prolonged hunger and thirst, resting problems, thermal stress and diseases. The magnitude of the welfare consequences is likely to increase over longer journeys.

As unweaned calves are more susceptible to long journeys, the assessment of fitness for transport is a critical point. Calves with wet or inflamed nibbles, lame, with respiratory disease signs, dehydrated or underfed are not fit for transport and should not be transported.

Based on knowledge on calves when kept on farm, unweaned calves need between 10 and 20 % of BW as temperate milk or milk replacer daily, with 16-22 MJ (3-6 weeks of age) and 160-240 g crude protein. On European farms, dairy calves in the age of 2-5 weeks are typically fed manually twice per day with an interval of 12 h, if they are not fed by automatic milk feeders. Even though unweaned calves are fed liquids (milk), they still need water. Long transports where calves are not properly fed before departure or during the resting period challenge the welfare and health especially of those unweaned calves that have low body reserve and immunological weakness.

Calves have a behavioural and physiological need to ingest their milk by sucking. The position of the head during milk drinking is essential to prevent liquid from flowing into the developing rumen. To secure best welfare liquid feed should be provided with rubber teats placed at 50-75 cm high. After milk feeding calves need at least 3 h of rest for a proper digestion. Improper digestion increases de risk of diarrhoea. Electrolytes as pre-transport diet do not fulfil the calves' nutritional requirement.

On the basis of farm practice, 12 h may be recommended as maximum interval between milk meals, but this needs validation under transport conditions.

Therefore, during the journey calves should have enough space and of adequate quality in terms of surface texture, dryness and hygiene, lie down, stand up and turn around without hindrance. If space allowance is reduced too much, calves cannot rest properly, resulting in fatigue. Physical space requirements increase with increasing body weight and can be calculated using the formula $A = k \times (BW)^{2/3}$, with a k between 0.027 and 0.047 for animals resting properly and change position, if required.

At any time during the journey (stationary or moving) the temperature range have to be maintained from 5 °C to 25 °C. In winter, pre-warm/air conditioning vehicles by using heaters/AC prior to loading. During hot weather and delays, provide water manually and spray the floors with water when risk of heat stress and only with low environmental humidity. In case of engine failure have a generator to keep running the ventilation.

Transport of dairy beef calves after weaning could mitigate the negative welfare consequences occurring during transport and might help to reduce post transport morbidity and mortality.

## Particular welfare needs in the transport of pregnant females

In the EU pregnant females who are 90% or more through their gestation period are considered unfit and should not be transported. Females in the last 10% of gestation are considered vulnerable, i.e. they present physiological weaknesses or pathological process that prevent them from being transported without undue suffering. This length of the pregnancy represents 255 of 284 days in cattle, 135 of 150 days in sheep, 139 of 155 days in goats and 104 of 115 in sows at the time of arrival at the place of destination. The major critical point on transport of pregnant female is the ascertaining of the state of pregnancy. A simple method to determine the length of pregnancy is through documents with the insemination or mating date. In case of natural insemination, the first day the male are put together with the female might be considered as the date of conception.

There are alternatives for pregnancy diagnosis. Some of them are invasive, expensive and/or not applicable in commercial conditions, while others are more practical approaches, but the determination of the gestational age is not accurate. Determination of gestational age should be possible with ultrasonography. However, the available data do not currently allow reliable benchmarks to be derived during late pregnancy. Further research is needed to develop an appropriate method to determine the gestational age during late pregnancy when the date of insemination or matting is unavailable.

Scientific evidence is lacking to determine the fitness for transport according to the stage of gestation. Further research is needed to establish the gestational age at which females are at particular risk of suffering poor welfare during transport and if their transportation should be avoided.

Further research is needed to determine the appropriate space allowance, partitions, ceiling height, bedding material and maximum journey duration for transporting pregnant females for each livestock species.

# INTRODUCTION

The European Parliament ([1]), the European Court of Auditors ([2]) as well as academic analysis and NGO's reports have noted that, despite the adoption and entry into force of Council Regulation (EC) Nº 1/2005 on the protection of animals during transport ([3]), the degree of progress in the implementation of many provisions by Member States has been insufficient to meet the European goals. The European Implementation Assessment of October 2018 on the Regulation (EC) 1/2005 highlighted the long-distance transport of unweaned animals and the ascertaining of the state of pregnancy of live animals as two of the most important issues which remain unsolved.

On 19 June 2020 the European Parliament decided to set up the Committee of Inquiry on the Protection of Animals during Transport (ANIT) to investigate alleged violations in the application of EU law on the protection of animals during transport and related operations within and outside the EU, including by air, road, rail and sea. The work of the ANIT Committee is focused on how EU rules are being implemented by Member States and whether the EU Commission is enforcing them properly ([4]). On this basis, the ANIT Committee wishes to commission a research project on the subject of 'Particular welfare needs in animal transport: unweaned animals and pregnant females' with a view to provide authoritative and timely information to its Members.

The aim of this research project is to analyse the particular welfare needs in the transport of unweaned animals (focused on calves) and pregnant females (focused on cattle, sheep, goats and sows).

1 Sections 2.5 and 2.6 of European Implementation Assessment on the Regulation (EC) Nº 1/2005 on the protection of animals during transport and related operations (pages 23 to 25).

2 European Court of Auditors, Special report Nº 31/2018 - Special report No 31/2018: Animal welfare in the EU: closing the gap between ambitious goals and practical implementation (europa.eu), 14 November 2018.

3 Council Regulation (EC) Nº 1/2005 of 22 December 2004 on the protection of animals during transport and related operations (consolidated text).

4 About | ANIT | Committees | European Parliament (europa.eu)

# 1. PARTICULAR WELFARE NEEDS IN THE TRANSPORT OF UNWEANED CALVES

KEY FINDINGS

- Unweaned calves between 2 and 4 weeks of age experience an immunological gap due to the shift between passive and active immunity, and therefore they are more susceptible to long journeys than weaned calves.
- Calves with wet or inflamed nibbles, lame, with respiratory disease signs, dehydrated or underfed should not be transported.
- To meet the basic physiological and behavioural needs, unweaned calves need between 10 and 20% of BW as temperate milk or milk replacer daily with 16-22 MJ and 160-240 g crude protein.
- On the basis of farm practice, 12h may be recommended as maximum interval between milk meals, but this needs validation under transport conditions.
- Electrolyte solution does not meet nutritional requirements of unweaned calves and cannot be considered part of feeding requirement.
- The effective temperature inside the vehicle should range between 5 and 25 ºC.
- Transport beef calves after weaning could mitigate the negative welfare consequences and might help to reduce post transport morbidity and mortality.

## 1.1. Literature review

To our knowledge, the transport of unweaned lambs and kid goats over long journeys is not a common practice in the EU. According to the European Council Regulation (EC) No 1/2005, calves may be transported for up to eight hours from the age of 10 days and for more than eight hours from 14 days. Therefore, the focus of this study is on unweaned calves of 2-4 weeks of age, transported over long journeys (>8h), regardless of the means of transport (road and sea transport). In the EU, the number of unweaned male and female calves that are not kept for replacement are around 1.5 million/year (Eurostat, 2019). These calves, considered a by-product of the dairy farm, are separated from their dams after calving and colostrum is provided by bottle or tube. However, the amount and quality of colostrum and the time of administration are often compromised (Renaud et al., 2018). Afterwards, calves are allocated in individual hutches and fed with milk replacer twice daily without reaching their nutritional needs (around 20 % of their BW) as a normal commercial management. After 14 days those calves are collected form the dairy farm and transported to auction market or concentration centre where after grouping are transported for long distance to be raised for veal or beef production. At this age, calves experience an immunological gap due to the shift between passive and active immunity (Hulbert and Moisá, 2016). At destination farm, those calves are liquid fed for 6-8 weeks before weaning following a restricted milk replacer program (around 2-3 L twice daily of 125 g milk replacer/L). The feeding management varies between veal and dairy beef calves. Veal calves are fed exclusively with milk replacer while beef calves are progressively introduced to solid feed. In this case, the restricted milk replacer program is used to enhance solid feed consumption.

In the EU, unweaned calf welfare during transport is protected by the Regulation (EC) 1/2005. Although it focuses on requirements such as fitness for transport, minimum age, transport duration, feeding intervals, transport temperature range or transport conditions such as space allowance and bedding, the welfare of those calves may still be compromised. Roadknight et al. (2021) cited in their review of transport of young calves that the challenges to be address include energy deficit (Knowles et al., 1997a; Fisher et al., 2014), fatigue (Todd et al., 2000; Jongman and Butler, 2014), cold (Knowles et al., 1997a; Fisher et al., 2014), stress due to handling, social mixing (Wilcox et al., 2013; Masmeijer et al., 2019), discomfort (Jongman and Butler, 2014), illness (Stafford et al., 2001) or injury (McCausland et al., 1977). Calf mortality during transport tends to be low, however, mortality rates following transport can be high, usually as a result of disease (Knowles, 1995). Age and weight are among the most important factors contributing to the ability of the calves to manage transport stress.

The literature review to examine and assess the particular welfare needs of unweaned calves during transport has been grouped into two phases: the preparation for transport and the journey itself.

### 1.1.1. Preparation for transport

According to Regulation (EC) 1/2005 animals that are injured or that present physiological weaknesses or pathological process shall not be considered fit for transport unless they are slightly injured or ill and transport would not cause additional suffering. Additionally, it mentions that unweaned calves are not considered to be fit for transport for long journeys if they have less than 14 days.

***Calf immunity, age and weight***

In the dairy industry, calves not kept for replacement are considered by-products. Often the amount and timing of colostrum feeding is not adequate if provided (Renaud et al., 2018). Poor colostral immunity has been associated to a high incidence of BRD and lower daily weight gain after transport (Pardon et al., 2015; Roadknight et al., 2021).

