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Summary

Across the globe, millions of cattle are transported to slaughter every year. The transport process includes a number of different phases (e.g., loading and unloading), during which several hazards can potentially lead to various negative welfare consequences for the animals.

One group of cattle that has received limited scientific attention in relation to transport is cull dairy cows. Dairy cows are culled for a variety of reasons and many of these are health related. They may be considered a particularly vulnerable group of animals in relation to transport as their clinical condition is often weakened by repetitive reproduction cycles under intensive farming conditions. The capacity of cull cows to cope with transport will likely decline faster than that of younger cattle. However, data comparing the clinical condition of cull cows before and after transport are scarce. Such data are essential in order to determine whether the clinical condition of cull dairy cows deteriorates during transport and to identify potential hazards for such deterioration.

The transport of animals within the European Union is covered by the EU Transport Regulation. The regulation contains no specific guidelines for cull animals, but states that an animal must be fit for transport and that injured, sick or otherwise compromised animals are not fit for transport. Slightly ill or injured animals may, however, be transported if said transport will not cause additional suffering. Nonetheless, what constitutes a fit animal, how to assess fitness for transport and how to define a slight illness or injury are only vaguely described in the literature, while "suffering" has no scientific definition. It is therefore very much a matter of individual interpretation of the words and assessment of the animal.

Despite the important roles of farmers, livestock drivers and veterinarians in ensuring animal welfare during transport, very little research has focused on these three professional groups in relation to assessing fitness for transport. A lack of common interpretations may lead to compromised animal welfare as well as legal uncertainty.

This thesis focused exclusively on the transport of cull dairy cows to slaughter by road (<8 h) in Denmark and the assessment of fitness for transport. The work includes two studies: Study 1 focused on describing the clinical condition of cull dairy cows before and after transport. The study was designed as an observational cross-sectional study and included 411 cull dairy cows transported to slaughter in Denmark by truck. All cows underwent a clinical examination on farm before loading and again after unloading at the slaughterhouse. Transport distance, duration and stops

along the way were recorded, and additional production data were retrieved from the Danish Cattle Database. I compared and statistically analysed the clinical condition of the cows before and after transport in order to identify potential risk factors for a deterioration in clinical condition.

The results showed that cull dairy cows constituted a very diverse group of animals in terms of age, productivity and health. The cows were culled for several different reasons, including reproductive failure, low milk yield, udder health and lameness, and presented with a wide range of deviations from the clinical norm – the majority had a least one deviation. Furthermore, the results showed that even short a transport time (<8 h, mean 187 min.) was a straining experience for cull dairy cows. They were at risk of becoming lame or more lame and developing milk leakage and wounds. Risk factors were related to the individual cow rather than the transport itself. The risk factors for becoming lame or more lame were digital dermatitis in the hind feet, a body condition score (BCS) lower than 2.75, early lactation (<100 DIM), late lactation (>300 DIM) and pelvic asymmetry. Risk factors for milk leakage were early lactation (<100 DIM) and distance transported (>100 km). Risk factors for wounds could not be determined in this study.

Study 2 focused on lameness scoring and assessing the fitness for transport in relation to lameness in dairy cows. The objective was to investigate the level of agreement among and within three groups of professionals involved with the transport of cows in Denmark: farmers, veterinarians and livestock drivers. The study was based on an online questionnaire with 30 short video recordings of walking cows seen from the side. The recordings featured both non-lame cows and cows with varying degrees of lameness from mild to severe, and participants were asked to assess the degree of lameness and fitness for transport.

The results showed that *within* the professional groups, the levels of agreement for scoring lameness, unweighted and weighted kappa values, were moderate within all three groups. When assessing fitness for transport, the levels of agreement within the veterinarians and the livestock drivers were moderate, and the level of agreement within the group of farmers was fair. The levels of agreement *between* pairs of groups when scoring lameness, unweighted as well as weighted, were moderate for all pairs. When assessing fitness for transport, the level of agreement between veterinarians and livestock drivers was moderate, and the levels of agreement between farmers and veterinarians and between farmers and livestock drivers were fair.

Based on the results of these two studies, we can conclude that: 1) cull dairy cows are a particularly vulnerable group of animals with regard to transport and even relatively short journeys may cause a

deterioration in their clinical condition, 2) assessment of fitness for transport is a complex matter that different groups of professionals may not agree upon, even when using a simplified version taking only lameness into consideration.

Sammendrag (Danish summary)

På verdensplan transporteres årligt flere millioner kreaturer til slagtning. Transportprocessen indeholder et antal forskellige faser, fx læsning og aflæsning, hvor adskillige risikofaktorer potentielt kan medføre en række negative velfærdskonsekvenser for dyrene.

Malkekøer bliver sat ud og sendt til slagtning af flere forskellige årsager, hvoraf de fleste er relateret til koens sundhedstilstand. Udsætterkøer, som de kaldes, har i meget begrænset omfang været genstand for videnskabelige undersøgelser. I forbindelse med transport kan udsætterkøer anses for at være særligt skrøbelige, da deres kliniske tilstand ofte er svækket efter gentagne kælvninger og laktationer under intensive produktionsforhold. Udsætterkøer vil sandsynligvis have sværere ved at modstå belastningen ved transport end yngre, raske slagtedyr. Data, der sammenligner den kliniske tilstand før og efter transport, er dog desværre meget mangelfulde. Den type data er yderst vigtigt for at kunne afgøre om udsætterkøers kliniske tilstand forværres under transport og for at kunne identificere mulige risikofaktorer knyttet til en sådan forværrelse.

I den Europæiske Union er dyr under transport beskyttet af EU Transportforordningen. Forordningen indeholder ingen specifikke bestemmelser for udsætterdyr, men det anføres, at ethvert dyr skal være egnet til transport og at tilskadekomne og syge dyr er ikke egnede til transport. Lettere tilskadekomne eller syge dyr kan dog transporteres, hvis transporten ikke vil tilføje dem yderligere lidelse. Men hvad der præcis forstås ved et egnet dyr, hvordan transportegnethed skal vurderes og hvordan man skal definere lettere syg eller tilskadekommen er kun meget vagt beskrevet i forordningen. Desuden har begrebet "lidelse" ingen videnskabelig definition. Derfor er det i høj grad et spørgsmål om individuel fortolkning af ordene og vurdering af dyret. På trods af dyretransportchaufførers, landmænds, og dyrlægers vigtige rolle i forbindelse med at sikre dyrs velfærd under transport har meget få videnskabelige studier beskæftiges sig med disse tre faggrupper i forhold til vurdering af transportegnethed. Manglende fælles fortolkning kan lede til dårlig dyrevelfærd og mangel på retssikkerhed.

Denne afhandling fokuserede udelukkende på transport af udsætterkøer med lastbil til slagtning (< 8t) i Danmark og vurdering af transportegnethed. Det videnskabelige arbejde afhandlingen bygger på omfatter to studier:

Studie 1 fokuserede på at beskrive udsætterkøers kliniske tilstand før og efter transport. Studiet var designet som et observationelt cross-sectional studie og inkluderede 411 køer transporteret til

slagtning i Danmark. Alle køer fik en fuld klinisk undersøgelse før læsning og igen efter ankomsten til slagteriet. Transportdistancer, transportvarigheder og stop undervejs blev registreret og supplerende data blev hentet fra den danske kvægdatabase. Den kliniske tilstand før og efter transport blev sammenlignet og analyseret statistisk med henblik på at identificere potentielle risikofaktorer for forværrelse af den kliniske tilstand.

Resultaterne viste, at udsætterkøer var en meget forskelligartet gruppe af dyr med hensyn til alder, produktivitet og sundhedstilstand. Køerne blev sat ud på grund af en række forskellige årsager, hvoraf de væsentligste var reproduktionsproblemer, lav mælkeydelse, dårlig yversundhed og halthed. Køerne havde en lang række forskellige afvigelser fra det klinisk normale, hovedparten havde mindst én afvigelse. Yderligere viste resultaterne, at selv korte transporter (<8t, gns. 187 min) var belastende for udsætterkøerne. De risikerede at blive halte eller mere halte, udvikle mælkeløb og får sår. Risikofaktorerne var knyttet til den enkelte ko snarere end til transporten. Risikofaktorer for at blive halt eller mere halt var digital dermatitis på bagbenene, huldscore lavere end 2,75, tidlig laktation (<100 dage efter kælvning), sen laktation (>300 dage efter kælvning) og skævt bækken. Risikofaktorer for mælkeløb var tidlig laktation (<100 dage efter kælvning) og transportdistance (>100 km). Risikofaktorer for sår kunne ikke bestemmes i dette studie.

Studie 2 fokuserede på halthedsvurdering af malkekøer og vurdering af transportegnethed i forbindelse med halthed. Formålet var at undersøge graden af overensstemmelse indenfor og imellem tre faggrupper, der arbejder med transport af kvæg i Danmark: Dyretransportchauffører, landmænd og dyrlæger. Studiet baserede sig på en online-spørgeundersøgelse med 30 korte videooptagelser af gående køer set fra siden. Optagelserne viste både ikke-halte køer og køer med varierende grader af halthed fra mild til alvorlig og deltagerne blev bedt om at vurdere graden af halthed og dernæst, om dyret var egnet til transport eller ej.

Resultaterne viste, at *inden for* grupperne var graden af overensstemmelse for halthedsvurdering moderat i alle tre grupper. For vurdering af transportegnethed var graden af overensstemmelse moderat i gruppen af dyrlæger og gruppen af chauffører, mens den kun var rimelig i gruppen af landmænd. Graden af overensstemmelse *mellem* grupperne (parvis sammenligning) for halthedsvurdering var moderat for alle par. For vurdering af transportegnethed var graden af overensstemmelse moderat mellem dyrlæger og chauffører, mens den kun var rimelig mellem dyrlæger og landmænd, og mellem chauffører og landmænd.

På baggrund af resultaterne fra studie 1 og 2 kan det konkluderes, at 1) udsætterkøer er en særligt skrøbelig gruppe af dyr med hensyn til transport, endda også transporter af kort varighed kan føre til en forværrelse af deres kliniske tilstand, 2) vurdering af transportegnethed er en kompleks opgave som forskellige faggrupper ikke nødvendigvis er enige om, selv i en forenklet situation, hvor kun halthed tages i betragtning.

List of original papers

The research conducted as part of this PhD project has resulted in three scientific papers, all of which are published. This thesis is a synopsis based on the three papers, which can be found in Section 8.

Paper I:

A descriptive study of the clinical condition of cull dairy cows before transport to slaughter

Kirstin Dahl-Pedersen, Mette S. Herskin, Hans Houe and Peter T. Thomsen

Livestock Science, vol. 218, pp. 108-113, 2018; DOI 10.1016/j.livsci.2018.11.001

Paper II:

Risk factors for deterioration of the clinical condition of cull dairy cows during transport to slaughter

Kirstin Dahl-Pedersen, Mette S. Herskin, Hans Houe and Peter T. Thomsen

Frontiers in Veterinary Science, vol. 5, article no. 297, 2018; DOI 10.3389/fvets.2018.00297

Paper III:

Lameness scoring and assessment of fitness for transport in dairy cows: Agreement among and between farmers, veterinarians, and livestock drivers

Kirstin Dahl-Pedersen, Leslie Foldager, Mette S. Herskin, Hans Houe and Peter T. Thomsen

Research in Veterinary Science, 2018 vol. 119, pp. 162-166, 2018; DOI 10.1016/j.rvsc.2018.06.017

1. Introduction

Animal welfare, including during transport, is of increasing concern to society, industry stakeholders, politicians and authorities, and it is a research topic of growing interest to the scientific community (Keeling, 2005; Marahrens et al., 2011; Freire and Nicol, 2019).

Around the world, farm animals such as cattle, sheep, pigs, and poultry are transported in great numbers for a variety of reasons. One important reason is transport for slaughter. Approximately 300,000,000 cattle are slaughtered every year, which illustrates the enormous extent of global cattle transport (Ritchie et al., 2017).