Unweaned calves are transported from the dairy farm to a rearing facility between 2 and 4 weeks of age. At this age, calves experience an immunological gap due to the shift between passive and active immunity (Hulbert and Moisá, 2016). Antibodies from the colostrum are low, and it is when calves build its own antibody responses to environmental microbiota (Hulbert and Moisá, 2016). Therefore, the transport of unweaned calves at this age matches a very vulnerable moment for the immunity of the calves as reported by the higher mortality rates in calves transported younger than 3 weeks of age (Staples and Haugse (1974).

In addition, most of the time age and body weight (BW) are related. However, low weight due to different conditions itself could be a critical factor in young calves. Masmeijer et al. (2019) observed that low BW calves (< 46 kg of BW and 2 to 4 month of age) even after 2 h of transport showed leucocytosis and more pro-inflammatory stage compared with high body weight calves (> 46 kg of BW). (Windeyer et al., 2014) reported that small calves are at a higher risk of post transport mortality than larger calves at arrival at the veal farm. Scott et al. (2020) associated light weight at transport to a decreased weight gain thereafter.

Knowing that there is a failure of passive transfer due to the poor colostral management of the calves at the dairy farms, it may be doubtful that those calves are in a good immunological condition to be transported with less than 5 weeks of age. From 6-8 weeks of age, the active immunity might be enough developed to face the transport challenges.

***Feeding***

Based on knowledge on calves when kept on farm, calves need between 10 and 20% of BW as temperate milk or milk replacer daily (Khan et al., 2011), with 16-22 MJ (3-6 weeks of age) and 160-240 g crude protein (Drochner et al., 2008). On European farms, dairy calves in the age of 2-4 weeks are typically fed manually twice per day with an interval of 12 h, if they are not fed by automatic milk feeders. Liquid feeding of unweaned calves requires the observation, and often the handling, of each individual animal. It also requires attention to hygienic presentation of the feed which must be made up to the correct temperature and solution strength in order to avoid digestive problems. Even though unweaned calves are fed liquids (milk), they still need water (Jensen and Vestergaard, 2018). Electrolytes as pre-transport diet does not fulfil the calves' nutritional requirement of the calves.

Calves have a behavioural and physiological need to ingest their milk by sucking (a teat or rubber teat) and not drink from a surface (De Passillé (2001). In addition, the position of the head during milk drinking is essential to prevent liquid from flowing into the developing rumen (Heinrichs, 1985; Brammertz, 2014). On-farm drinkers are often placed at 50-75 cm, but the exact height of the teat to secure best welfare is to our knowledge not known.

After milk feeding calves need at least 3 h of rest for a proper digestion (Marahrens and Schrader, 2020). Improper digestion increases de risk of diarrhoea. During digestion, calves must therefore have enough space to lie down and rest (Marahrens and Schrader, 2020). While nowadays it is not technically possible to feed calves on the truck, they should be fed with milk replacer between 3 and 6 h before transport to allow them to digest and get the maximum energy available to cope with the fasting period of the journey.

Calves are often sold and placed into collection centres before long distance transportation and do not always receive an appropriate quantity and quality of feed (milk replacer) and water during that time. Marcato et al. (2020) found that 70% of the calves were dehydrated (based on skin elasticity) already before the journey starts. Feeding electrolytes as pre-transport diet does not fulfil the calves' nutritional requirement and increases creatinine kinase (CK) and lactate compared with calves fed milk replacer (Marcato et al., 2020) and it might be indicative of calf tissue damage, hypoxia, fatigue and exhaustion during transport (Chacon et al., 2005; Averós et al., 2008) due to a poor feeding pre transport strategy. Instead, feeding milk replacer before transport is a good strategy to reduce energy depletion or hypoglycaemia (Schaefer et al., 1997; Marcato et al., 2020). Higher nutrient and energy content of milk seemed to protect calves against the effects of transport on nutrient mobilization and thus on BW loses (Marcato et al., 2020). However, caution should be taken also with milk replacer as suddenly changes on the quantity and quality of the milk replacer during all these phases may cause intestinal problems. Marcato et al. (2020) observed a reduced BW and increased presence of liquid faeces in the first 3 weeks post-transport in calves fed milk replacer at the concentration centre, due to the transport (e.g prolonged hunger, fatigue) and the management stress after (Devant and Marti, 2020).

***Lesions, wounds and overall health***

Umbilical problems have been reported to affect between 20 to 27 % of the calves transported (Pempek et al., 2017; Renaud et al., 2018; Marquou et al., 2019), and diarrhoea between 6.3 % and 14 % (Pempek et al., 2017; Renaud et al., 2018; Marquou et al., 2019). Therefore, these authors found in a study in auction markets in Quebec that before starting the journey 43 % of male dairy calves had at least 1 health abnormality performed and 7.7% of the calves had a generally unhealthy appearance.

These studies were performed in North America and no similar studies have been reported in the EU to ascertain the fitness for transport of the unweaned calves. The welfare consequences of the transport of calves with umbilical problems and other lesions have not been studied.

***Handling stress***

Young calves increase cortisol to response to transportation (Fell and Shutt, 1986; Grigor et al., 2001; Masmeijer et al., 2019). Cortisol peaks at the beginning of the journey might indicate that loading is an acutely stressful moment in the whole marketing and transportation process (Fell and Shutt, 1986; Kent and Ewbank, 1986; Grigor et al., 2001). Additionally, young calves have not learned to herd and follow other calves and therefore the handling of those animals become more difficult (Jongman and Butler, 2013) increasing the risk of poor handling (Roadknight et al., 2021).

### 1.1.2. During journey

During journey unweaned calves may experience negative welfare consequences such as prolonged hunger and thirst, resting problems, thermal stress and diseases. The magnitude of the welfare consequences is likely to increase over longer journeys. There is a relationship between death and transport distance and duration (Cave et al., 2005; Boulton et al., 2020). Roadknight et al. (2021) suggested that reducing transport distances and duration may reduce mortality and improve calves' welfare during and after transportation.

***Prolonged hunger and thirst***

Prolonged hunger occurs when the calf has been unable to meet its physiological requirements for energy, proteins and specific nutrients. This results in a catabolic energy metabolism leading to hunger and over time to impaired bodily functions (micro-nutrient deficiency). Feed restriction during the time at the sale yards and concentration centres together with the fasting period during transport means that prolonged hunger and thirst is one of the main welfare concerns during transport (Todd et al., 2000; Fisher et al., 2014). The Regulation (EC) 1/2005 states that unweaned calves, after nine hours of travel, be given a rest period of at least one hour is sufficient in particular if provided with liquid and if necessary, fed. After this resting period, they may be transported for a further nine hours. After the journey time of 19 h, animals must be unloaded, fed and watered and be rested for at least 24 h.

Todd et al. (2000) showed changes in metabolic measures after 12 h of feed withdrawal in calves aged 5-10 days, and Thomas et al. (2001) reported increased occurrence of behavioural indicators of hunger in newborn calves fed in 12 h intervals compared to animals fed in 4 h intervals. The same consequence might occur also in older calves with similar feeding intervals.

The minimum milk allowance to meet their requirements for maintenance and growth (3.75 L/d for a 50 kg calves) and avoid signs of hunger has been estimated to be 15% of BW/day (Thomas et al., 2001; Jensen and Holm, 2003; de Paula Vieira et al., 2008; Herskin et al., 2010). The most common feeding management at the farm is to feed calves twice daily in intervals of 12 h before reducing to one feeding a day previous to weaning although there are farm that feed calves three times a day but is less common. Liquid feeding in one meal or long intervals between two daily milk feedings limits milk intake and increases stress due to hunger (Stanley et al., 2002; Kehoe et al., 2007; Hulbert et al., 2011; Saldana et al., 2019; Jongman et al., 2020).

The exact need of feeding during transport is not known. It might be higher than on farm, due to the energy demands from being on a moving truck. However, prior studies (Knowles et al., 1997a; Knowles et al., 1999) involving physiological indicators of metabolism and liquid balance did not suggest differences between transported and control calves kept on-farm. A recent report summarizing 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) states that they have not been able to find studies establishing minimal or maximal intervals between successive liquid feeding, but intervals lower than 6 h may reduce milk intake due to the presence of curd in the abomasum form previous liquid feeding (Miyazaki et al., 2019). Further research on energy and liquid demand during long transports needs be carried out.

Journey duration, related to fasting period increases CK concentrations in blood indicating muscle activity, damage and fatigue (Todd et al., 2000; Fisher et al., 2014) and causes hypoglycaemia and high morbidity (Mormede et al., 1982). After 14 to 24 h of fasting, glucose concentration in young calves decreases (Todd et al., 2000; Fisher et al., 2014). Marcato et al. (2020) observed lower concentration of glucose in 18 h compared with 6 h transported calves and found that 12% of the calves had hypoglycaemia (plasma glucose < 2.8 mmol/L) after 6 to 12 h of transport. Negative energy balance described as an increase of non-esterified fatty acid (NEFA) and betahydroxybutirate (BHB) have also been observed (Todd et al., 2000; Fisher et al., 2014; Marcato et al., 2020) due to transport and fasting. Knowles et al. (1997a) observed a high plasma glucose concentration in calves that received 1L of glucose/electrolyte solution at eight-hour intervals during a 24 h journey. However, these authors suggested minor benefits to justify the unloading and feeding the calves compared to fasting them for the whole journey. Roadknight et al. (2021) proposed based on their review to feed the calves before transport as a more logically feasible option than mid-journey feeding. Furthermore, Devant and Marti (2020) pointed out that feed restriction during 19 h of transport may cause alterations in the intestinal permeability of those calves causing endotoxin infiltration (Bischoff et al., 2014) activating an inflammatory response.

No specific ABMs to assess prolonged hunger have been identified. Suggested non-specific hunger measures could be cross-sucking, vocalization and competition for milk, if available. However, they have been validated under farm conditions, and need to be validated under transport conditions.

In a recent review, Jensen and Vestergaard (2018) concluded that even though un-weaned calves are fed liquids (milk), they still need water. Calves' diet is based on liquid feeding, and they need constant access to drinking water for early development of the rumen (Jensen and Vestergaard, 2018). Prolonged thirst during transport occurs when the calf is unable to meet its physiological requirements for water. Dehydration has been observed after transport (Atkinson, 1992; Knowles et al., 1997a; Todd et al., 2000; Fisher et al., 2014; Pempek et al., 2017). When sever, it could 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.

Unweaned calves are suction drinkers and therefore for the physiological act of sucking they need a teat to take into their mouth. During transport (in concentration centres and on the trucks), calves might encounter unfamiliar drinkers that impede them to drink properly. Teat needs to be a flexible and of rubber to enable sucking with a negative and positive pressure phase triggering the abomasal groove reflex (Khan et al., 2011). This should be taken in account at the time of providing water or liquid feed during transport as well the temperature of the liquid feeding.

***Resting problems***

The balance maintenance in transit involves great physical effort (Terlouw et al., 2008). Therefore, during the journey calves should have enough space and of adequate quality in terms of surface texture, dryness and hygiene, lie down, stand up and turn around without hindrance. If space allowance is reduced, calves cannot rest properly, resulting in fatigue.

Whether animals will lie down is dependent on the transport conditions (driving quality, road conditions and suspension characteristics of the transport vehicle), journey length, space allowance and if it is comfortable to lie down (e.g. bedding). The lying time varies between 33 and 94 % in relation to the total journey in calves from 1 to 4 weeks of age depending of transport duration and space allowance (Kent and Ewbank, 1986; Knowles et al., 1997a; Knowles et al., 1999; Eicher and Morrow, 2000). Knowles et al. (1997b) found that calves spent approximately 50 per cent of the time lying during 24 hours of transport. During cold weather the amount of the journey spent lying increased to 80-90 per cent (Knowles et al., 1999).

The space allowance is the space provided per animal. Regulation (EC) 1/2005 point out that 50 kg calves require 0.30-0.40 $m^2$/animal. Apart from the size and weight of the animal, the minimum requirement also depends on various other factors that include ambient conditions (environmental temperature, adequate ventilation, relative humidity), ability of the animals to thermoregulate effectively and need for animals to lie down, to be watered or to be fed. Physical space requirements increase with increasing BW, and can be calculated using the formula (Petherick and Phillips, 2009):

$$A = k \times (BW)^{2/3}$$

where A is the floor area covered by the calf; k is a constant value that depends on the calf posture; and BW is individual body weight. This formula is useful to provide standardized space allowance recommendations across all cattle regardless of their weight (EFSA Panel on Animal Health and Welfare (AHAW), 2011; González et al., 2012). For a medium-sized calf with a body weight of 110 kg, k = 0.019 for standing animals equates to 0.44 $m^2$, k = 0.027 for lying equates to 0.63 $m^2$ and k = 0.047 for changing position equates to 1.096 $m^2$ (EFSA Panel on Animal Health and Welfare (AHAW), 2020). Therefore, the space allowance required for 50 kg BW calves with a k-value of 0.027 (to allow animals to lie simultaneously) will be 0.37 $m^2$ within the range of the requirement of the legislation. Additional space to change position from standing to lying or vice versa is also needed. In this case a k-value of 0.047 equates to 0.64 $m^2$. Space allowance should be revised to mitigate the welfare consequences derived from the restriction of movement and resting problems in transit.

***Cold and heat stress***

Regulation (EC) 1/2005 states that sufficient ventilation shall be provided to ensure that the needs of the animals are fully met taking into account in particular the number and type of animals and expected weather conditions. At any time during the journey (stationary or moving) the temperature range have to be maintained from 5 °C to 25 °C (+/– 5 °C).

Calves are homoeothermic 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 thermoneutral zone of young calves varies with age, weight, environmental conditions and other stressors ranging from 15 °C to 25 °C (Davis and Drackley, 1998). Thermal stress can manifest as two extremes: heat stress or cold stress, and will start to appear when the climatic conditions are such that the temperature regulation of the animals cannot be achieved by non-evaporative physical processes alone or without thermogenesis (Aggarwal and Upadhyay, 2012). Thermal stress intensity may vary from very light (e.g. start of sweating) to very severe (e.g. multiorgan failure and death; Silanikove (2000).

Below the lower critical temperature, the animals rely on thermogenesis to maintain the core body temperature. Animal's heat production is the result of the metabolic rate of body tissues, the metabolism of the brown adipose tissue, physical activity and the feeding heat increment (Vermorel et al., 1983). However, it should be taken into account that the unweaned calves transported already have low body fat reserves and possible failure of passive transfer that make them more susceptible to thermoregulate (Hulbert and Moisá, 2016). An ABM to assess cold stress is shivering (rapid twitching of muscle groups anywhere on the body).

Webster et al. (1978) showed that veal calves over 80 kg have considerable ability to cope with prolonged exposure to cold however, calves lighter than that (younger) did not present evidence of adaptation to cold environment (Webster et al., 1978). Additionally, these authors indicated that in very young calves, cold has no effects on growth and food conversion efficiency until the air temperature is below 5°C. However, intermittent exposure to temperatures around 0 °C increases heat loss and consequently food energy requirements around more than 25 %. Cold weather has been associated with highest post-transport mortalities in Canada and North Dakota (Staples and Haugse, 1974; Winder et al., 2016). Calves transported in winter took 16 h to regained pre-transport BW and 72 h to regained BW compared to non-transported group compare to calves transported in summer that took 8 h and 48 h, respectively (Knowles et al., 1999). Related to cold stress several factors have to be accounted such as wind chill (i.e. when air temperature is 10 °C and wind speed is 32 °C, it feels 0 °C; (Richardson, 2001), wet hair coat (e.g. environmental temperature rises the lower critical level from 0 °C to 14°C; (Richardson, 2001) or insufficient bedding (as a potential strategy to reduce calves' heat loss).

Some preventive measures can be taken at the time of transportation, e.g. the coldest hours of the day should be avoided for transportation. Cold stress in the truck can be controlled by partially closing the ventilation openings (boarding) in order to reduce air flow and by adding a minimum of 5 cm layer of Styrofoam to the vehicle top ceiling (Gonyou and Brown, 2012) and bedding on the truck floor such as woodchips, sawdust, cellulose or straw (Schwartzkopf-Genswein et al., 2012) dry and with high ability to soak up fluids. When bedding is sufficiently deep, calves can nest and trap a boundary layer of warm air reducing the lower critical temperature (Nordlund, 2008). Long straw of 20 cm provides great isolation in calves up to 6 weeks of age and is recommended in cold environment conditions (Sutherland et al., 2013).

As temperature rises, calves will start feeling warm. Above the upper critical temperature, calves will activate the thermoregulatory evaporative heat loss processes (vasodilatation, sweating and panting). When the temperature continues to rise in the hot zone, coping mechanisms to maintain homeothermy are unsuccessful, body temperature continues to rise, and fitness can be impaired. Heat stress in calves have been described at 25.5 °C and it means that calves increase dehydration, reduce feed intake and affects the immune system (Bentley, 2015), consequently calves drink more water to maintain normal hydration and energy requirements for maintenance increases 20-30 %, reduce movement, and increases breathing (Bentley, 2015). Severely heat stressed calves are recognisable from the open mouth breathing progressing to protruding tongue as the body temperature steadily rises (Gaughan and Meder, 2013).

In summer sand or sawdust bedding is more appropriate as they will not retain as much heat as the straw. A crucial preventive measure is to avoid transportation of cattle during the hottest hours of the day and to increase the space allowance. These measures are particularly relevant if the truck is not equipped with a forced ventilation system.

***Diseases***

Transport of calves and the related operations (e.g. mixing animals from different origins, fasting, handling and journey) may impair health conditions and increase morbidity. Calf morbidity is ranged for 12 to 43 % in North American auction markets and veal farms (Pempek et al., 2017; Marquou et al., 2019; Wilson et al., 2020). Between 33% and 96% of young calves are treated with antimicrobial during the first weeks after the transport at the rearing farm (Seppä-Lassila et al., 2018; Marcato et al., 2020). Mortality rates range from 0.06 to 0.7 % during the 24-30 h after arrival (Cave et al., 2005; Thomas and Jordaan, 2013). Furthermore, post-transport diarrhoea is not uncommon. Recently, Morrison et al. (2019) reported diarrhoea in approximately one third (31 %) of unweaned Holstein calves within three weeks after transport. Calves most probably cope better with transport over long journeys after weaning at an appropriate age and after appropriate management.

In addition to the age at which calves are transported, the length of time that marketing takes is also important. Mormede et al. (1982) found less post-transport disease amongst calves whose marketing took only 13 hours rather than 37 hours.

## 1.2. Data related to unweaned calves registered in TRACES and EUROSTAT

Transport between MS is recorded in TRACES, whereas transport within MS is recorded nationally. However, the quality of these within-MS transport data varies between MS. **Table 1** shows the number of cattle under 80 kg (excl. purebred for breeding) imported and exported for the individual MS in 2019 (EUROSTAT). This category is considered to be the best proxy for the import and export of unweaned dairy calves, because beef calves of this weight most likely stay suckling with their mothers at this stage and therefore are not sold. It includes both male and female calves. Within the EU, around 1.5 million unweaned calves were transported across MS annually. The main exporters were Germany (44 % of all calves exported in the EU), France (18 %) and Ireland (8 %). **Table 1** also shows that an estimated 7 %

of the calves born is exported to another MS. This is estimated assuming that one calf is born per dairy cow per year. In 2019, between 0 and 34 % of the calves born in a MS were exported, with the highest percentage (not numbers) for Estonia (34 %), Luxembourg (28 %), Czech Republic and Slovakia (both 19 %), Lithuania (18%) and Germany (17 %).