The entire transport process includes several different phases: preparation at the place of departure (including milking if lactating), separation from the herd, waiting in holding pen, loading, the journey itself with stops along the way, possible reloading, unloading, and penning or lairage at the destination. During this process, several hazards can potentially lead to a number of welfare consequences for the animals. These risk factors include but are not limited to handling during loading and unloading, space allowance, mixing of unfamiliar animals, type of vehicle, type of road, driving style, noise, ambient temperature and weather conditions, rest stops, access to feed and water, and the duration of the journey. Thus, *"It is clear that the effect of road transport is a multi-factorial problem where a combination of stressors rather than a single factor is responsible for the animal's wellbeing"* (Schwartzkopf-Genswein et al., 2012). Some of these hazards and their potential consequences on different aspects of animal welfare have been investigated in experimental as well as observational studies, but much remains to be elaborated. It is specified in both legislation and guidelines from around the world (e.g., the OIE guidelines) that animals must be "fit for transport". However, there is currently no clear definition of exactly what constitutes a "fit" animal or how to assess fitness.

The European Commission issued a report in 2002 entitled, "The welfare of animals during transport (details for horses, pigs, sheep and cattle)" written by the Scientific Committee on Animal Health and Animal Welfare (Anonymous, 2011c). The report gave a thorough review of the then current scientific literature regarding the transport of farm animals and highlighted a number of areas where further research was needed. One of these areas was the "*effects of transport on the welfare of older breeding pigs, older dairy cows and older ewes*". However, until very recently, "older dairy cows" have not received much scientific attention. A recent report from EFSA, "Welfare of Cattle during Transport", concludes that the most important animal welfare issue for

cull dairy cows in connection with transport is their fitness for transport, and recommends that the concept "fitness for transport" should be properly defined (Nielsen et al., 2022). The European Commission is currently preparing a revision of the animal welfare legislation in connection with the "Farm to Fork Strategy", which includes animal transport in order "*to align it with the latest scientific evidence, broaden its scope, make it easier to enforce and ultimately ensure a higher level of animal welfare*" (Anonymous, 2020). In December 2023, the European Commission released a proposal for the revision of the transport legislation with a number of specific objectives, one which is to "*improve the condition of transport of vulnerable animals*" (Anonymous, 2023b). Cull animals are not specifically mentioned, however, and a clearer definition of fitness for transport is not provided, but the proposal does include a maximum journey time for animals transported for slaughter of 9h (Anonymous, 2023b).

Cull dairy cows are dairy cows that are removed from the herd, most often to be slaughtered. Dairy cows are culled for a number of reasons and culling is largely based on production considerations. Some of the most common reasons for culling are: reproductive failure, low milk yield, lameness, metabolic disorders and mastitis (Smith et al., 2000; Ahlman et al., 2011; Chiumia et al., 2013; Pinedo et al., 2014; Armengol and Fraile, 2018; Workie et al., 2021), and there is often more than one reason for culling for each individual (Bascom and Young, 1998). Over the past decades, the average percentage of culled dairy cows per year has been reported to range from 23–36% (Smith et al., 2000; Ahlman et al., 2011; Chiumia et al., 2013; Nor et al., 2014; Pinedo et al., 2014; Armengol and Fraile, 2013; Nor et al., 2014; Pinedo et al., 2014; Armengol and Fraile, 2013; Nor et al., 2014; Pinedo et al., 2014; Armengol and Fraile, 2013; Nor et al., 2014; Dinedo et al., 2014; Armengol and Fraile, 2013; Nor et al., 2014; Pinedo et al., 2014; Armengol and Fraile, 2018). Therefore, millions of cull dairy cows are transported to slaughter each year. Within the EU, around 5 million dairy cows are culled and slaughtered per year (Nielsen et al., 2022), and in Denmark alone, approximately 165,000 cull dairy cows are transported to slaughter per year (Anonymous, 2023a). Specific transport times are not published.

During transport within the European Union, animals are covered by the "Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/97" (henceforth referred to as "the EU Transport Regulation"). However, this regulation contains no specific guidelines for cull animals, which may be a matter of concern as cull dairy cows can be considered a particularly vulnerable group of animals. Their clinical condition is often weakened by repetitive reproduction cycles under intensive farming conditions, and it is therefore conceivable

that transport is a more straining experience for cull dairy cows than it is for other groups of cattle (Nielsen et al., 2011). Cull dairy cows are thus a particularly important group of animals to investigate further to ensure that their welfare is not compromised during transport. This thesis focused exclusively on the transport of cull dairy cows by truck to slaughter in Denmark under EU regulations, with an emphasis on animal welfare consequences, legal aspects, and practical implications of the term "fitness for transport". As Denmark is a small country, only journeys with a duration of less than 8 hours were included.

Aim and hypotheses

The overall aim of the work presented in this thesis was to contribute to the body of scientific evidence that can be used to refine transport legislation and thereby contribute to better welfare for cull dairy cows during transport.

The working hypotheses were that fitness for transport is a complex term to characterize and assess and different groups of professionals therefore might not agree upon its definition; that transport might cause a deterioration of the clinical condition of cull cows; that an assessment of fitness for transport of cull cows should not only take the conceptual complexities into account but also account for any such deterioration as well as different views about priorities from different groups of professionals.

These working hypotheses were investigated by answering the following research questions:

- What are the clinical characteristics of cull dairy cows before transport? (Paper I)
- Should cull dairy cows be considered particularly vulnerable with regard to transport? (Paper I and II)
- Can short journeys (<8 h) by road cause a deterioration of the clinical condition of cull dairy cows? (Paper II)
- Can characteristics of the individual cow be risk factors for such deterioration? (Paper II)
- Can characteristics of the journey be risk factors for such deterioration? (Paper II)
- Are farmers, livestock drivers and veterinarians in agreement when assessing fitness for transport? (Paper III)

Outline of the thesis

The work presented in the thesis was part of a larger project on animal transport and the assessment of fitness for transport, carried out in cooperation between Aarhus University and the University of

Copenhagen and funded by the Danish Veterinary and Food Administration. This collaboration also yielded studies on transport of broilers and cull sows, but the present thesis focused exclusively on the transport of cull cows.

The thesis investigated the afore-mentioned hypotheses through two studies, Studies 1 and 2. The thesis consists of a background section (Chapter 2) with a review of the current literature on the transport of cull dairy cows. To date, research focusing on the transport of cull dairy cows has been scarce and studies involving other types of cattle e.g., beef cattle are therefore included in this section in order to gain a broader perspective on the transport of cattle and potential welfare consequences. The concept of animal welfare, reasons why dairy cows are culled, and legislation related to animal transport are also addressed in this chapter. An overview of the materials and methods used for Studies 1 and 2 is given in Chapter 3 and results are presented in Chapter 4. For further details, please refer to Papers I–III. In Chapter 5, the results from the studies are critically reviewed, put into perspective in relation to current knowledge, and limitations and uncertainties are discussed. Conclusions drawn from the results are summarized in Chapter 6.

2. Background

Challenges related to animal transport extend into many different areas including animal welfare, legislation, food safety, biosecurity, trade policies and product traceability. This section gives an introduction to different animal welfare views and transport legislation and reviews the current scientific literature on cattle transport with an emphasis on cull cows.

Animal welfare

Animal welfare is a term widely used by consumers, producers, authorities and within the scientific community. However, animal welfare is a complex term without a fixed definition. Different people have different views on animal welfare based on their values (Mason and Mendl, 1993; Fraser et al., 1997; Heise and Theuvsen, 2018). Fraser et al. (1997) suggest that there are three different views on animal welfare. The first focuses on "basic health and functioning", where welfare assessment is based on whether an animal is free from injury and disease and able to grow and reproduce as expected. The second view focuses on "naturalness of life", where welfare assessment is based on whether animals have the opportunity to express natural behaviour (e.g., nest building) and live in a natural environment (e.g., with access to pasture). The third view focuses on "affective states", where welfare assessment is based on whether animals experience pleasant or unpleasant feelings (e.g., joy, fear, thirst). These three views on animal welfare are not mutually exclusive, nor do they fully overlap.

The main welfare view used in this work is the basic health and functioning view with a focus on the clinical condition of cull cows. In a transport situation, clinical signs like lameness and wounds can be – more or less – directly observed and assessed, making them practical and useable. However, it is important to keep in mind that the assessment of clinical signs is somewhat subjective. Prior studies have shown that the more severe the clinical sign, the easier it is to recognize (Whay et al., 2003; Espejo et al., 2006; Thomsen and Baadsgaard, 2006; Alawneh et al., 2012), that different professional groups may assess the potential pain associated with various clinical conditions differently (Thomsen et al., 2012) and that age and gender also affect how people assess pain (Raekallio et al., 2003; Remnant et al., 2017). Different professional groups and individuals might therefore assess fitness for transport quite differently.

Life of a dairy cow

In modern dairy farming, the life of a dairy cow follows a rather fixed pattern. A heifer calf will be raised either for slaughter or to join the dairy herd as a pregnant heifer. After a number of calvings and lactation cycles – ideally one per year – the cow will be culled and sent to slaughter.

Culling

Dairy cows are continuously culled, i.e., removed from the herd. Cull cows are often replaced by younger, healthier, and genetically supreme individuals. The process of culling and replacement aims to keep the herd as healthy, productive, and profitable as possible. For decades, culling rates have been reported to range from 23–36% (Esslemont and Kossaibati, 1997; Bascom and Young, 1998; Smith et al., 2000; Nor et al., 2014; Pinedo et al., 2014; Armengol and Fraile, 2018). In Denmark alone, approximately 165,000 cows are culled annually (Anonymous, 2023a).

Much research has focused on the reasons and related risk factors for culling, as reviewed by Beaudeau et al. (2000) and more recently by Compton et al. (2017). The decision to cull is complex and involves several factors e.g., lactation stage, health status, milk prices and the availability of replacement heifers (Bascom and Young, 1998; Haine et al., 2017). The decision process can vary from farm to farm, and reasons for culling are somewhat subjective. Farmers may state primary, secondary and tertiary reasons for culling, with the order of importance depending very much on the specific priorities of the farmer (Bascom and Young, 1998). Dairy cows are often culled due to health and production issues. The most common culling reasons include reproductive failure, low milk production, udder health issues and lameness (Esslemont and Kossaibati, 1997; Ahlman et al., 2011; Chiumia et al., 2013; Nor et al., 2014; Armengol and Fraile, 2018; Workie et al., 2021). A large proportion of the dairy population is culled annually for health-related reasons, and deciding whether a cull cow is fit for transport must therefore be a frequent task for farmers, livestock drivers and veterinarians working in the dairy sector. However, as previously stated, fitness for transport is a concept that is subject to interpretation by the individual, and more knowledge about how different groups of professionals assess the clinical condition of a cull cow in relation to fitness for transport is needed.

Transport procedures

In Denmark, farmers send their cull cows directly to slaughter rather than via auctions or markets. The farmer will often send a small number of cull cows at a time, and in order to fill the truck, the livestock driver must pick up cows from several farms on their way to the slaughterhouse. The

farmer will have assessed the fitness of the individual cows in advance, and the driver will assess fitness during handling prior to loading. Since Denmark is a relatively small country, journey times rarely exceed 8 hours. Upon arrival at the slaughterhouse, all cows are subject to a mandatory ante mortem inspection that includes an assessment of fitness for transport by a veterinarian from the Danish veterinary authorities, i.e., the veterinarian must assess whether or not the cow was fit before loading. This inspection is usually done as the cows are unloaded. Hereafter, the cows will either go directly to the slaughter line or wait in lairage. Lactating cows are prioritized to go to slaughter without delay to avoid having to milk them. For detailed information on slaughter procedures, please refer to the EFSA report "Welfare of Cattle at Slaughter" (Nielsen et al., 2020).

Legislation regarding animal transport

Within the European Union, animals are legally covered during transport by the EU Transport Regulation (Anonymous, 2005). In addition, journeys fully completed within Danish borders are regulated by two national laws: the "Statutory Order on Animal Welfare" (Anonymous, 2021) and the "Statutory Order on the Protection of Animals during Transport" (Anonymous, 2006), a law that supplements the EU Transport Regulation.

Canada, the United States, New Zealand, and Australia also have national legislation on the protection of animals during transport that share some similarities with the EU legislation as recently reviewed by Duval et al. (2024). The EU Transport Regulation is the one of the strictest in terms of e.g., duration of transport, as it dictates that adult bovines can only be transported for 14 hours, after which the animals must rest for 1 hour while given access to water and, if necessary, feed (on the truck). After this one-hour rest, the animals can be transported for another 14 hours before they must be unloaded, offered water and feed and be allowed to rest for no less than 24 hours. However, loading and unloading are additional hazards and thus constitute a conundrum when protecting the cull dairy cows.

In many parts of the world, however, no legislation regarding animal transport exists at all. The World Organisation for Animal Health has published a list of general recommendations for the transport of animals by sea, land and air (in section 7 "Animal Welfare" in the Terrestrial Animal Health Code), applicable all over the world (Anonymous, 2011b). These recommendations are not legally binding, nor are the many voluntary guidelines that have been developed by stakeholders, e.g., "Practical Guidelines to Assess Fitness for Transport of Adult Bovines" (Anonymous, 2012).

Fitness for transport and assessment of fitness

The concept of fitness

Fitness for transport is a central concept when discussing animal transport and its welfare implications, yet it has received very limited scientific attention (Grandin, 2001; Cockram, 2019). The term "fitness for transport" is included in the OIE recommendations, in many stakeholders' guidelines and in national legislations. In the EU Transport Regulation, the term "fit" is mentioned in article 3:

"General conditions for the transport of animals

No person shall transport animals or cause animals to be transported in a way likely to cause injury or undue suffering to them. In addition, the following conditions shall be complied with (...) (b) the animals are fit for the journey; (...)"

In Chapter I, Annex I, Technical Rules, the term "fitness for transport" is described in more detail:

"FITNESS FOR TRANSPORT

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

2. Animals that are injured or that present physiological weaknesses or pathological processes shall not be considered fit for transport and in particular if:
(a) they are unable to move independently without pain or to walk unassisted;
(b) they present a severe open wound, or prolapse;
(c) they are pregnant females for whom 90% or more of the expected gestation period has already passed, or females who have given birth in the previous week;
(...)."

(Letters d-g are not included as they are not applicable to cull dairy cows).

Points 1 and 2 are probably intended to be very clear: animals must be fit to travel, while injured, sick or otherwise compromised animals are not fit for transport. However, several of the terms lack a clear definition, e.g., "severe open wound" and Point 3 only adds to the complexity:

"3. However, sick or injured animals may be considered fit for transport if they are:
(a) slightly injured or ill and transport would not cause additional suffering; in cases of doubt, veterinary advice shall be sought;
(...)".

The phrase "slightly ill or injured" is not further defined or exemplified in the text, thus relying on one's individual interpretation of the words and assessment of the animal. In addition, the key word "suffering" has no clear scientific definition (Weary, 2014). Dawkins (1980) suggested "a wide range of unpleasant emotions", but this again depends on the interpretation of the word.

Cull dairy cows or other cull animals are not mentioned specifically in the EU Transport Regulation, but it is specified that lactating females not accompanied by their offspring shall be milked at intervals of no more than 12 hours.

Assessment of fitness

According to the EU Transport Regulation, the duty to assess the fitness for transport of all cows before loading falls upon the animal keeper (i.e., the farmer) and the transporter (i.e., the livestock driver). Farmers and livestock drivers share legal responsibility for the animals' fitness and veterinary advice must be sought in cases of doubt. This means that farmers, livestock drivers and veterinarians all play very important roles in ensuring animal welfare during transport, based on an accurate assessment of fitness for transport. It is widely accepted that assessing fitness for transport is not straightforward and clear thresholds for fitness are lacking (as reviewed by Grandin (2016) for cull sows), meaning that guidelines and regulations do not always protect unfit animals from being transported (Cockram, 2019). Differences in education, training, experiences, cultural background, tradition, economic considerations, and time constraints may result in differing opinions of what makes a cow "fit" or "unfit". Likewise, there might be differences in opinion about what constitutes "suffering", "unnecessary suffering" and "undue suffering" – all terms used in the EU Transport Regulation.

Despite the important roles of farmers, livestock drivers and veterinarians, very little research has focused on these three professional groups in relation to the assessment of fitness for transport. Herskin et al. (2017) examined knowledge about and experience with fitness for transport among Danish livestock drivers transporting cattle: 35% of the livestock drivers stated that they were frequently in doubt about whether a particular cow was fit for transport. When asked two specific questions regarding the legislation on fitness for transport, only 52% of the livestock drivers answered both correctly (Question 1: May a cow be transported 10 days after calving? Correct answer: No. Question 2: What are the requirements for transporting cows under special care? Correct answer: Separated from other animals and with extra bedding). Recently, Dahl-Pedersen (2022) assessed knowledge of and experience with fitness for transport among Danish cattle

producers and found that 39% of the respondents found it difficult to understand the rules regarding fitness for transport either often or very often, while 32% reported that only sometimes or rarely were they able to assess whether the clinical condition of a slightly ill or injured animal might deteriorate during transport and 87% reported that lameness was the most difficult clinical condition to assess with regard to fitness for transport (Dahl-Pedersen, 2022).

In 2011, the European Commission issued a report that evaluated the impact of the EU Transport Regulation titled "Report form the Commission to the European Parliament and the Council on the impact of Council Regulation (EC) NO 1/2005 on the protection of animals during transport" (Anonymous, 2011a). It concluded that there was poor compliance such as overstocking of vehicles and the transport of unfit animals, and that a common interpretation of the requirements was lacking in several areas including fitness for transport. This was also underlined by annual inspection reports from the EU member states to the EU Commission of their inspections of live animal transport. These reports were publicly accessible on the European Union's website up until recently and documented that a large proportion of non-compliance cases relate to the lack of fitness for transport. The reports can however no longer be found on the website.

It seems evident that "fit for transport" lacks clear definition as a legal term and that animal welfare may therefore be compromised during transport.

Transport studies

General welfare consequences

Animal transport is widely acknowledged as being stressful for animals. It involves many different phases and stressors (Broom, 2003) and in the words of Ljungberg et al. (2007), transport is, "*a series of stressful events*". Several studies have tried to evaluate different aspects of transport, single factors, or combinations of factors through both experimental and observational studies, and many of these will be discussed below.

Blood chemistry

Many of the studies looked at changes in blood chemistry in relation to transport. In particular, levels of cortisol, adrenaline and lactate and creatine kinase activity have been investigated as indicators of stress and exhaustion. However, these physiological measures must be interpreted with caution as they lack specificity (Mason and Mendl, 1993; Dawkins, 2003). For instance, cortisol (sometimes referred to as "the stress hormone") is released in response to stressful events, but also

during exercise, mating and when waiting for food. It may also fluctuate during the day (Dawkins, 1980; Broom and Johnson, 2019).

Loading

Cattle are known to be neophobic, i.e., showing avoidance and a reluctance to approach when presented with novel stimuli such as unfamiliar humans or new objects (see for instance Hemsworth et al. (1996) and Herskin et al. (2004) for behavioral studies of this). Loading onto trucks involves many novel stimuli including unfamiliar humans and sounds, separation from the herd, navigating paths, climbing the ramp and entering the truck and mixing with unfamiliar animals. It is therefore not surprising that many studies find the loading process particularly stressful for cattle. Warriss et al. (1995) investigated journeys of varying duration (up to 15 h) and found increased plasma cortisol concentrations in young steers after loading and during the first part of the journey. Cortisol concentrations returned to normal as the journeys continued, perhaps indicating that the loading process is particularly stressful for the animals. Creatinine kinase activity increased progressively with duration, indicating muscular fatigue (Warriss et al., 1995). Pettiford et al. (2008) reported similar results. Maria et al. (2004) examined the time spent and behavioral events (e.g., falls, vocalization, slips, reversals) during loading and unloading and compared these to concentrations of cortisol and lactate and creatine kinase activity. The study concluded that loading was more stressful than unloading and that the number of behavioral events at loading correlated with higher levels of cortisol. Booth-McLean et al. (2007) showed that during short-haul transport (of up to 3 h), finished steers had a lower heart rate during road travel compared to loading and unloading, and suggested that loading and unloading are more stressful than the driving phase. It should however be noted that heart rate will naturally increase with movement. Palme et al. (2000) tried to avoid any additional stress related to blood sampling and measured fecal cortisol metabolite concentrations in relation to transport stress. Two groups of cows were investigated; one group was loaded and then transported for 2 hours, another group was loaded and then kept stationary for 3 hours. Significantly elevated fecal cortisol metabolite concentrations were observed for both groups, but to a lesser extent in the stationary group, indicating that loading, confinement, and unloading is in itself stressful, but vehicle movements add to the stress. However, cattle can potentially become habituated to loading. In a previous study, higher levels of cortisol and adrenaline were found in calves subjected to loading and simulated transport, particularly during the first 5 minutes after loading. The calves were then loaded and "mock transported" three times 10 days apart, after which a significant reduction in cortisol and adrenaline response was observed.

These results suggest some degree of habituation to the process of being loaded and transported (Locatelli et al., 1989).

Distance and duration

Journeys are becoming longer in distance and duration due to small local slaughterhouses closing down and an increasing international trade of live animals (Ljungberg et al., 2007; Miranda-de la Lama et al., 2014; Dahl-Pedersen and Herskin, 2020; Franks and Peden, 2022). Although a long journey may not be detrimental to animal welfare in itself, it is clear that the longer the duration of the journey, the longer the exposure to other stressors (Nielsen et al., 2011). Adult cattle prefer not to lie down while being transported, but they may lie down after 24 h due to fatigue (Knowles et al., 1999). Bulitta et al. (2015) carried out an experimental study looking at transport of different durations up to 12 h. Results showed that cortisol, glucose and lactate concentrations and creatinine kinase activity significantly increased during transport, thus indicating a stress response, and increasing muscular fatigue. Swaying, restlessness, and loss of balance increased with an increased duration of transport. In many cases, a loss of balance was seen when cornering (Bulitta et al., 2015). Booth-McLean et al. (2007) found that 3 hours of transport resulted in increased lying time post-transport, thus indicating some degree of fatigue.

Space allowance

Space allowance refers to the area available to each animal during transport. This can be expressed in m² per animal or as kg body weight per m². The EU Transport Regulation includes space allowance tables for different animal species and different modes of transport, e.g., "heavy cattle" with an approximate body weight of 550 kg should have a space allowance of 1.3–1.6 m² during road transport (Anonymous, 2005). Optimal space allowance from an animal welfare perspective will allow animals to stand and lie down in a natural position, to support each other without harming each other, to thermoregulate and, if feed and water are provided during transport, to eat and drink. What exactly constitutes optimal space allowance is dependent not only on the weight of the animals but also their condition, weather conditions and the duration of the journey. It is financially desirable to load animals as densely as possible without any negative effects on the quality of meat and hides, yet suboptimal space allowance can potentially have a detrimental effect on animal welfare. It might seem counterintuitive that too much space can be problematic, but this can increase risk that the animals will move around excessively and initiate aggressive interactions. With too little space, there is a risk that lying animals are not able to rise again. It is possible that the effect of either too little or too much space may be greater in larger groups and/or compartments

(Gonzalez et al., 2012b). Eldridge and Winfield (1988) investigated how different space allowances influenced the level of bruising, carcass weight and risk of injury and found that a medium space allowance was associated with a lower risk of bruising compared to smaller and larger space allowances. In contrast, Tarrant et al. (1992), found an increase in struggles, falls, bruises and creatinine kinase activity the higher the stocking density.

Driving events

During transport, cattle must maintain their balance through different driving events such as cornering and acceleration. Their ability to do so depends on the space allowance, partitions between animals, flooring in the truck, driving style and type of road. Cockram and Spence (2012) examined the effect of acceleration, minor roads, major roads, and motorways (3 h journeys). The type of road had a considerable influence on the speed and events during driving leading to a loss of balance, sliding, a struggle to regain stability and falls. Loss of balance occurred five times more frequently on minor and major roads compared to motorways, and this was most often observed during cornering, followed by acceleration and braking. Stockman et al. (2013) compared the effect of different driving styles on behaviour and found that steers exposed to "stop-start driving" were scored by observers post-transport as "restless", "agitated" and "scared", while steers exposed to different conditions during transport: steers that were transported on a non-grip flooring were scored by observers post-transport as "agitated", "restless" and "anxious" compared to steers transported on grip flooring, which were scored as more "calm", "comfortable" and "relaxed".

Livestock drivers

In addition to driving style, the livestock driver's level of experience has an impact on animal welfare. Valadez-Noriega et al. (2018) found that livestock drivers with more than 6 years of truck driving experience had a lower risk of being involved in road accidents compared to livestock drivers with less truck driving experience. Gonzalez et al. (2012b) reported that the proportion of compromised animals (lame, non-ambulatory or dead) declined with more truck driving experience. Training for livestock drivers varies widely across the world. In North America, training is offered by the large livestock transport companies (Schwartzkopf-Genswein and Grandin, 2019). In a Mexican study by Valadez-Noriega et al. (2018), the majority of drivers stated that they received no formal training, but were taught by either family members or colleagues. In contrast, in a Danish study by Herskin et al. (2017), all drivers had formal training and a Certificate of Competence, as

required by EU law, which entails training and examination in e.g., animal physiology, animal behaviour and practical aspects of animal handling (Anonymous, 2005).