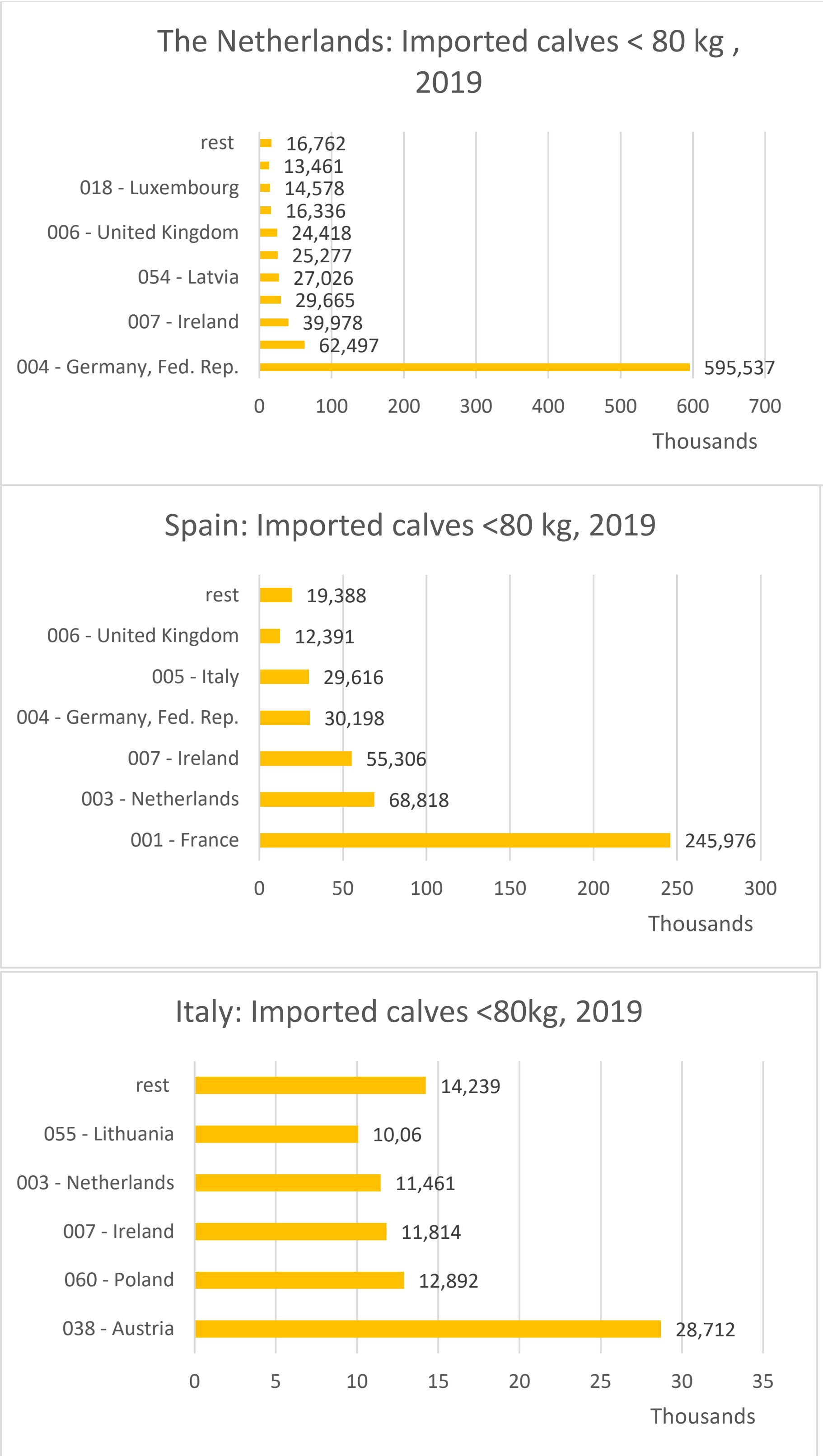
Sixteen MS imported calves, but nine of these fewer than 10.000 calves. The main importers were The Netherlands (57 % of all imported calves), Spain (30 %) and Italy (6 %). In these MS, calves production capacity exceeds the domestic production of calves. Figure 1 shows that in 2019, the vast majority of calves imported in The Netherlands originated from the neighbouring MS Germany (69 %) and Belgium (7 %). Between 2011-2014 these imports were 22.8 % and 1.4 % respectively, indicating that there has been a significant shift to imports from neighbouring MS. Most of the remaining calves came from MS in the eastern part of the EU. Most calves imported in Spain came from neighbouring MS France (53 %), followed by The Netherlands (15 %) and Ireland (12%) (**Figure 1**). For Italy, most calves came from neighbouring MS Austria (32 %), followed by Poland, Ireland, Netherlands, and Lithuania (all from 11 to 15 %). These numbers show that for all the three major importers of calves, the most important country of origin is a neighbouring MS, but that all three MS also import substantial numbers of calves from more distant MS.

**Table 1: Number of dairy cows and number of calves exported and imported in 2019 by EU MS.**

| | | Number of calves *1,000 (Eurostat code 01022910 - Live cattle of a weight ≤ 80 kg (excl. pure-bred for breeding) | | |
|---|---|---|---|---|
| | Number of dairy cows = number of calves born *1,000(1) | Export(2) | % of born calves exported(3) | Import(2) |
| **Belgium** | 538 | 32 | 6 | 11 |
| **Bulgaria** | 227 | | | |
| **Czech Republic** | 361 | 70 | 19 | |
| **Denmark** | 563 | 35 | 6 | 0 |
| **Germany** | 4,012 | 675 | 17 | 0 |
| **Estonia** | 85 | 29 | 34 | |
| **Ireland** | 1,426 | 130 | 9 | 5 |
| **Greece** | 86 | | | |
| **Spain** | 813 | 3 | | 462 |
| **France** | 3,486 | 273 | 8 | 17 |
| **Croatia** | 130 | | | 0 |
| **Italy** | 1,876 | 3 | | 89 |
| **Cyprus** | 35 | | | |
| **Latvia** | 138 | 15 | 11 | |
| **Lithuania** | 241 | 42 | 18 | 5 |
| **Luxembourg** | 54 | 15 | 28 | 0 |
| **Hungary** | 243 | 9 | 4 | 6 |
| **Malta** | 6 | | | |
| **Netherlands** | 1,590 | 95 | 6 | 866 |
| **Austria** | 524 | 36 | 7 | |
| **Poland** | 2,167 | 21 | 1 | 48 |
| **Portugal** | 234 | 16 | 0 | |
| **Romania** | 1,139 | 10 | 0 | 16 |
| **Slovenia** | 101 | | | |
| **Slovakia** | 126 | 23 | 19 | 0 |
| **Finland** | 259 | | | |
| **Sweden** | 301 | 1 | 0 | 0 |
| **UK** | 1.867 | 12 | 1 | |
| **Total** | **21,197** | **1,545** | **7%** | **1,525** |

(1) Source: Eurostat; (2) Source: Comext. A value '0' means less than 500, empty means no export / import; (3) Assuming one calf born per dairy cow per year. Since in a number of countries also females not used for replacement are exported the fraction exported of all new born calves is calculated.

**Figure 1**: Origin of calves of less than 80 kg imported in 2019 in The Netherlands, Spain, and Italy.



## 1.3. Overview of the main current protocols/guidelines implemented in the EU Member States and third countries improving the welfare of unweaned calves transported

As mentioned before transport of unweaned calves falls under the EU Regulation (EC) 1/2005. National contact points of EU Member States and some third countries were to request information regarding protocols or guidelines that have been implemented by the Competent Authorities and Industry in their countries targeted to improve the welfare of type of these animals during transport. We received feedback from Norway, Iceland, Sweden, Estonia, Croatia, Belgium, Greece, Luxembourg, Latvia, Germany, Spain, Slovenia and United Kingdom, confirming our previous assumption. None of these countries have specific protocols/guidelines for long transports of unweaned calves.

In Germany, in March 2020 the Friedrich-Loeffler-Institute (FLI) published the Technical Requirements for long-distance transport of unweaned calves (Marahrens and Schrader, 2020) with the objective to assist the authorities of the Federal States responsible for granting permissions of long distance transport of calves in approving the means of transport. Those technical requirements are focused in feeding the calves and the following essential requirements must be fulfilled:

- Milk or milk replacer should be administered by a flexible rubber teat.
- Animals must obtain feed already with the first sucking act.
- Milk or milk replacer must be administered at 30 ºC.
- Acidification or liquid feeding should be avoided.
- All calves must be able to feed at the same time and the full energy and protein content necessary per meal.
- Guidance to the feeding system must be provided by the keeper.
- The capacity of the storage must comply with the total number of transported calves.

In the Netherlands, specifications about the drinking systems for transports longer than 8 h it has been established. The water provided should not have additives and be heated (warm water). The teats in the lorry should be synthetic (rubber), flexible and durable and provide easy water release. The hight should be between 50 and 100 cm above the floor and 10 cm below the ceiling. It states that the number of teats is 1 per 3.5 $m^2$ of floor space and a minimum of 2 teats for compartment and the way that they are attached do not hurt the calves.