Weather and temperature

Weather conditions and ambient temperature may also have an impact on welfare during transport, as reviewed by Schwartzkopf-Genswein et al. (2012). Cattle trucks rarely have climate control systems and have only limited ventilation options. During extreme weather conditions, this may pose a risk to animal welfare both in terms of cold stress and heat stress.

Transport of cull cows

Different types of cattle (calves, fattened cattle, cull cows, etc.) might have very different clinical conditions, and their ability to cope with the stressors related to transport may also vary as a result. As suggested by Nielsen et al. (2011) and Cockram (2019), cull cows may be more vulnerable than younger, healthier cattle due to their clinical condition. Grandin (2001) proposed that the most serious problems related to a lack of fitness for transport concern cull breeding stock. Likewise, Schwartzkopf-Genswein et al. (2012) proposed that transporting cull cattle entails some of the most important welfare issues in animal transport. However, until Study 1 was carried out, knowledge of the actual clinical condition of cull cows prior to transport had not been described in detail.

Cows are culled for a variety of reasons, the majority of which are health related. With that in mind, cull cows might be considered a particularly diverse and vulnerable group of animals due to their clinical condition. They may be weakened by repetitive reproduction cycles, or they may be injured or ill. The animal's capacity to cope will likely decline faster for cull cows than for younger cattle, especially when stressors are combined or when the impact of an individual stressor is increased. However, until now, there has been a lack of data comparing the clinical condition of cull cows before and after transport. Most studies on cattle transport have focused on young stock like steers and heifers. Few studies include cull cows and even fewer include their clinical condition prior to transport. Most of the current studies including cull cows are from North America and may include transport conditions not directly comparable to Danish or European conditions. In the US for instance, cull dairy cows are sometimes transported for several days and through markets due to very different regulations and trading patterns (Edwards-Callaway et al., 2019).

A number of studies from around the world have reported on the clinical conditions of cull dairy cows as they arrive at either markets or slaughterhouses. In general, there is little to no information about the clinical condition of the animals prior to transport included in these studies, but the reported clinical findings clearly demonstrate that cull dairy cows as a group are characterized by several health issues.

Body condition score, lameness, udder issues and other clinical manifestations

Ahola et al. (2011) carried out a survey of more than 12,000 cull dairy cows sold through markets in the US. They reported a mean BCS of 2.6, but 13% were emaciated (BCS =1) or near emaciated (BCS =1.5), 45% had a lameness score >1 (on a scale of 1–5) and, more specifically, 4% had a lameness score of 4 (lame) or 5 (severely lame), 12% had extra-large udders, 3% had mastitis and 0.4% had foot abnormalities. A very concerning figure was the 3% of visibly sick cows arriving at the markets, as characterized by lethargy, extreme weakness, significant panting, ears down and/or extremely gaunt. In addition, the 1.5% of the cows that were classed as "no-sale", meaning that buyers would not buy these animals at any price. The reason for this was not evaluated in the study, but the authors believed it was due to one or more major visible defects.

Similar results, including 37% of cows with visible defects and 4.5% extremely emaciated cows were reported by Nicholson et al. (2013), who conducted a survey of more than 2,000 cull dairy cows at 23 US slaughter plants. Heuston et al. (2017) evaluated the condition of close to 1,000 cattle arriving at auctions and slaughterhouses in Canada and found that 22.5% of cattle arriving at slaughterhouses were in an overall compromised condition based on their lameness scores, respiratory signs, BCS and heavy lactation, compared to 1.4% for auction cattle. Stojkov et al. (2020a) evaluated 6,300 cull dairy cows arriving at livestock markets in Canada and concluded that as many as 30% of the sold cows had poor fitness for transport due to a low BCS (<2), lameness and udder inflammation and/or engorgement. A study from Chile reported that 52% of 237 cull cows arriving at a slaughterhouse had more than one health problem, with low BCS, udder issues and lameness being most prevalent (Sánchez-Hidalgo et al., 2019). Meanwhile, a Scottish study reported 31% of 383 cull dairy cows were lame and 71% had a BCS of 2.5 or less when arriving at a market (Rosen et al., 2020), and a Columbian study reported that 75% of 137 cull dairy cows showed signs of disease, 98% had a BCS of 1 or 2, 28% had mastitis and 22% were lame when arriving at the slaughterhouse (Romero et al., 2020).

A study of cull beef cows by Goldhawk et al. (2015) evaluated some aspects of the cows' clinical condition before and after 14 hours of transport. The study included the American Meat Institute's Compromised Animal Score ("Excellent", "Acceptable", "Not Acceptable" and "Serious Problem")

used to assess animal condition before and after transport. The Compromised Animal Score is not a scientific scale but a risk management scale and is based on an assessment of the entire load rather than individual animals. Out of 12 truckloads assessed, ten loads had injuries and poor udder condition that were not present before transport. Six of the 12 loads had a higher number of lame cows after transport than before. In total, three cows became non-ambulatory. The injuries observed after transport included cuts and grazed skin, expected to result from interactions with the trailer. In contrast, Warren et al. (2010) conducted a study in Canada of younger beef cattle (steers, heifers and bulls) and found that after transport, 79 of 50,000 animals were lame, and five animals were unable to move (non-ambulatory) or dead. Their clinical condition prior to transport was unknown. Warren et al. (2010) concluded that *"there were very few visible animal welfare concerns associated with the transportation of slaughter cattle in the population sampled*".

In another Canadian study, livestock drivers were asked to complete surveys during their journeys. Almost 300,000 cattle transported for more than 400 km were included. Results showed that calves and cull cattle were more likely to become non-ambulatory or die during the journey than feeders and fat cattle (Gonzalez et al., 2012b). Compared to the studies by Goldhawk et al. (2015) and Ahola et al. (2011), Gonzalez et al. found very few lame animals after transport – a total of 36 out of almost 300,000 animals (calves, feeders, fat cattle and cull cattle combined). The severity of lameness was not reported, and it is possible that the livestock drivers who assessed the lameness used different cut-off values for lame vs. non-lame than those used in the other studies. In a more recent study, Stojkov et al. (2020b) compared the lameness, BCS and udder condition of cull dairy cows at three points in time: when added to the culling list, when leaving the farm and upon arrival at the slaughterhouse. They found that the overall fitness for transport declined while the cows were on the waiting list and again during transport.

Lesions and bruises

Rezac et al. (2014) evaluated a range of gross pathological lesions in 1,400 cull dairy and beef cow carcasses (87% Holstein cows) at a US slaughterhouse. They found that 32% had liver abscesses, 35% had rumen epithelial abnormalities, 34% had pulmonary lesions and 54% had bruises. They also identified that many Holstein cows seemed to be too tall for the trailers, resulting in bruises on their hips and backs. Strappini et al. (2012) evaluated more than 250 cow carcasses in a Chilean slaughterhouse and found that 92.2% had bruises and that the cows that came via markets had more bruises than those that came directly from farms. Risk factors for bruising include rough handling during loading and unloading (Strappini et al., 2013).

Feed and water deprivation

Cattle are often deprived of feed and water during transport, and in some cases also before transport, and it is possible that adverse effects will be influenced by other transport-related stressors (Hogan et al., 2007). Gonzalez et al. (2012a) investigated body weight loss (shrinkage) in fattened cattle, feeders, calves, and cull cattle for journeys >400 km. Gonzalez et al. found that fattened cattle had the lowest shrinkage compared to feeders, calves and cull cattle, indicating that fattened cattle were more able to cope with transport stress compared to the other types of cattle. Considering that many cull cows are lactating and therefore have higher requirements for feed and water than non-lactating animals, feed and water deprivation may have a more profound adverse effect on this group of animals during transport (Hogan et al., 2007; Vogel et al., 2011; Stojkov et al., 2020b).

Mortality

Animals dying during transport can result from a total failure to cope with the associated stressors and demonstrates extremely poor welfare. Vecerek et al. (2006) investigated cull dairy cow mortality rates during transport to slaughter in the Czech Republic. More than 1,000,000 cows were included in the study. The mean mortality rate was 0.04%, and mortality rates were correlated to distance, i.e., the mortality rate increased to 0.2% for journeys longer than 300km. These results were confirmed by Malena et al. (2007), who compared mortality rates for different types of cattle during transport to slaughter in the Czech Republic. Results showed that cull dairy cows had a higher risk of dying during transport than calves and fattened cattle. Mortality rates were higher for longer journeys (>300 km) compared to shorter journeys. A Canadian study of journeys >400 km also showed that the likelihood of dying increased with transport time and that it increased sharply when cattle spent more than 30 h in the truck (Gonzalez et al., 2012b).

To sum up, cattle transport entails many potential stressors. Existing literature has established that transport can be both stressful and exhausting for cattle, and cull dairy cows are likely to be less capable of coping than younger and healthier individuals. Although the welfare implications related to animal transport have gained more scientific attention in recent years, there is still a need for further research. It is clear from the reviewed studies that while much previous research describes the clinical condition of cull dairy cows after transport (although in varying levels of detail), the clinical condition of cull cows before transport has not been investigated. However, knowledge of the clinical condition before transport is essential in order to determine whether the clinical condition has deteriorated during transport and to identify potential hazards.

Based on the existing literature, it seems clear that there is great potential to improve animal welfare during the pre-slaughter period via timely culling and better preparation of cows for transport, e.g., drying-off and/or fattening. How farmers, livestock drivers, veterinarians and other personnel assess fitness for transport has gained very limited scientific attention and more knowledge is needed about their views on animal welfare, practical experiences, and decision-making processes.

3. Materials and methods

Two studies were conducted as part of this thesis. The following two chapters provide an overview of the study design and a discussion of the methods and results. For a more thorough description, see Papers I–III.

Study 1 (Papers I and II)

Study 1 had three objectives. The first was to describe to the clinical condition of Danish cull dairy cows before and after transport to slaughter. The second objective was to determine whether transport could cause their clinical condition to deteriorate. The third objective was to identify possible risk factors for any potential deterioration in clinical condition. The investigated risk factors included both factors relating to the individual cow (e.g., lameness) and factors relating to the journey (e.g., duration).

Study design, participants, and selection of cows

The study was an observational cross-sectional study of 411 cull dairy cows transported by truck to slaughter in Denmark. All cows had a clinical examination on the farm before being loaded onto trucks and were re-examined after being unloaded at the slaughterhouse. The clinical examinations before and after transport were all carried out by the same veterinarian. Loading, transport and unloading all took place under typical conditions for commercial Danish cattle transport. The transport distance, duration and stops along the way were recorded.

Four hauliers, 20 dairy farmers and one cattle slaughterhouse participated in the study. The hauliers were recruited with help from two Danish transport organizations, DTL (Dansk Transport & Logistik, 1019 Copenhagen, Denmark) and ITD (International Transport Danmark, 6330 Padborg, Denmark). The hauliers all transported cull cows to the largest Danish cattle slaughterhouse, Danish Crown Beef (6670 Holsted, Denmark), and operated in different geographical areas of Denmark. This was important in order to ensure that journeys of varying duration and distance were included in the study. Dairy farmers were recruited via the hauliers. All participants were offered full anonymity.

The participating farmers were instructed to follow their normal culling routines and hence decided themselves which cows to cull. The cows were included in the project if they met the inclusion

criteria: cows, dairy breeds, fit for transport according to EU Transport Regulation. However, in order to include animals that were only borderline fit for transport in the study, the Danish Animal Experiments Inspectorate issued an ethical permit allowing the inclusion of animals of varying levels of fitness. Exclusion criteria were: heifers, beef cattle, last tenth of pregnancy or within 2 weeks after calving, housed in tie stalls and cows unfit for transport. All reasons for culling, ages and lactation stages (except the last tenth of pregnancy or first 2 weeks after calving as prohibited by legislation) were allowed. Cows from both conventional and organic farms could be included.