In Australia, unweaned calves transport is regulated by the Australian Animal Welfare Standards-Land Transport of Livestock (Animal Health Australia (AHA), 2012). Calves younger than 5 days only can be transported for 6 h. The maximum time off water for calves 5–30 days old travelling without mothers is 18 h. In addition, bobby calf or unweaned calves between five and 30 days old must be protected from cold and heat. They must be in a good health, alert and able to rise from a lying position, have been adequately fed milk or milk replacer on the farm within six hours of loading. They should be assembled and transported to ensure delivery in less than 18 h from last feed with no more than 12 h spent on transports and have an auditable and accessible record system that identifies the calf last fed. They must have a minimum of 23 kg. In addition, bobby calves under 30 days old must have sufficient space in the livestock compartment to lie down all calves at the same time on their sternums. Direct marketing should be used when possible. Calves should not be consigned through saleyards that do not have holding facilities suitable for calves. A person must not use a dog to move a bobby calf during the transport process.

New Zealand is regulated by Transport within New Zealand- Code of Welfare (New Zealand Government, 2018), and states that the duration of travel for young animals (up to 14 d of age) should not be longer than 12 h and calves less than one week of age should be fed within two hours prior to travel. Minimum transport age is 4 full days (96 h) and defines fitness for transport and protection of the calves based on environmental conditions.

Canada has recommendation and they are stated in the Code of Practice for the Care and Handling of Farm Animals: Transportation (National Farm Animal Care Council, 2020). This code of practice is under revision.

## 1.4. Main issues linked to the transport of unweaned calves which need addressing

The main issues linked with the transport of unweaned calves are the following:

- Age at transport: during the second and fourth week of age unweaned calves are typically transported and experience an immunological gap that compromises animal health and welfare during and after transport.
- Fitness for transport: unweaned calves are more susceptible to long transports making the assessment of fitness for transport a critical point.
- Transport duration and hours of fasting: unweaned calves can only be fed with milk or milk substitutes. Electrolyte solution does not meet nutritional requirements and cannot be considered part of feeding requirement. Long transports where calves are not properly fed before departure or during the resting period challenges the welfare and health specially of those unweaned calves that have low body reserve and immunological weakness. Further research on energy and liquid demand during long transports needs be carried out. In calves, the interval from a milk meal and until gastric emptying is to our knowledge not known.
- Feeding, watering, and resting during long transport: calves can only be successfully fed (with milk or milk replaced) and watered after unloading and this should take place at a control post, market or assembly centre. Provision of water, milk or milk replacer and resting times needs to be addressed based on calves needs before and during transport, at assembly centres, markets and control posts. There is a lack of studies on resting time in unweaned calves on the truck and at control posts.
- Feeding and drinking devices in the truck: milk or milk replacer is not provided on the truck due to the technical difficulties with the current drinking system. Drinking is not ensured for all calves due to inappropriate devices (type and quantity). On-farm drinkers are often placed at 50-75 cm, but the exact height of the teat to secure best welfare is to our knowledge not known.
- Space allowance in the truck: if space allowance is not enough to lie down, stand up and turn around without hindrance, unweaned calves will not rest properly, resulting in fatigue. The lack of studies makes difficult to address the best space allowance based on the animal, the journey

duration, and the environmental conditions. Future research is needed to address the time calves need to spend lying and the position during the journey.

- Transportation with lower and upper critical temperature: unweaned calves are very susceptible to extreme temperatures that can be manifested by heat or cold stress heat and will start to appear when the climatic conditions are such that the temperature regulation of the animals cannot be achieved by non-evaporative physical processes alone or without thermogenesis.
- The fitness for transport and the welfare status during the journey and at arrival should be assessed and monitored through ABMs.

## 1.5. Best animal welfare practices as regards transport of unweaned calves and the major conditions for proper enforcement

The following best practices aim to improve the fitness of the unweaned calves for transport and promote the health and welfare during the journey and at destination. They are based on either scientific knowledge, scientific literature, or experiences. The health and welfare status should be assessed at each phase of the transport to prevent and correct hazards and mitigate the negative welfare consequences. ABMs, such as injuries, panting or shivering can be a very useful tool to monitor the health and welfare of the animals before, during and after transport.

Nevertheless, the better practice is to only transport dairy beef calves after weaning. At this age calves are capable of ingest solid feed and have a better immunological system to cope with the challenges of the long journey. In case of veal calves where liquid feeding is part of their diet until slaughter the best practices would be increase transport age at 6-8 weeks and reduce journey hours to avoid long transportation. However, further research should be performed to know how calves cope with long transports at these ages.

Preparation for transport:

Unweaned calves with wet or inflamed nibbles, lame, with respiratory disease signs, dehydrated or underfed are not fit for transport and should not be transported.

To meet the basic physiological and behavioural needs, calves should be fed 20-30% of their body weight as temperated milk or milk replacer daily and 16-22 MJ and 160-240 g crude protein.

Calves need 3h of rest after a milk meal. During this period, it might be preferable not to load or unload calves to a means of transport. However, further research on the effect of handling after milk meals on proper digestion is needed.

Electrolyte solution does not meet nutritional requirements and cannot be considered part of feeding requirement. Fed the calves with milk and not electrolyte solution based on their daily requirements.

Provide liquid feed with rubber teats at the assembly centres, markets, control posts. Make sure calves are familiar with the drinking system to supply milk, milk replacer of the right temperature (e.g. use rubber teats).

Even though unweaned calves are fed liquids (milk), they still need water. Calves should be offered water ad libitum in addition to the milk.

When calves are handled in a group, limit the group size to 10 – 15 calves during loading. Calves can be loaded with individual help (one hand before the head, the other hand on the back) to guide them on the ramp.

During journey:

On the basis of farm practice, 12 h may be recommended as maximum interval between milk meals, but this needs validation under transport conditions. To accommodate the need for proper ingestion, the height of the sucking device has to meet the need for fluid not to go into the rumen. New mechanical technology should be developed to feed the calves inside the truck.

Water should be provided all the time with a system with rubber teats. The exact height needed in not known, but is probably at least 50 cm. Resting time should be enough to allow all calves to have access to the drinker devices.

During long journeys adequate space should be provided to stand up, lie down and turn around without hindrance. Space allowance should be calculated through the formula $A = k \times (BW)^{2/3}$, where A is the floor area covered by the calf; k is a constant value that depends on the call posture and behaviour; and BW is the bodyweight of the individual animal. It is recommended to use a k value of between 0.027 and 0.047.

Provide always appropriate bedding (e.g. straw) which guarantees comfort appropriate to the number of animals, the journey time, the weather and the absorption of urine and faeces. Adequate bedding material should be dry with high ability to soak up fluids. Enough bedding allow for more comfort and facilitate the resting of animals. During cold days, bedding depth should be increased to 20 cm. In warm days sand or sawdust is recommended as it will dissipate heat.

The deck should have a side protection in order to avoid the animals' legs getting trapped between the deck and the side wall.

The effective temperature inside the vehicle should range between 5 and 25°C. The automatic control of mechanical ventilation by the monitored temperature of a control system is technically feasible and beneficial in animal transport. In winter, pre-warm/air conditioning vehicles by using heaters/AC prior to loading. During hot weather and unexpected delays, provide water manually and spray the floors with water when risk of heat stress and only with low environmental humidity. In case of engine failure there should be an emergency generator, so the fans can be kept running and the temperature, air flow and oxygen can be controlled.

## 1.6. Policy recommendations

Based on the available evidence, the study sets out the following policy recommendations in relation to the transport of unweaned calves:

- Due to the higher susceptibility to the transport conditions, unweaned calves need specific protocols for the assessment of their fitness for transport. Calves with wet or inflamed nibbles, lame, with respiratory disease signs, dehydrated or underfed should not be transported.
- Feed (milk replacer) should be provided to the calves between 4 and 6 h before loading according to calf needs (minimum 20 % of their BW).

- Water should be provided in addition to the milk until the time of loading.
- Electrolyte solution should not be provided as a feeding strategy.
- Feeding devices should allow the calves to suck.
- The feeding and watering frequency, amount (MJ) and temperature during transport (from last feeding before loading on the farm of origin to end of the journey) should be recorded and monitored during the journey to take remedial actions if needed.
- Maximum transport duration should have in account the time the last feeding. On the basis of farm practice, 12 h may be recommended as maximum interval between milk meals, but this needs validation under transport conditions.
- Nowadays, feeding on the truck often faces technical difficulties with current drinking system. Drinking cannot always be ensured for all calves due to inappropriate devices (type and quantity). Further research and technology development should be carrying out to prevent prolonged hunger and thirst in unweaned calves during transport.
- The lack of studies makes difficult to address the optimal space allowance based on the animal and environmental conditions. Future research is needed to address the time calves need to spend lying and the position during the journey.
- Protocols during transportation with low and high critical temperature should be put on place to prevent calves do not exceed the limits stablished by the regulation.
- ABMs should be developed and used to assess the fitness for transport and the welfare of the animals during the journey, at arrival and afterwards at destination.
- Due to the immunological gap at the actual age of transport, consider transporting calves after weaning for dairy beef calves, and additionally to increase transport age at 6 to 8 weeks avoid long transports for veal calves. If the transport age increases, actions should be taken to ensure calves receive proper care at the origin farm.

# 2. PARTICULAR WELFARE NEEDS IN THE TRANSPORT OF PREGNANT FEMALES (FOCUSED ON CATTLE, SHEEP, GOATS AND SOWS)

KEY FINDINGS

- Confirmation of the date of insemination or mating should be obtained before the transport of pregnant females to ensure that the stage of gestation can be ascertained.
- A simple method to determine the length of pregnancy is through documents with the insemination or mating date. Ninety percent of gestation represents about 255 days in cattle, 135 days in sheep, 139 in goats and 104 in sows.
- Alternatively, determination of gestational age could be possible with ultrasonography. However, the available data do not currently allow reliable benchmarks to be derived during late pregnancy.
- Further research is needed to establish the gestational age at which females are at particular risk of suffering poor welfare during transport.

## 2.1. Literature review

In the EU, pregnant females can be transported before the 90% of gestation period between EU MS and imported/exported from/to third countries for breeding purposes. Pregnant females are also transported for slaughter due to health and welfare issues, management advantages and economic necessity/benefits, as well as due to the lack of supervision of breeding (specially in extensive production systems), absence or failure of pregnancy diagnosis and poor record keeping or loss of information in the trading chain (EFSA Panel on Animal Health and Welfare (AHAW), 2017). Nonetheless, only few studies have evaluated how the transport can affect the welfare of pregnant females.

The literature review to examine and assess the particular welfare needs of pregnant females is presented in two phases: the preparation to transport and the journey itself.

### 2.1.1. Preparation to transport

***Ascertaining of the state of pregnancy***

The Regulation (EC) 1/2005 requires that no animal shall be transported unless it is fit for the intended journey. Females in the last 10% of gestation are considered vulnerable, i.e. they present physiological weaknesses or pathological process that prevent them from being transported without undue suffering. Heavily pregnant cattle are at higher risk of health and welfare problems during and after transport, being more susceptible to abortion or early delivery, heat stress, dehydration, injury and metabolic disease (Government of Western Australia, 2020). Therefore, pregnant females who are 90% or more through their gestation period are considered unfit and should not be transported. The major critical point on transport of pregnant female is the ascertaining of the state of pregnancy. Despite the

Regulation and guidelines are clear regarding the unfitness condition of pregnant females that are 90% or more through their gestation period to be transported, the determination of pregnancy stage before the transport still remains to be resolved.

A simple method to determine the length of pregnancy is through documents with the insemination or mating date. The length of the pregnancy may not be longer than 255 days in cattle, 135 days in sheep, 139 in goats and 104 in sows at the time of departure. In line with that, the White Paper published by the Eurogroup for Animals (2021) also proposes that "*prior to approval for the transportation of pregnant bovines, swine, rabbits and ovine, the gestation stage of these animals must be assessed, and the organiser must provide documentation to the competent authority on the insemination/mating approximate date, and the last gestation check carried out by the veterinarian on the farm not longer than two weeks before the expected planned departure. In case of natural insemination, the first day the male was put together with the female shall be considered as the date of conception. In case of continuous coexistence of the males with the females, only the latter applies*".

There are different alternatives for pregnancy diagnosis in livestock that can be used in cases of unavailability of the documents with insemination or mating date. Some of them are invasive, expensive and/or not applicable in commercial conditions (e.g. laparotomy, laparoscopy, vaginal biopsy, radiography). Transabdominal palpation, abdominal inspection, appearance of milk secretion, evaluation of increase in live body weight, and non-return to oestrus are more practical approaches, but they are more useful for pregnancy diagnostic and accuracy depends on gestation stage. In contrast, only few techniques are used to estimate pregnancy length, such as enlargement of udder, manual rectal palpation, assay of steroid hormone concentration, radiography and real-time ultrasonography. These techniques are briefly described below.

**Enlargement of udder:** The evaluation of udder has been considered as a cheap technique for pregnancy diagnose in sheep (Watt et al., 1984). Moderate development of the udder occurs in 96% of sheep between days 111 and 130 of gestation (81 and 96% of gestation length, respectively), and 84% of animals have enlarged udder after the 130 days of gestation (out of 135 days of gestation in total). These changes are even more evident in primiparous ewes (Watt et al., 1984). Therefore, udder evaluation could be as a practical method for diagnosis to detect the end of pregnancy; however, they are not an accurate technique to determine the exact gestation length. This technique could be extrapolated to goat and cattle, keeping the proper proportion of gestation length for these species.

**Manual rectal palpation**: Pregnancy status is routinely detected in cattle by inserting the hand into the rectum, palpating through the rectal and uterine walls for the amniotic vesicle, foetal membranes or cotyledons within the uterus (Garth Sasser et al., 1986). Rectal palpation is considered an accurate and safe method to detect pregnancy in cows from about 30 to 35 days of pregnancy, and few days earlier in heifers (Momont, 1990). Manual rectal palpation could also be used as a method to predict calving dates in dairy cows based on foetal ages (Matthews and Morton, 2012). However, the accuracy of this method reduces when cows are examined at a gestation stage later than 14 weeks (out of 40 weeks of cattle gestation length), i.e., manual rectal pregnancy diagnosis should be scheduled within 15 weeks after their most recent artificial insemination. Therefore, they might not be useful to detect, prior transport, if a female is over 90% of pregnancy.

**Assay of steroid hormone concentration**: Progesterone and estrone sulphate concentration is also used to early detect pregnancy in small ruminants (Karadaev, 2015). Progesterone concentrations are analysed in blood, milk and faecal while estrone sulphate is analysed in blood (Karadaev, 2015). According to Tsang (1978), estrone sulphate could be detected in blood plasma of goats and sheep about 70th gestation day (0.1-0.3 ng/mL), which gradually increasing until 2 days before the parturition (1-9 ng/mL). However, it is important to note that 70th day of gestation correspond to approximately 50% of the gestation length of goats and sheep, which could not be useful to determine, prior to transport, if a female is over 90% of pregnancy.

**Radiography**: As reviewed by Karadaev (2015), the radiography is a technique applicable with more accuracy after the 80th post breeding day in goats because the foetal skeleton is mineralised and then visualised on the radiographic images. This method could be used to measure the foetus length and, consequently, the gestation length, however the equipment is expensive, requires technical skills, are not applicable in commercial conditions and has potential risks for both operator and the animal.

**Real-time ultrasonography**: Real-time ultrasonography is a practical and efficient approach for pregnancy diagnoses and for monitoring foetal growth in ewes (Jones et al., 2016; Petrujkić et al., 2016), goats (Erdogan, 2012; Karadaev, 2015) and cows (Buczinski, 2009; Hunnam et al., 2009) via transrectal and transabdominal ultrasonography scans. The limitation of the transrectal ultrasound to pregnancy diagnosis is because it becomes more difficult after certain stage of gestation, as most structures required to diagnose are beyond the reach of a transrectal probe (Hunnam et al., 2009). In ewes, it also might require a dorsal recumbent position of the animal, which could indicate a limitation in field conditions (Wurst et al., 2007).

Transabdominal ultrasonography is known as an accurate and rapid method. It is an alternative to assess structural developments and measure foetal length, which are important indicators of gestational age and breeding date (Jones et al., 2016). In cattle, Hunnam et al. (2009) determined the gestation age by transabdominal ultrasonography, measuring the diameter of thoracic, abdominal and umbilical, and placentome length and placentome height, however, gestation period evaluated was up to 190 days. Lazim et al. (2016) reported a high correlation between gestational age and foetal measurements (especially heart width) during the 2nd and 3rd trimester of gestation, including cows with more than 250 days of gestation. In fact, this was the first study evaluating the correlation between heart width and cows gestational age using ultrasonographic information in cattle. Transabdominal ultrasonographic assessment of the foetus on the last third of pregnancy is done with the cow standing and tied by the head Buczinski (2009).

Transabdominal ultrasonography is commonly used for pregnancy diagnosis in small ruminants, which provides critical information during early gestation, such as estimations of foetal number and gestation age (Jones et al., 2016). Petrujkić et al. (2016) reported that transrectal ultrasonography proved the most confirmative method in foetal age determination in ewes but the ewes were in their 1st third of gestation period (<63 gestation day). From the 2nd trimester of gestation of small ruminants, transrectal ultrasonography becomes inadequate because the pregnant uterus descents into the cranio-ventral direction, making the transabdominal scan more appropriate (Erdogan, 2012).

The limitation associated with the transabdominal ultrasonography technique is due to the fact that the measurement of foetal length depend on foetal positioning in utero and limited by equipment capacity (Jones et al., 2016). Also, the earlier the ultrasound is done, the more accurate it is at estimating the offspring's due date (Buczinski, 2009). In cows, the assessment of specific foetal parts is limited due to its large size, which makes impossible to observe the whole foetus as in human and ovine foetus (Buczinski, 2009).

Therefore, further investigation of the sensitivity and improvement of the transabdominal ultrasonography technique to determine gestation age of pregnant females in late pregnancy is needed.

The characterization of foetal age can also be determined by external features (e.g. presence of hair and teeth) or linear measures (e.g. crown-rump length, nose-to-tail length or large bones length) of the foetus. The EFSA Panel on Animal Health and Welfare (AHAW) (2017) suggested that external morphological features are better indications than linear dimensions due to the differences between breeds in mature body weight and size and the individual variation within breeds. These methods are useful to determine the length of pregnancy in case of violation of the Regulation (EC) 1/2005; however, they can only be applied in the slaughterhouse after the transport and the slaughter of the dam.

***Fitness for transport***

The effect of transport on pregnant females has been evidenced by changes in physiological parameters in different stages of pregnancy.

**In early pregnancy stage:** An increase in cortisol concentration was reported in pregnant beef cows transported for 4 to 6 h approximately 14 days after artificial insemination (Merrill et al., 2007). In early pregnant heifers (2 to 3 months pregnancy), serum cortisol increased after transportation for 100 and 200 km and return to baseline concentrations (as before the transport) at 24 h after transportation (Kang et al., 2017). These animals also showed a reduction in serum triglyceride concentration which contrasted with an increase in non-esterified fatty acid (NEFA) concentration (Kang et al., 2017). The stress associated with the transport also increased the number of innate immune cells in these heifers, as it was demonstrated by the increase in granulocyte-to-lymphocyte ratio and the percentage of monocytes after a transport over 200 km (Kang et al., 2017). To our knowledge, this is the only study that evaluated the effect of the transport on blood progesterone concentration in pregnant animals. Further to the lack of the transport effect on this parameter, this study also reported that the cows had normal calving and that all calves grew normally (Kang et al., 2017). Finally, there was no effect on the pregnancy rate of beef cows transported approximately 14 days after artificial insemination (Merrill et al., 2007).