Data collection

Clinical examinations

All cows had an individual clinical examination at the farm before loading. The examination lasted approximately 15 minutes per cow and included: assessment of general condition; body condition scoring on a scale of 1 to 5 with .25 increments, where 1 = emaciated, 5 = obese (Ferguson et al., 1994); inspection and palpation of coat and skin and assessment of wounds (definition of wound: lesion at least 1x1 cm penetrating all layers of skin); assessment of hydration level; inspection and palpation of body and limbs; inspection and palpation of udder; assessment of peripheral blood circulation; measuring of rectal temperature, respiration rate and heart rate; auscultation of lungs, heart and rumen; locomotion scoring on a scale of 1 to 5, where scores of 1 and 2 were considered not lame and scores of 3 to 5 were considered lame (Thomsen et al., 2008). The cows were allowed to walk freely for the locomotion scoring and were observed walking a short distance (5–10 m). At the slaughterhouse, the cows were unloaded and separated into a holding pen, where they could move freely. The second clinical examination took place while the cows were in the holding pen, see Figure 1. The examination included a visual inspection of general condition, locomotion, wounds, and milk leakage. See Figure 2 for a schematic visualization of the steps included in the data collection.



Figure 1. Examples of cows ready for clinical examination. To the left is a cow in its home pen, fixated in head locks. To the right is a cow after unloading at the slaughterhouse, not fixated and therefore a little more difficult to examine.

Transport to slaughter and associated recordings

The trucks used in the study were all approved for transporting cattle and all livestock drivers were certified to transport cattle according to Danish legislation (Anonymous, 2006, 2015). General rules regarding the transport of animals were complied with at all times, see Figure 3 and 4. For all journeys, the veterinarian who followed the trucks to the slaughterhouse recorded the distance, duration, number of stops and duration of stops.



Figure 2. A schematic visualization of the different practical steps of the data collection: Step 1: clinical examination at the farm, Step 2: loading at the farm, Step 3: transport, Step 4: unloading at the slaughterhouse, Step 5: clinical examination at the slaughterhouse.



Figure 3. Farmer and livestock driver working together on loading cows.



Figure 4. A typical view inside a truck loaded with cull cows. They all stand perpendicular to the direction of driving and are not tied. Partitions divide the space into smaller units to prevent the cows from moving to much around and reduce the risk of falling.

Additional data from the Danish Cattle Database

Data from the two clinical examinations were supplemented by production data obtained from the Danish Cattle Database (SEGES, 8200 Aarhus N, Denmark). The supplementary data included age, parity, days in milk (DIM), milk yield and previous veterinary treatments.

Data analysis

Data were analysed in SAS (version 9.4, SAS Institute Inc., Cary, NC). In Paper I, PROC MEANS and PROC FREQ functions were used to obtain descriptive results. In Paper II, McNemar's test (PROC FREQ) was used to evaluate differences in the proportion of cows with a certain clinical finding before and after transport. An analysis of risk factors for the deterioration of clinical findings included three groups of explanatory variables: 1) the clinical condition of the cows before transport; 2) factors related to the journey (e.g., distance); 3) production-related factors (e.g., DIM). A step-wise procedure was used. For Step 1, the association between the outcomes and explanatory variables from all three groups of explanatory variables were screened one by one using a univariable logistic regression model (PROC GLIMMIX). For Step 2, the association between each of the outcomes and variables selected in step one (i.e. with $P \le 0.25$) were evaluated using a multivariable logistic regression model. The model was reduced using backwards elimination.

Study 2 (Paper III)

This study focused on lameness scoring of dairy cows and the associated assessment of fitness for transport. The objective was to investigate the level of agreement within and among three groups of professionals involved in transporting cows in Denmark, namely farmers, veterinarians, and livestock drivers.

Study design and participants

The study was based on an online questionnaire (Surveyxact, Rambøll Management Consulting, Aarhus, Denmark) with video recordings of cows walking. In total, 19 farmers, 19 veterinarians and 17 livestock drivers participated. All participants were emailed a unique link to the survey.

Data collection

The questionnaire contained 30 short video recordings of walking Holstein cows as seen from the side. Each video recording lasted between 4 and 11 seconds, and each cow walked approximately 10 m. Both lame and non-lame cows were included in the 30 recordings, and the lame cows had

varying degrees of lameness from mild to severe. The recordings were shown in an arbitrary order with respect to the degree of lameness.

Participants were first asked to assess each cow and decide whether the cow was "not lame", "mildly lame" or "lame". They were then asked to assess whether or not the cow was fit for transport (short journeys of less than 8 h duration) with regard only to the degree of lameness. Participants were provided with definitions of lameness categories before they viewed the recordings. However, no specific threshold for lameness in relation to fitness for transport was given and participants had to judge this for themselves. Participants were briefly reminded about the legislation and were instructed to watch each video recording no more than three times. In order to proceed, each question had to be answered. The time required to answer the survey was approximately 10 minutes.

Data analysis

Weighted and unweighted versions of Cohen's kappa were used to measure interrater agreement between pairs of observers. The kappa statistic measures the obtained degree of agreement beyond chance.

Lameness was rated on an ordinal scale with categories 0 (not lame), 1 (mildly lame) and 2 (lame). Two approaches were used to investigate 1) whether within-group agreement differed between groups, and 2) whether agreement between groups differed between pairs of groups (i.e., three pairwise combinations: veterinarians/livestock drivers, livestock drivers/farmers, farmers/veterinarians).

Approach 1 – agreement within groups. Kappa was calculated between all pairs of individuals within each professional group (veterinarians, livestock drivers and farmers). To compare withingroup agreement between groups, these kappa values were then used as response in a linear mixed model with a fixed effect of group and two random effects for subject repeatability (one for the first subject and one for the second subject within a pair).

Approach 2 – agreement between groups. For each pair of professional groups (veterinarians/ livestock drivers, livestock drivers/farmers, farmers/veterinarians), kappa was calculated between all pairs of individuals with one from each of two groups, i.e., each veterinarian was compared to each livestock driver and so on. These kappa values were then passed on to linear mixed models to estimate the mean agreement between each pair of groups and to estimate and test the three contrasts. A significance level of 5% was applied, i.e., differences were interpreted as statistically significant when the corrected p-value was below 0.05.

4. Results

For a more detailed description of the results, please refer to the included Papers I–III and Chapter 5.

Study 1

Study 1 (Papers I and II) aimed to describe the clinical condition of Danish cull dairy cows before and after transport to slaughter and determine whether transport could cause their clinical condition to deteriorate and if so, to identify possible risk factors. The study included a total of 411 cull dairy cows of the following breeds: Danish Holstein (68%), Red Danish Dairy (14%), Danish Jersey (8%) and crossbreeds (10%). All cull cows were transported to slaughter by truck between January 2015 and January 2016.

Paper I – description of the clinical condition of cull dairy cows before transport

Production data

Production data (age, parity, DIM, milk yield and treatment for disease) were obtained from the Danish Cattle Database. The mean age was 4.7 years (range 2.0–12.4, median 4.7), the mean number of parities was 2.9 (range 1–10, median 3.0), the mean number of DIM was 270 (range 15–871, median 244), the mean milk yield in kg milk per day was 20.6 (range 0.0–50.0, median 21.3).

Of the 411 cows, 191 (46%) had received treatments for one or more diseases during the six-month period before culling. The 234 treatments were grouped into five categories: lameness, mastitis, metabolic disorders, reproductive disorders and other. Lameness was the most frequently treated disease (52% of the treatments), followed by mastitis (27% of the treatments).

Clinical examinations

The clinical examination included 50 different clinical measures. Detailed results of the clinical examinations are shown in Table A.

Table A.

The 411 cull dairy cows included in this study were clinically examined on-farm before transport to slaughter. All examinations were performed by the same veterinarian while the cows were retained in head locks, with the exception of locomotion score, for which they were released and allowed to walk freely. The table shows the number and percentage of cows for which the clinical findings deviate from a normal condition.

The table is reprinted with permission from Paper I (A descriptive study of the clinical condition of cull dairy cows before transport to slaughter, *Livestock Science*, 2018. DOI 10.1016/j.livsci.2018.11.001, license: https://creativecommons.org/licenses/by-nc-sa/4.0/).

Clinical sign	Definition of	Number (%) of cows		
	"not clinically normal"	not clinically normal		
General condition	Not bright, alert, and responsive	3 (1)		
Vocalization	Present	0 (0)		
Ear movement	Absent	0 (0)		
Cud chewing	Absent	18 (4)		
Grinding of teeth	Present	1 (<1)		
Nose cleaning behavior	Absent	21 (5)		
Muscle tremors	Present	3 (1)		
Sunken eyes	Present	0 (0)		
Respiratory rate, breath/min	Above 30 breaths/min	106 (26)		
Rectal temperature, °C	Above 39.5°C	0 (0)		
Heart rate, beats/min	Above 80 beats/min	202 (49)		
Body condition score	<2.75 or >3.75	153 (37)		
Hair coat	Dull, rough, or shaggy	51 (12)		
Wounds at least 1x1 cm	Present	90 (22)		
Elasticity of the skin	Reduced	5 (1)		
Lymph nodes	Enlarged	48 (12)		
Respiration, rhythm, and quality	Non-rhythmic and/or superficial or	10(2)		
Respiration, mythin, and quanty	abdominal	10(2)		
Coughing	Present	3 (1)		
Nasal discharge	Present	0(0)		
Auscultation of lungs	Non-vesicular lung sounds	38 (9)		
Mucous membranes	Not moist and pink	4 (1)		
Capillary refill time	Prolonged	2(<1)		
Skin temperature	Cold	2(<1) 2(<1)		
Jugular vein fill	Filled more than normal			
		20 (5)		
Pulsation of jugular vein	Present	$\frac{10}{2}(2)$		
Jugular vein refill	Prolonged	3(1)		
Oedema	Present	2(<1)		
Auscultation of heart	Heart murmur	17 (4)		
Auscultation of rumen	No contraction of rumen	3(1)		
Withers pinch test	Failure to flex ventrally	50 (12)		
Rectal prolapse	Present	0(0)		
Vaginal discharge	Present	4(1)		
Vaginal prolapse	Present	0 (0)		
Vaginal tearing	Present	0 (0)		
Swelling, tenderness, redness of udder	Present	83 (20)		
Milk leakage	Present	4(1)		
Muscle condition front legs	Below normal	0 (0)		
Muscle condition hind legs	Below normal	32 (8)		
Hair loss, lesions, swelling front legs	Present	10 (2)		
Hair loss, lesions, swelling hind legs	Present	126 (31)		
Bursitis front legs	Present	8 (2)		
Bursitis hind legs	Present	20 (5)		
Arthritis front legs	Present	3 (1)		
Arthritis hind legs	Present	8 (2)		
Digital dermatitis front feet	Present	24 (6)		
Digital dermatitis hind feet	Present	77 (19)		
Overgrown claws front feet	Present	7 (2)		
Overgrown claws hind feet	Present	9(2)		
Pelvic asymmetry	Present	16 (4)		
Locomotion score	Score of 3 or higher	126 (31)		

Of the 50 clinical measures, there were eleven where 10% or more of the cows deviated from the norm: respiration frequency, heart rate, BCS, coat, wounds, lymph nodes, wither pinch test, swollen, tender and red udder, hair loss, lesions and swelling of the hind legs, digital dermatitis in the hind feet and locomotion score. See Figure 5 for examples.

The BCS ranged from 2.0 to 5.0 (median 3.0); 65 cows (16%) had a BCS <2.75 and 88 cows (21%) had a BCS >3.75.

The locomotion score ranged from 1 to 4 on a scale of 1 to 5, with 176 cows (43%) receiving a score of 1, 109 cows (26%) receiving a score of 2, 69 cows (17%) receiving a score of 3, and 57 cows (14%) receiving a score of 4. Cows with scores of 3 and 4 were recorded as *lame* (31% in total). Sixteen cows (4%) had pelvic asymmetry, 11 of these were lame (scores of 3 or 4).

Wounds were found on 90 cows (22%). More than half of these cows (57%) had more than one wound. A total of 184 wounds were recorded, primarily located on the legs (see Table B). Tail fractures were found in 22 cows (6%), all of these were old and had healed. Hock lesions in the form of hairless spots, swelling and/or wounds were found on 126 (31%) of the cows. Coat quality was poor, i.e., dull, rough, and shaggy in 51 cows (12%).

Table B.

Localization of 184 wounds found on 90 out of 411 cull dairy cows clinically examined on-farm before transport to slaughter.

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Location of wounds	Frequency	Percentage
Legs	101	55%
Body and tail	61	33%
Udder	16	9%
Head and neck	6	3%



Figure 5. Examples of clinical manifestations found prior to transport. To the left is a cow with both medial and lateral hock lesions. To the right is a cow with a red, swollen udder with pus oozing from a fistula.