**In the medium stage of pregnancy:** An increase in cortisol concentration was reported in pregnant beef cows (approx. 139 ± 5 days of gestation) transported for 48 km (Lay Jr et al., 1996). Glucose level increased significantly in pregnant heifers (approx. 5 months of gestation) and decreased afterwards, which could be associated with the deprivation of food and water (Lambooy and Hulsegge, 1988). Similar pattern was reported for the serum creatine kinase (CK) concentration in pregnant cows (approx. 185 days of gestation) during long journeys lasting 3 or 4 days and 48 hours after transport (Fisher et al., 1999). These animals also experienced a concerned decline in serum magnesium concentration, and a stabilisation or recovery occurred only after 1 or 2 days of BW increases (Fisher et al., 1999).

**In late pregnancy:** Cortisol concentration also increased in goats transported for 20 km during the last five weeks of gestation (Duvaux-Ponter et al., 2003), and in ewes transported in isolation during their last 6 weeks of pregnancy (Roussel et al., 2006). Pregnant goats transported for 20 km during the last five weeks of gestation (Duvaux-Ponter et al., 2003) had an increase in glucose level and non-esterified fatty acid (NEFA) concentration. The transport of goats and ewes transported during their last five or six weeks of gestation did not affect their gestational length (Duvaux-Ponter et al., 2003) and litter size (Roussel et al., 2006), respectively.

Previous studies have shown that animals become accustomed to the stress caused by the transport when it is repeated over the gestation (Lay Jr et al., 1996), which can indicate that the stress caused by the transport is more associate with the novelty of the procedure rather than the pregnancy state. This psychological habituation was indicated by a decrease in serum cortisol (Lay Jr et al., 1996) and serum glucose concentrations during a repeated transport of pregnant cows (Price et al., 2015).

Finally, earlier studies showed that the stressful situations experienced by pregnant animals during transport in different pregnancy stage have detrimental effects on offspring's behavioural and physiological responses (Duvaux-Ponter et al., 2003; Roussel et al., 2006; Roussel-Huchette et al., 2008; Littlejohn et al., 2016; Littlejohn et al., 2017; Littlejohn et al., 2018; Littlejohn et al., 2019; Baker et al., 2020; Cilkiz et al., 2020; Littlejohn et al., 2020).

### 2.1.2. During the journey

***Space allowance, partitions, ceiling height and bedding material***

As previously stated, pregnant females can be transported in the EU before the 90% of gestation period. The Regulation (EC) 1/2005 defines that pregnant cattle must be allowed 10% more space than those given to no-pregnant animals during transport by sea. Regarding the transport of heavily pregnant sheep, the regulation requires the increase of the minimum area per animal from 0.20 to 0.40 $m^2$/animal for sheep with less than 55 kg BW and from 0.30 to 0.50 $m^2$/animal for sheep with more than 55 kg BW. In goats, the regulation requires the increase of the minimum area per animal from 0.20 to 0.40 $m^2$/animal for goats with less than 35 kg BW and from 0.30-0.40 to 0.50 $m^2$/animal for goats with more than 35 kg BW. These values did not differ for transport by rail or by road. For the transport of sows, the regulation does not distinguish between pregnant and no-pregnant animals.

According to the Regulation (EC) 1/2005, adult animals do not need to be transported separately if they are used to live in groups, which is the case of cattle, sheep, goats and sows. However, no special mention is done in relation to pregnant females. Lambooy and Hulsegge (1988) evaluated a 24 h transport of pregnant heifers (5 months into gestation), hauled either loose or penned in pairs and separate gates during the transport, with similar stocking density (300-350 kg/$m^2$). The penned heifers spent more time lying down, drank more water and lost less weight when compared to those loose in the compartment. However, in the case of this study, heifers were not accustomed to stand between gates, and it appeared to be insufficient room for the remaining animal to stand, resulting in more skin lesions.

Furthermore, ceiling height to transport livestock should be at least 20 cm above the withers height of the tallest animal to allow them to stand with their heads up in a natural and comfortable position (EFSA Panel on Animal Health and Welfare (AHAW), 2011). A previous study examining a long distance transport (approx. 27 h) reported that pregnant heifers remained quiet and calm and did not head-butt the ceiling with a clearance of 40 cm above their withers, which contrasted with no-pregnant adult dairy cattle that head-butted the ceiling when it was set at 10, 15 or 20 cm above their withers (Lambooij et al., 2012).

***Journey duration***

To our knowledge, the Regulation (EC) 1/2005 does not specify different maximum duration to transport pregnant females. The reduction in bodyweight of pregnant females was already reported after long (e.g. 24 and 3-4 days including resting stops) (Lambooy and Hulsegge, 1988; Fisher et al., 1999) and short (e.g. 1-2 hours) journeys (Duvaux-Ponter et al., 2003; Price et al., 2015). However, to our knowledge, there is no study that compared the effect of different transport duration on the welfare of pregnant females.

***Prolonged hunger and thirst***

A study showed that the access to feed and water during the overnight resting stops can help the pregnant cows to recovery a hydration and muscle status caused by the lack of water and food during transport (7 h road transport, 12 h rest in a bare paddock with water and feed, 2.5 h road transport, 3 h rest in the truck, 5 h ferry transport, 20 min road transport, 12 h rest overnight in a bare paddock with access to water and hay) (Fisher et al., 1999). After a long journey of 4 days, pregnant cows did not seek water during the first 2h after arrival at the destination farm, which could indicate that the animals were not severely dehydrated or exhausted by the transport process, which shows the benefits from overnight rests, watering and feeding in terms of hydration and muscle status (Fisher et al., 1999).

## 2.2. Data related to pregnant female registered in TRACES and EFSA report

The category of pregnant females is not registered in TRACES and there is a lack of information regarding the prevalence of pregnant females transported in livestock species. When an infringement is detected, i.e. a pregnant female over 90% of the gestation period is transported, it is registered under "unfit animal", without any additional specification.

To our knowledge, the only EU data available on the transport of pregnant female is reported by the EFSA Panel on Animal Health and Welfare (AHAW) (2017) regarding the animal welfare aspects in respect of the slaughter or killing of pregnant livestock animals. The Panel estimated the median percentages of animals slaughtered in the last third of gestation as 3%, 1.5%, 0.5%, 0.8% and 0.2% for dairy cows, beef cattle, pigs, sheep and goats, respectively.

## 2.3. Overview of the main current protocols/guidelines implemented in the EU Member States and third countries improving the welfare of pregnant females transported

The legislation in force in the majority of EU Member States does not go beyond the Regulation (EC) 1/2005, and when it exists, the majority of national guidelines do not provide additional recommendation or information regarding the transport of pregnant animals. Indeed, we have contacted the national contact point in EU Member States and some third countries to request information regarding protocols or guidelines that have been implemented by the Competent

Authorities and Industry in their countries targeted to improve the welfare of type of these animals during transport. We received feedback from Norway, Iceland, Sweden, Estonia, Croatia, Belgium, Greece, Luxembourg, Latvia, Germany, Spain, Slovenia and United Kingdom, confirming our previous assumption. The few protocols or guidelines from countries that provide further information about the transport of pregnant females are listed and described below.

The Animal Transport Guides Project (2017) guidelines indicate as “best animal welfare practices” that sows should not be transported in the last third of duration of the pregnancy period (more details about best practices are commented in section 2.5). Besides that, in Germany, there is a national trade that ban to deliver for slaughter any mammal, except sheep and goat, which is in the last third of the gestation period. The prohibition shall not apply if the killing of such an animal (1) has been prescribed or ordered in accordance with animal health regulations, or (2) is required in individual cases on veterinary grounds and there are no overriding reasons of animal welfare against the slaughter do not stand in the way. In the last case, the veterinarian shall immediately hand over a certificate to the animal keeper, which states the requirements of the veterinary surgeon including the indication determined by the veterinary surgeon. The keeper shall keep the certificate for at least three years.

***Ascertaining of the state of pregnancy of live and slaughtered females***

The guideline published by the Ministero della Salute (2008) from Italy indicates the number of days that correspond to “90% of gestation” in cows (255 of 284 days), sheep (135 of 150 days), goats (139 of 155 days) and sows (101/106 of 113/118 days). The guidance published by the Swedish Board of Agriculture (2016) for control authorities does mention that the gestation period of sheep is about 145 days so these animals must not be transported during their last 14 days before lambing. Similar period of 14 days before the expected delivery date is indicated for goats and sows.

Despite mentioning that highly pregnant animals unusually enter to slaughterhouses, the Swedish guidance for control authorities (Swedish Board of Agriculture, 2016) make notes on how to determine the length of pregnancy in case of violation of the Regulation (EC) 1/2005. To assess how mature is a calf, foetus, the Swedish guideline states: (1) Hair - during the last weeks of pregnancy, the coat develops so that the foetus gets normal fur instead of just short hairs; (2) Teeth - the foetus’ front teeth have broken through and are clearly visible during the last week before birth; (3) Size - by measuring the distance from the top of the head to the tail-root of the foetus: 60 cm = about 205 days = 6.5-6.7 months old; 70 cm = about 220 days = 7.2-7.5 months old; 80 cm = about 240 days = 7.8-8.0 months; 90 cm = about 265 days = 8.5-8.7 months old; 95 cm = about 275 days = 9 months old. This means that if the foetus measures more than 80-85 cm from the top of the head to the tail-root, there is a suspect that less than 28 days remain until the day of calving and information should be sent to the county administrative board with suspicion of violation of transport rules. In severe cases, e.g. when the cow/heifer has calved during transport or at the slaughterhouse, prosecution may be relevant. This guideline also mentions how to proceed when a cow is slaughtered, and late pregnancy is suspected. In summary, it is recommended that once the cow has been stun and bled, the calf should be left in the womb for at least 15 min. In this case, the foetus will have low oxygen tension in the blood and will not be physiologically “awake”, experiencing low capacity for consciousness or pain sensations. Similar than for calves, the guideline states how to assess the maturity of a lamb: (1) Hair – the foetus is fully developed and covered with fur, and the hooves are complete but soft; (2) Teeth – usually does not break through until de lambs start sucking; (3) Size – by measuring the distance from the top of the head to the tail-root of the foetus: 15-35 cm = about 3-13 weeks; 35-40 cm = about 14-18 weeks; 40-48 cm = about 19-21 weeks.