Paper II – risk factors for deterioration of the clinical condition of cull dairy cows during transport

Culling reasons

The primary reasons for culling given by the farmers were: reproductive failure (28% of the cows), low milk yield (26%), udder health (15%) and lameness (13%). The remaining cows (18%) were culled due to a number of different reasons.

Transport to the slaughterhouse

The study included 49 journeys with a mean distance of 129 km (range 20–339 km); 156 cows (38%) were transported less than 101 km, 173 cows (42%) were transported between 101 and 200 km and 82 cows (20%) were transported more than 200 km. The mean journey duration was 187 min (range 32–510 min). The study was designed to include journeys of up to 8 hours (480 min), which is the legal maximum duration for cull cows in Denmark, but due to a delay in unloading at the slaughterhouse, six cows experienced a journey of 8.5 hours (510 min). In total,

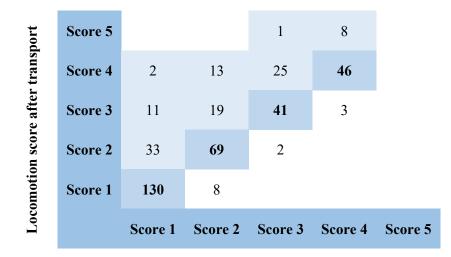
164 cows (40%) were transported for less than 121 min, 144 cows (35%) were transported between 121 and 240 min and 103 cows (25%) were transported for more than 240 min, including the six above-mentioned cows. The median number of stops along the way was 2 (range 0–6 stops) and the mean total duration of stops was 48 min (range 0–155 min). In five cases, a cow was transported with special provisions, i.e., segregated from the other cows for protection.

Unfit for transport

Nine cows arrived at the slaughterhouse in a condition that could be judged as unfit for transport according to the EU Transport Regulation. They were all lame (scores of 3 and 4) when loaded and were severely lame (score 5) when unloaded.

Clinical variables

Three variables changed significantly during transport: locomotion score, milk leakage and wounds. See Figure 7 for examples. Significantly more cows were lame after transport than before (41% after vs. 31% before, P<0.0001). Of the cows that were not lame prior to loading (n=285, Scores of 1 and 2), 15.8% became lame, while the severity of lameness increased in 26.6% of the cows that were already lame prior to loading (n=126, Scores of 3 and 4). Overall, 19% of the cows became lame or more lame during transport. Figure 6 shows locomotion scores before and after transport.



Locomotion score before transport

Figure 6.

Number of cull dairy cows with a given locomotion score before (x-axis) and after (y-axis) transport to slaughter, N = 411. The diagonal line in bold represents cows with the same scores before and after transport. Numbers above the diagonal line represent cows that scored higher after transport; numbers below the diagonal line represent cows that scored lower after transport.

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Results from the statistical analysis showed that deterioration of locomotion was significantly associated with the clinical condition of the cow and the following production-related factors: lactation stage, BCS, digital dermatitis in the hind feet and pelvic asymmetry. No association was found between factors related to the journey itself (e.g., distance and duration) and a reduction in locomotion scores, see Table C.

Table C.

Results from a logistic regression evaluating risk factors for the deterioration in locomotion score during transport of 411 cull dairy cows to slaughter.

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Risk factor	Odds ratio	95% confidence interval	<i>P</i> -value
Lactation stage			0.01
Early lactation (<100 DIM)	1.9	0.9-4.1	
Mid lactation (100–300 DIM)	1		
Late lactation (>300 DIM)	2.6	1.3-5.1	
Body condition score			0.001
≤2.50	3.7	1.7–7.9	
2.75-3.75	1		
≥4.00	0.7	0.3–1.4	
Digital dermatitis in the hind feet			0.01
No	1		
Yes	3.0	1.6-5.7	
Pelvic asymmetry			0.001
No	1		
Yes	4.9	1.4–16.3	

Significantly more cows had milk leakage after transport than before (17% after vs. 1% before, P<0.0001). Milk leakage was significantly associated with lactation stage and transport distance. Cows in early lactation (<100 DIM) and cows that were transported more than 100 km had significantly higher odds of milk leakage, see Table D.

Table D.

Results from a logistic regression evaluating risk factors for deterioration of milk leakage during transport of 411 cull dairy cows sent to slaughter.

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Risk factor	Odds ratio	95% confidence interval	<i>P</i> -value
Lactation stage			0.04
Early lactation (<100 DIM)	2.9	1.3-6.6	
Mid lactation (100–300 DIM)	1.3	0.7–2.6	
Late lactation (>300 DIM)	1		
Distance of journey			0.006
>200 km	10.2	2.2-46.9	
101–200 km	8.6	2.1-34.4	
<101 km	1		

Significantly more cows had wounds after transport than before (34% after vs. 22% before, P<0.0001), including 103 new, bleeding wounds and nine older wounds where the scab had torn off during transport. The new wounds were primarily located on the hips, hocks, and fetlocks. No significant association was observed between the increase in the proportion of cows with wounds and the included risk factors.

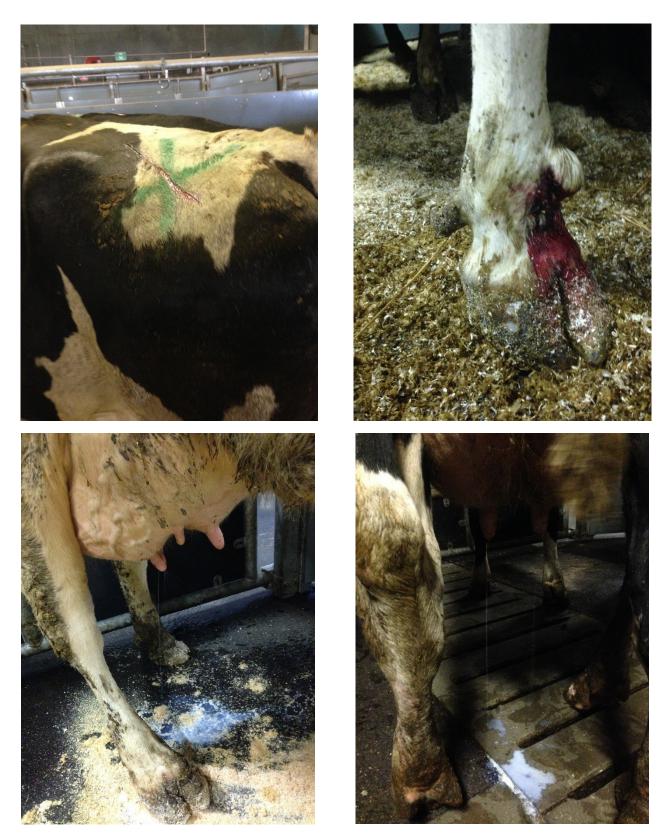


Figure 7. Examples of clinical manifestations found after transport. Top left is a cow with a traumatic wound to the back inflicted during transport. Top right is a cow with a traumatic wound to the fetlock inflicted during transport. Bottom left and right shows cows with milk leakage after transport potentially due to lack of milking immediately prior to loading.

Study II

Study II (Paper III) aimed to investigate the level of agreement within and between groups of farmers, veterinarians and livestock drivers in terms of assessing dairy cows' fitness for transport in relation to lameness.

Paper III – agreement among and between farmers, veterinarians, and livestock drivers

Agreement within groups: The levels of agreement for scoring lameness, unweighted and weighted kappa values, were moderate within all three groups. For assessment of fitness for transport, the levels of agreement within the veterinarian and the livestock driver groups were moderate, and the level of agreement among the farmers was fair, see Table E.

Table E.

Estimated agreement (shown as mean kappa) within three groups of professionals: farmers, veterinarians and livestock drivers when asked to score lameness and assess fitness for transport of dairy cows from 30 video recordings of walking cows. For lameness scoring, both unweighted and weighted kappa values are shown.

The table is reprinted with permission from Paper III (Lameness scoring and assessment of fitness for transport in dairy cows: Agreement among and between farmers, veterinarians and livestock drivers, *Research in Veterinary Science*, 2018. DOI 10.1016/j.rvsc.2018.06.017, license: https://creativecommons.org/licenses/by-nc-sa/4.0/).

Measurement	Group of professionals	Mean	95% confidence
		kappa	interval of kappa
Lameness, unweighted	Farmers	0.42	0.37 - 0.47
	Veterinarians	0.49	0.44-0.54
	Livestock drivers	0.48	0.42-0.53
Lameness, weighted	Farmers	0.50	0.45-0.55
_	Veterinarians	0.57	0.52-0.61
	Livestock drivers	0.55	0.50 - 0.60
Fitness for transport	Farmers	0.35	0.25 - 0.44
*	Veterinarians	0.53	0.43-0.62
	Livestock drivers	0.53	0.43-0.63

Agreement between pairs of professional groups: The levels of agreement between pairs of groups when scoring lameness – unweighted as well as weighted – were moderate for all pairs. In terms of assessing fitness for transport, the level of agreement between veterinarians and livestock drivers was moderate, and the levels of agreement between farmers and veterinarians and between farmers and livestock drivers were fair, see Table F.

Table F.

Estimated agreement (shown as mean kappa) between three pairs of groups (farmers/veterinarians, farmers/livestock drivers, veterinarians/livestock drivers) when asked to score lameness and assess fitness for transport of dairy cows from 30 video recordings of walking cows. For lameness scoring, both unweighted and weighted kappa values are shown.

The table is reprinted with permission from Paper III (Lameness scoring and assessment of fitness for transport in dairy cows: Agreement among and between farmers, veterinarians and livestock drivers, *Research in Veterinary Science*, 2018. DOI 10.1016/j.rvsc.2018.06.017, license: https://creativecommons.org/licenses/by-nc-sa/4.0/).

Measurement	Pairs of groups	Mean	95% confidence
		kappa	interval of kappa
Lameness, unweighted	Farmers/Veterinarians	0.41	0.36-0.47
-	Farmers/Livestock drivers	0.44	0.39-0.50
	Veterinarians/Livestock drivers	0.45	0.40-0.50
Lameness, weighted	Farmers/Veterinarians	0.50	0.45-0.55
-	Farmers/Livestock drivers	0.52	0.47 - 0.58
	Veterinarians/Livestock drivers	0.53	0.48 - 0.58
Fitness for transport	Farmers/Veterinarians	0.39	0.30-0.48
*	Farmers/Livestock drivers	0.37	0.28-0.46
	Veterinarians/Livestock drivers	0.52	0.43-0.61

In most cases, no group scored lameness or fitness for transport in a systematically different way than the other groups. Overall, disagreement among the groups appeared to be random, but there were a number of interesting exceptions. A higher proportion of veterinarians scored a cow as being lame compared to livestock drivers and farmers in eight out of the 30 lameness evaluation cases. A lower proportion of farmers scored a cow as being unfit for transport compared to livestock drivers and veterinarians in five out of 30 cases.

5. General discussion, perspectives, and limitations

The two studies are among the first to describe the clinical condition of cull dairy cows before and after transport, and to examine how farmers, livestock drivers and veterinarians assess dairy cows' fitness for transport in relation to lameness.

Study 1: Clinical condition of Danish cull dairy cows before and after transport to slaughter

The objective of this study was: 1) to describe the clinical condition of cull dairy cows prior to transport, 2) to determine whether transport can lead to a deterioration of the clinical condition of cull cows, and 3) to identify possible cow- and transport-related risk factors for any potential deterioration.

The major findings were: 1) cull cows constitute a very diverse group in terms of clinical condition; 2) there is a risk of cull dairy cows' clinical condition deteriorating during journeys shorter than 8 h; 3) risk factors for deterioration are primarily related to the individual cow and not to the transport.

A diverse group

Overall, the cull dairy cows included in the study constituted a very diverse group of animals in terms of age, productivity, health, BCS and reasons for culling. The farmers listed more than 25 different reasons for culling and only one out of four cows had no deviations from the clinical norm. None of the cows included were emaciated and their BCS ranged from 2.0 to 5.0. This is in contrast to a number of North American studies, where up to 13% of the cows were emaciated (Ahola et al., 2011; Nicholson et al., 2013; Stojkov et al., 2020a). The results suggest differences in culling strategies and cull cow management between Denmark and North America. The mean age was 4.9 years. Such short lifespans have both financial and welfare implications. Dairy cow longevity is a focal issue in modern dairy farming (Horn et al., 2012; De Vries and Marcondes, 2020), where sustainability is an important issue (von Keyserlingk et al., 2013; Schuster et al., 2020).

To the author's knowledge, the clinical condition of cull dairy cows prior to transport has not previously been described in detail. Results from this study may serve as a basis for future discussions of whether cull dairy cows, with their many different health issues, are sufficiently protected by the current legislation – or lack thereof. Future studies should include direct

comparisons of cull cow fitness to that of other types of cattle, as well as studies of how different clinical conditions affect cull cows during transport.

Lameness

Lameness is scored based on e.g., back arching, weight bearing, leg placement and head bobbing, and often on a scale of 1 to 5, where 1 is not lame and 5 is severely lame. Several systems for lameness scoring have been described in the literature (Sprecher et al., 1997; Flower and Weary, 2006; Thomsen et al., 2008). I found 126 (31%) lame cows (i.e., Lameness Scores of 3 and 4), which was not surprising since lameness is a major issue in dairy farming. Research conducted over the previous decades has reported a mean prevalence ranging from 20% to 36% (Espejo et al., 2006; Leach et al., 2010; Fabian et al., 2014; Solano et al., 2015; Cutler et al., 2017; van Huyssteen et al., 2020). Lameness is a common (though not necessarily always the main) reason for culling. Booth et al. (2004) found that survival in the herd decreased for cows that became lame in the first half of lactation, and the risk of being culled due to lameness is likely to be greatly underestimated as lameness has a detrimental effect on production and fertility. Barkema et al. (1994) studied the impact of lameness on production, fertility and culling in Dutch dairy herds and found that 26% of the lame cows that were culled were culled due to lameness. The time between the farmer's decision to cull and when the cow is actually transported may be longer for cows culled due to lameness than for cows culled due to al., 2020).

I found a significant increase in the number of lame cows after transport. Of the cows that were lame before transport, 26.6% were more lame after transport, while 15.2% of the cows that were not lame before transport became lame after transport. This is in contrast to a study by Thomsen and Sorensen (2013), where non-lame dairy cows were subjected to short journeys (mean 115 km/84 min) and then returned to their home farm. The study showed no significant change in locomotion score after transport. However, these cows were not cull cows and it is possible that they were in an overall better clinical condition than the cows in the present study. Other studies have also found an increase in lameness after transport, but the journeys included in these studies were of much longer durations (Ahola et al., 2011; Gonzalez et al., 2012b; Goldhawk et al., 2015). One interesting observation that I made was that the farmers often did not consider the lame cows to be lame. This was not quantified, but the observation is in accordance with many other studies showing that farmers find it difficult to identify lame cows (Whay et al., 2003; Espejo et al., 2006; Cutler et al., 2017; Jensen et al., 2022), and the less severe the lameness, the fewer cows they can

identify (Alawneh et al., 2012). This can become problematic if lame cows are loaded for transport and their condition deteriorates as a consequence.

Risk factors for increased lameness scores were digital dermatitis in the hind feet, BCS lower than 2.75, early lactation (<100 DIM), late lactation (>300 DIM) and pelvic asymmetry. Both digital dermatitis (Plummer and Krull, 2017; Capion et al., 2018) and low BCS (Randall et al., 2015) may result in lameness, possibly due to a reduction in the fat content and thickness of the digital cushion (Green et al., 2014; Wilson et al., 2021). In both cases, it is possible that the strain of transport may intensify the pain. Cows in early and late lactation may be under different kinds of physiological stress than cows in mid-lactation, possibly contributing to a greater risk of developing lameness. Severe pathological conditions such as fractures of the pelvis or coxofemoral luxation may cause a cow to develop pelvic asymmetry and lameness to varying degrees (Aiello and Moses, 2016), but it is possible that the opposite can also happen, with lameness causing pelvic asymmetry without such severe underlying pathology. Exactly how pelvic asymmetry affects cows during transport remains unclear, but the results showed that out of 16 cows with pelvic asymmetry, 11 became lame or more lame from being transported. Further research is therefore needed to clarify the welfare implications of pelvic asymmetry and potential suffering related to transport. Future studies should also investigate whether transport with special provisions can prevent additional suffering and injuries in lame cows, including cows with pelvic asymmetry.

As discussed in Chapter 2, it has been suggested that loading is stressful for the cows, but exactly how demanding loading is for lame cows is not known. Future studies of lameness in relation to transport should investigate how much the loading itself affects the lameness score of cull cows.

Udder health

Signs of mastitis (i.e., tender, swollen and red udders) were observed in 20% (83) of the cows. Mastitis and other udder health issues are common reasons for culling (Ahlman et al., 2011; Workie et al., 2021) and these numbers are therefore not surprising. As described by Hart (1988), sickness behavior (e.g., decreased feed intake and activity levels) is well documented in cows with mastitis (Medrano-Galarza et al., 2012; Fogsgaard et al., 2015; Sepúlveda-Varas et al., 2016). Interestingly, reduced lying time has also been documented (Siivonen et al., 2011; Medrano-Galarza et al., 2012; Fogsgaard et al., 2015). Reduced lying time is not part of the normal sickness behaviour pattern – on the contrary, animals tend to lie more when they are sick (Hart, 1988). The reduced lying time might be associated with pain, where cows with mastitis find it uncomfortable or painful to lie on the infected udder. Even mild cases of clinical mastitis can result in reduced lying time and increased restlessness and kicking during milking (Medrano-Galarza et al., 2012). Fogsgaard et al. (2015) found that local clinical udder signs and behavioural changes persisted a week after antibiotic treatment. Given that mastitis can cause pain and sickness behaviour in dairy cows, it can be speculated whether cows with mastitis are sufficiently protected by the current legislation from suffering during transport. According to the current EU Transport Regulation, mastitis itself does not make a cow unfit for transport. However, it could be that cows with mastitis should be considered "slightly ill" and therefore only fit for transport if the journey will not cause "additional suffering".

After transport, I observed a significant increase in the number of cows with milk leakage. Omission of milking leads to an accumulation of milk in the udder, which in turn may result in milk leakage. Milk leakage does not make a cow legally unfit for transport per se but may be an indication of a need to be milked. The EU Transport Regulation states that lactating animals not accompanied by offspring must be milked immediately prior to loading and thereafter at intervals of no longer than 12 hours. However, it appeared that many of the cows in the study were milked hours before loading as part of normal milking routines on the farm, rather than immediately before loading. The mean milk yield of the transported cows was 20.6 kg milk/day, but some yielded much more – up to 50 kg milk/day. To the best of my knowledge, there has been no investigation into how omission of milking might add to the stress of transport. However, drying-off is a routine management practice in dairy production and has been investigated in a number of studies. In modern dairy production, cows are dried off when they are producing what a cow in 1975 would have produced at peak lactation (Zobel et al., 2015).

Bertulat et al. (2013) identified increased udder pressure after abrupt cessation of milking, and highyielding cows were five times more likely to leak milk than low-yielding cows. The study also demonstrated that the concentration of faecal glucocorticoid metabolites increased, indicative of a stress response. Kohler et al. (2016) studied the effect of a prolonged milking interval (24 h) on the health and well-being of dairy cows in early and mid-lactation. The results showed increased udder pressure, milk leakage, decreased eating time, oedema in the subcutaneous udder tissue and increased hind limb abduction while walking, possibly to avoid touching the udder. The most pronounced effect was found in early lactation cows. Silanikove et al. (2013) found that the abrupt cessation of milking resulted in neutrophilia in milk as a response to distress, in addition to a considerable engorgement of the udder and vocalization suggesting that the cows were in pain.

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O'Driscoll et al. (2011) studied the effect of reducing milking frequency from twice to once per day and found higher udder firmness scores and milk leakage. Cows also spent less time lying down, possibly because internal pressure in the udder might be intensified by external pressure on the udder when lying down. However, O'Driscoll et al. (2011) found it unlikely that omission of a milking would be a significant welfare problem for dairy cows. Chapinal et al. (2014), whose study also showed that an abrupt cessation of milking resulted in reduced lying time, came to a different conclusion. As lying is a highly prioritized behaviour for cows, they suggested that omission of a milking might compromise the welfare of cows.

The animal welfare implications related to omission of milking demand further scientific clarification, as reviewed by Zobel et al. (2015). It is evident, however, that omission of milking can result in a number of adverse effects, including increased udder pressure, and it is therefore conceivable that it is, at least transiently, stressful and associated with pain – regardless of whether the omission is in connection with dry-off or with transport. Indeed, there is a possibility that the additional stress of transport will exacerbate the discomfort.

Risk factors for milk leakage in this study were early lactation (<100 DIM) and distance transported (>100 km). Bertulat et al. (2013) and Kohler et al. (2016) also identified early lactation as a risk factor, which seems reasonable as milk production peaks during the first part of lactation. The observed effect of distance is perhaps rather an effect of time from milking to second clinical examination as the two confound each other. Many of the cows that were transported more than 100 km were loaded during the very early hours of the morning (e.g., at 3 a.m.) for logistical reasons and were not milked immediately prior to loading.

Future research should focus more thoroughly on the potential negative effects of udder-related issues such as very large udders, mastitis, and omission of milking on the welfare of dairy cows during transport and, by extension, on whether cull dairy cows with udder-related issues are sufficiently protected by the EU Transport Regulation.

Wounds

I found wounds on more than one fifth of the examined cows, and more than half of these cows had two or more wounds. The wounds were mainly superficial skin lesions covered by a scab and located on the legs. Such skin lesions are common in dairy cows (Veissier et al., 2004; Kielland et al., 2009). In particular, a high prevalence of hock lesions related to inappropriate housing design is frequently reported (Kester et al., 2014), and Kielland et al. (2010) found that dairy farmers who

scored high in empathy and positive attitude toward the cows had lower levels of hock lesions in their herds compared to farmers who scored lower in empathy. Although skin lesions may be a reflection of animal welfare problems at the farm, they do not constitute a violation of the EU Transport Regulation in terms of fitness for transport. The EU Transport Regulation states that an animal with a "severe open wound" is considered unfit for transport, but it does not define exactly what this term means. Nevertheless, when characteristics such as location, width and depth were taken into account, none of the recorded lesions were considered "a severe open wound" by the examiner.

After transport, I found a significant increase in the number of cows with wounds, as a total of 103 new, bleeding wounds were recorded. Many other studies have included bruises rather than wounds (Warriss, 1990; Tarrant et al., 1992; Jarvis et al., 1996; Strappini et al., 2012; Strappini et al., 2013; Sánchez-Hidalgo et al., 2019). Bruises are different from wounds because they are only visible on the de-hided carcass, but the occurrence of both wounds and bruises may indicate issues related to animal handling and/or the design and use of facilities and equipment, thus indicating reduced animal welfare. Cull cows that pass through markets often become more bruised than cows moved directly from farm to slaughterhouse, possibly due to repeated loading and unloading and additional handling (Strappini et al., 2012). Bruises may also result from events at the slaughterhouse, in particular in the stunning box (Jarvis et al., 1996; Strappini et al., 2013). Studies from other countries may be not fully comparable to Danish conditions regarding e.g., handling procedures, but it is evident that there are several stages during the transport process where cows could potentially be harmed (Warriss, 1990). In this study, the cows were examined before entering the stunning box. Loading, unloading and passage through cattle chutes at the slaughterhouse were generally carried out in a calm manner and it seems unlikely that the wounds were inflicted at these stages. Instead, I suspect that the majority of wounds were inflicted while the cows were on the trucks.

Just like the wounds recorded before transport, the new wounds recorded after transport were not regarded as severe and would not result in the cows being judged unfit for transport. However, the EU Transport Regulation states that animals must not be transported "in a way likely to cause injury", and the legal implications of these injuries are therefore somewhat unclear.

Future research should include filmed behavioural observations during periods of driving, being stationary, loading and unloading in order to identify possible risk factors for the infliction of wounds.

Duration of journey

An interesting finding of this study was that even journeys of short duration might have a negative impact on the clinical condition of cull dairy cows. Studies from the US and Canada have also shown a deterioration of the clinical condition, but after journeys of longer durations (Ahola et al., 2011; Gonzalez et al., 2012b, a; Goldhawk et al., 2015). The present study showed that the clinical condition of many of the included cows deteriorated during transport even though some journeys were as short as half an hour (mean duration 187 min). The duration itself was not identified as a risk factor, so whether a cow was transported for 2 or 5 hours was not crucial in terms of their clinical condition after transport. It is possible that for some cows, the different phases that make up every journey (e.g., climbing up and down the loading ramp) are enough to have a negative impact on their clinical condition, regardless of the duration. As mentioned in Chapter 2, several studies have proposed that loading is a particularly stressful part of a journey. More research is needed to clarify the effect that the different phases of the transport process have on the clinical condition of cull dairy cows.