***Handling pregnant female during transport***

The World Organization for Animal Health also released guidelines for transport of animals by land to facilitate and align the assessment of fitness to transport animals across countries (OIE, 2019). In this document, females in late pregnancy are considered at particular risk of suffering poor welfare during transport, requiring special conditions such as in the design of facilities and vehicles and the length of the journey, and require additional attention during transport. Nevertheless, the guidelines does not detail the requirements related to the design of the mean of transport, the length of the journey and the additional attention.

The Australian Animal Welfare Standards and Guidelines – Land Transport of Livestock (Animal Health Australia (AHA), 2012) considers females in third trimester of pregnancy as special classes of livestock, and that their transport should be carefully managed to minimise risk to impaired animal welfare. In general, the Australian guidelines recommend that water and resting periods should be provided prior transport of pregnant females if the travel time is expected to be of a long duration and approaching the maximum water-deprivation time for the class of livestock. During long distance travel, cows in the $6^{th}$ and $7^{th}$ and cows in the $8^{th}$ month (or later) of gestation (excluding the last two weeks of pregnancy) should not be deprived of water for more than 12 h and 4 h, respectively. Goats in the $3^{rd}$ month of pregnancy should not be deprived of water for more than 12 h, while water-deprivation should not exceed 8h in goats with more than four months pregnant.

The Australian guidelines (Animal Health Australia (AHA), 2012) refer that the transport of sows about to farrow or more than 80 days pregnant (approx. 80% of the gestation period) should be avoided and that the transport of sheep with more than three months pregnant (out of 150 days) should include additional space per animal in the vehicle, they should be segregate from other class of sheep and they should be provided with feed and water after unloading at the destination.

Finally, the "Code of Welfare: Transport within New Zealand" expands the basic of obligations of the Animal Welfare Act 1999 by setting minimum standards and recommending best practices (mentioned in section 2.5) for the care and management of animals in this country (New Zealand Government, 2018). As minimum standard for selecting and accepting animals for transport, the Code mentions that animals must not be transported if they are likely to give birth during the journey or be affected by metabolic complications of late pregnancy as a result of the journey. Also, the Code indicates that water and food must be provided for pregnant or lactating ruminants in 12 and 24 hours, respectively, timed from when water is first removed and within two hours of arrival unless the animals are sent for slaughter immediately.

As indicated in the New Zealander Code, the Animal Welfare (Care and Procedure) Regulation 2018 has a specific section regarding to the restriction on transporting animals in late pregnancy. It states that " *the owner of, and every person in charge of, a cattle beast, sheep, pig or goat that is in late pregnancy must no transport the animals, or allow the animal to be transported, unless (a) the animal is accompanied by a veterinary certificate that states that the animal is fit for transport, or (b) the animal is accompanied by a veterinary certificate that specifies conditions that must be complied with to manage the animal welfare risks associated with the transport and the owner, or person in charge, complies with all relevant conditions*". It also indicates the infringement fee in cases of a failure to comply with the national legislation.

## 2.4. Main issues linked to the transport of pregnant females which need addressing

The main issues associated with the transport of pregnant females are listed below:

- Fitness for transport of pregnant animals according to the stage of gestation.
- To ascertain if females in the last third of pregnancy (instead of 90% of gestation period) are already at particular risk of suffering poor welfare during transport and their transportation should be avoided.
- Confirmation of the insemination or mating date to ascertain the period of gestation.
- Accuracy and feasibility of the pregnancy stage test of live animals prior to transport.
- Determination of the appropriate space allowance, partitions, ceiling height and bedding material for transporting pregnant females for each livestock species.
- Determination of the appropriate maximum journey duration for transporting pregnant animals.

## 2.5. Best animal welfare practices as regards transport of pregnant animals and the major conditions for proper enforcement

The major condition for proper enforcement is again associated with the ascertaining of the pregnancy state before the transport. As indicated in the White Paper published by the Eurogroup for Animals, the gestation stage must be assessed prior transportation, through documentation of the insemination/mating approximate date and veterinary check on farm. For natural insemination, the date of conception would be consider the first day that male and female are put together (Eurogroup for Animals, 2021).

The Animal Transport Guides Project (2017) includes as “Better practices” that pregnant sows should not be in the last third of pregnancy when transported (Animal Transport Guides Project, 2017). This is in line with the Code of Welfare: Transport within New Zealand which recommends as best practice that animals that are pregnant should not be in the last third of pregnancy when transported and they should be transported for the shortest possible time (New Zealand Government, 2018). The White Paper on the revision of the Regulation (EC) 1/2005 recently published by the Eurogroup for Animals proposes to reduce the maximum gestation period that a pregnant female (bovines, swine, rabbits, poultry and bovines) can be transported and to forbidden the transport of females at 40% or more of the expected gestation period (Eurogroup for Animals, 2021).

As “Better practices” for the transport of pregnant females, the Animal Transport Guides Project (2017) also indicates that pregnant cattle require additional space (at least +10%) than those indicated by the Regulation (EC) 1/2005, i.e. they should be loaded in lower density. This extra space will allow pregnant females to get up in case they go down.

## 2.6. Policy recommendations

Based on the available evidence, the study sets out the following policy recommendations in relation to the transport of pregnant females:

- Research is needed to assess the fitness for transport of pregnant animals according to the stage of gestation and to establish the gestational age at which females are at particular risk of suffering poor welfare during transport, and if their transport should be avoided.
- The gestation stage should be assessed prior transportation through documentation of the insemination or mating approximate date and veterinary check on farm.
- Research should be carried out to develop accurate and feasible methods to determine the gestational age during late pregnancy when the date of insemination or matting is unavailable.
- Further research is needed to determine the appropriate space allowance, partitions, ceiling height, bedding material and maximum journey duration for transporting pregnant females for each livestock species.
- In the Regulation, there is need to clarify the terminology "heavily pregnant" and to specify the number of days which correspond 90% of gestation period.

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# ANNEX

## Approach and methodology of the "particular welfare needs of unweaned calves during transport"

The transport of unweaned animals was focused on unweaned calves. Lambs, kids and piglets are rarely transported before weaning for long journeys.

A literature search was conducted using the Web of Science database to identify available scientific information regarding the transport of unweaned calves. The search was conducted using combinations of terms covering three main components: livestock animals, unweaned calves, transport (**Table 1**). The search was conducted for years 1900-2021 and on the following indexes: SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI.

Search string used for the literature search for the transport of unweaned calves.

| **Search terms** | **Field searched** |
|---|---|
| ((transport) AND (calf OR calves) AND (welfare) | Title |
| (calf OR calves) AND (heat stress OR hyperthermia OR cold stress OR hypothermia OR thermal stress OR hunger OR starvation OR thirst OR dehydration OR handling stress) AND (welfare) | |
| (calf OR calves) AND (lameness OR respiratory OR illness) AND (welfare) | |

The academic literature, 'grey literature' and official reports regarding the transport of unweaned calves were also investigated.

## Approach and methodology of the “particular welfare needs of pregnant females during transport”

A literature search was conducted using the Web of Science database to identify available scientific information regarding the transport of pregnant females. The search was conducted using combinations of terms covering three main components: livestock animals, gestation/pregnancy, transport (Table 2). The search was conducted for years 1900-2021 and on the following indexes: SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI.

Search string used for the literature search for the transport of pregnant females.

| **Search terms** | **Field searched** |
|---|---|
| ((transport) NEAR/3 (pregnan* or gestat*)) | Title |
| AND | |
| ((Livestock* OR ((Farm OR farms OR farmed OR farming) NEAR/3 animal*) OR ruminant* OR cattle OR bovin* OR cow OR cows OR calf OR calves OR heifer* OR “Bos Taurus” OR (dairy NEAR/1 (herd* OR breed*)) OR weaner* OR yearling* OR stirk OR stirks OR springer* OR feeder* OR beef* OR swine OR (sus NEAR/1 (scrofa OR domestica OR domesticus)) OR pork OR porks OR porcine OR suidae OR pig OR pigs OR piglet* OR “sow” OR “sows” OR gilt OR gilts OR equidae* OR equus OR horse OR horses OR equine* OR yearling* OR mare OR mares OR pony OR ponies OR filly OR fillies OR ass OR asses OR mule OR mules OR donkey* OR ovis OR ovine* OR ewe OR ewes OR lamb OR lambs OR sheep* OR mouflon* OR hogget* OR ram OR rams OR tup OR tups OR Goat OR goats OR capra OR capras OR caprin* OR dam OR dams)) | Title |

A total of 54 references (2th March 2021) were found. After screening to identify relevance to the subject, 9 references were evaluated. Further, additional information was searched on web, using Google search and Google Scholar.

The academic literature, ‘grey literature’ and official reports regarding the transport of pregnant female were investigated. However, the information currently available is very limited.

This study provides a technical overview and policy recommendations of the welfare need during transport of unweaned animals and pregnant females. During long journeys unweaned calves may experience negative welfare consequences such as prolonged hunger and thirst, resting problems, thermal stress and diseases. Further research is needed to develop appropriate methods to determine the gestational age during late pregnancy when the date of insemination or matting is unavailable, as well as to establish the gestational age at which females are at particular risk of suffering poor welfare during transport.

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