Limitations

Study I was designed as an observational study in a commercial setting. In an observational study, the researcher does not intervene but can only observe. It would have been difficult to design an experimental study due to the limited existing knowledge of cull dairy cow transport. Instead, this observational study generated useful insights into the cull dairy cow population in Denmark and an understanding of the challenges related to transport. Future experimental studies can be designed more easily based on the results. One disadvantage of an observational study is that it leaves the researcher with little or no control over the events, e.g., it was not possible to control the loading density during transport or the speed at which the cows were removed from the holding pen at the slaughterhouse. However, I believe that the advantages outweigh this disadvantage.

All cows were selected by the farmers themselves. The farmers were instructed to maintain their regular culling routine and select animals for slaughter as they would normally. They were informed that an animal experimentation permit had been obtained to allow the transport of animals that were in a grey area between fit and unfit. There is a risk that some farmers were reluctant to select cows if they were unsure about their fitness, thus only selecting fit cows. There is also a risk of the opposite happening – that some farmers would select cows that they were sure were unfit as they saw the animal experimentation permit as an opportunity to legally transport cows they would otherwise have to euthanize. However, to the best of my knowledge, I did not experience either

scenario. The farmers adhered to their culling routines and the cows included in the study give a fair representation of the Danish cull dairy cow population.

Only one observer, an experienced veterinarian, performed all the clinical examinations during the study, which entailed some uncertainty. The absolute error of the clinical examination (i.e., the average bias in locomotion scoring, etc.) was unknown. However, the focus of the study was to compare clinical examinations before and after transport and the relative uncertainty between clinical examinations was most likely minor since the same observer carried out all examinations.

Although I attempted to film inside the trucks during transport, this was deemed impractical at the time and was aborted due to substantial technical difficulties combined with a tight logistic schedule. Filming could potentially have helped to understand when, why and how injuries like wounds on the legs were inflicted. Since the completion of this study, the technical solutions have been further developed and filming should be included in future studies.

The study only included short journeys <8 h, but cull dairy cows are often transported for a much longer duration in other countries. Even though this study cannot predict exactly how longer journeys will affect the clinical condition of cull dairy cows, results showed that even short journeys can have a negative impact and given that longer journeys will expose the cows to risk factors for a longer time, these results can be extrapolated to propose that longer journeys will pose an increased risk to animal welfare.

Study 2: Assessment of lameness and fitness for transport

The objective of this study was to evaluate the agreement among and between groups of farmers, livestock drivers and veterinarians when assessing lameness and fitness for transport. The main findings were: 1) moderate agreement on the lameness score, both within and between groups, while 2) agreement on fitness for transport was moderate within the livestock driver group and the veterinarian group, but only fair in the farmer group. Likewise, agreement on fitness was moderate between livestock drivers and veterinarians, but only fair between farmers and livestock drivers, and farmers and veterinarians.

Lameness scoring

It has previously been shown that farmers have difficulty identifying many of the lame cows in their own herds (Whay et al., 2003; Espejo et al., 2006; Alawneh et al., 2012; Cutler et al., 2017; Jensen

et al., 2022). This is problematic in relation to animal welfare and economic considerations (Kossaibati and Esslemont, 1997; Green et al., 2002), but also in terms of assessing fitness for transport. However, in a study where lameness assessment was based on video recordings (Garcia et al., 2015), farmers did not perform any worse than veterinarians. This could indicate that when farmers fail to identify their own lame cows, it is more a question of "stable blindness", i.e., farmers are so used to seeing lame cows that they are perceived as "normal", or it may reflect a lack of time or interest, rather than a lack of ability.

The least lame cows in the study were the ones that resulted in the lowest level of agreement within all three groups. It is well known that farmers find it easier to identify severely lame cows than slightly lame cows (Whay et al., 2003; Espejo et al., 2006; Alawneh et al., 2012). Similarly, Thomsen and Baadsgaard (2006) showed lower levels of agreement among veterinarians when assessing less severe cases of hock lesions, cutaneous lesions and lameness compared to more severe cases. Houe et al. (2002) showed that veterinarians differ less when assessing pathological measures like skin lesions compared to non-pathological measures such as teat shape. To the best of my knowledge, how livestock drivers assess lameness or other clinical conditions has not been described to date. It therefore seems that non-well-defined conditions and *slight* illness or injuries are difficult to agree upon – this is perhaps not surprising, but it is problematic in relation to assessing fitness for transport.

Fitness for transport

In terms of fitness assessment, farmers agreed less within their group than the other two groups. The farmers also agreed less with veterinarians and with livestock drivers than these two groups agreed with each other.

The concept of fitness for transport has gained limited scientific attention (Grandin, 2001), and little is known about how different groups of professionals assess fitness. However, assessment of fitness for transport remains a central link in the pre-slaughter logistic chain, with legal, financial and welfare ramifications. According to the EU Transport Regulation, it is legal to transport slightly ill or injured animals as long as it will not cause them to experience additional suffering. Therefore, it is crucial for those who assess fitness for transport to be able to recognize and evaluate signs of illness, injury and potential pain and take a whole range of extrinsic factors into consideration in order to assess whether the animal is fit (enough) and whether it will experience additional suffering – all before the animal is loaded. However, recognizing and evaluating pain and clinical signs in

animals is generally complex and cattle are no exception, as discussed in the previous section. Cattle often show subtle signs of discomfort, effectively hiding their pain (Weary et al., 2006; Remnant et al., 2017).

Reduced fitness is not always related to pain, for instance an emaciated cow that lacks the strength to keep standing during transport may not be in pain. However, pain evaluation is often necessary when assessing whether slightly ill or injured animals are fit for transport. Different approaches to pain evaluation may include measures of physiological responses (e.g., changes in cortisol levels), measures of body functions (e.g., feed and water intake) and measures of behaviour (Weary et al., 2006; McLennan, 2018). Examples of behavioural changes could include a change in the level of daily activity (O'Callaghan et al., 2003). In recent years, certain facial expressions known as "pain face" have gained attention as an alternative to other pain assessment methods (Gleerup et al., 2015; McLennan, 2018). Facial expressions certainly have advantages, e.g., it is a non-invasive and quick method, yet they can be difficult to interpret correctly in situations where the animal under observation is exposed to some kind of stressor, e.g., they are aware of the observer.

Each approach to pain evaluation thus has its limitations, and a standard pain evaluation method is yet to be developed for practical purposes. This lack of standardized pain evaluation method may result in disagreement among different observers. For instance, several studies have shown differences among veterinarians in terms of pain assessment and pain management (Raekallio et al., 2003; Huxley and Whay, 2006; Thomsen et al., 2010; Remnant et al., 2017). A Finnish study on veterinarians' attitudes to animal pain showed that as many as 40% agreed/somewhat agreed with the phrase "*it is difficult to recognize pain in animals*" (Raekallio et al., 2003). Thomsen et al. (2012) showed that dairy farmers and veterinarians agree on which diseases are painful, but with large individual differences. In a study by Kielland et al. (2010), 70% of the included dairy farmers agreed or fully agreed with the statement "*animals experience physical pain as humans do*", while 15% disagreed or fully disagreed.

Livestock drivers play an important role in ensuring animal welfare during transport. For example, Cockram and Spence (2012) showed that driving style can impact the stability and resting behaviour of animals during transport. However, very few studies have included livestock drivers, and there is a lack of knowledge about their attitudes to – and understanding of – animal welfare. A Mexican study investigating livestock drivers' attitudes, knowledge and practices regarding animal welfare showed that concern for animal welfare was not influenced by age or education, but by

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years of driving experience, with experienced drivers showing more concern (Valadez-Noriega et al., 2018). Another study showed an association between more years of livestock driving experience and a smaller number of animals arriving in a compromised condition (Gonzalez et al., 2012b) as well as a lower risk of being involved in a road accident (Valadez-Noriega et al., 2018). In a Danish study by Herskin et al. (2017), 30% of livestock drivers indicated that they were "sometimes" or "often" in doubt about the fitness of a dairy cow, and 5% were "very often" in doubt.

Why farmers agreed less on fitness assessment than livestock drivers and veterinarians is open to speculation, but a Canadian study presented interesting results about dairy farmers' perception of the effect transport would have on their animals "...*Canadian dairy producers generally exhibited strong confidence that their culled cows would arrive at slaughter in the same condition as they left*..." (Roche et al., 2020). In addition, in a Danish study of cattle farmers and fitness for transport, the farmers reported that lameness was the clinical condition that most often led to doubt about fitness for transport (Dahl-Pedersen, 2022). Livestock drivers on the other hand observe the animals both before and after transport and might have gained a unique understanding of the potential effect transport will have on certain clinical conditions.

Complex decisions

In the present study, the respondents were asked to assess fitness for transport in relation only to the lameness score. This is of course a very simplified task. In reality, a wide range of risk factors relating to both the transport and the clinical condition of the cow must be taken into account when assessing fitness for transport. Deciding whether a cull dairy cow is fit for transport is in fact quite a complex task, and there is a risk that animal welfare may be compromised due to incorrect assessments. Decision trees and guidelines can be helpful (Stojkov et al., 2018; Cockram, 2019), but paradoxically if the assessment is made too simple, animal welfare could also be at risk due to a lack of nuances in the decision-making process. Future studies must investigate the concept of fitness for transport further. If the results from this study can be confirmed through larger studies, there is a need for training, calibration, and exchange of knowledge across all the different professional groups involved in the pre-slaughter handling of cull dairy cows.

Timely culling

Studies from around the world stress the need to cull cows in good time (Ahola et al., 2011; Magalhaes-Sant'Ana et al., 2017; Stojkov et al., 2018; Edwards-Callaway et al., 2019; Sánchez-Hidalgo et al., 2019). The decision starts with the farmer at the dairy farm (Haine et al., 2017; Edwards-Callaway et al., 2019), but veterinarians also have a very important role to play in supporting ethical decisions that set the welfare of the animal above e.g., economic considerations. Pro-active culling strategies should be implemented (Stojkov et al., 2018) and timely euthanasia should be considered as the best option in some cases (Walker et al., 2020). Future studies should focus on how veterinarians and farmers can work together to make better culling decisions, perhaps including developing tools to ensure timely culling.

Limitations

Study 2 included a relatively small number of participants recruited by convenience sampling, and therefore does not describe a representative sample. However, people cannot be forced to participate, and sampling therefore cannot be absolutely random but must always include a certain level of convenience. Future studies should include larger numbers of (more) randomly sampled participants in order to confirm the findings.

The present study investigates Danish participants only. In Denmark, farmers, livestock drivers and veterinarians are all educated and trained, and animal welfare is a topic of concern across professions. It is conceivable that including participants of different nationalities and with other educational and cultural backgrounds would have led to different results. Since animal transport is an important aspect of trade all around the globe, it would also be relevant to include farmers, livestock drivers and veterinarians from other parts of the world in future studies.

The participants were asked to assess fitness for transport based on lameness scoring alone. This is an obvious limitation to the study since in reality many factors must be taken into account in relation to fitness assessment. However, since this was a novel research topic with little existing literature on which to base any assumptions, it was considered important to choose a simple study design in order to establish a starting point for further studies. Future studies could add to the complexity by providing the participants with more information about the individual cow and/or transport on which to base their assessments, and video recordings could be replaced by live assessments on farm. This would make the decision-making process more realistic but also much more complicated to analyse.

6. Conclusions

The global scope of animal transport is enormous and constitutes a major challenge to animal welfare. Although much research is still needed, the studies conducted during this PhD project have added substantial new knowledge to the field and deepened our understanding of several aspects of transporting cull dairy cows.

I have reached the following conclusions based on the research questions from Chapter 1:

- The clinical characteristics of cull dairy cows before transport can best be described as very diverse. Many cull cows are culled due to health issues, and they present with one or more deviations from the clinical norm in terms of e.g., lameness, udder issues or low BSC.
- Cull dairy cows should be considered particularly vulnerable with regard to transport since they may have several health issues before transport and even short journeys (<8 h) by road may lead to a deterioration in their clinical condition in terms of lameness, milk leakage and wounds.
- Risk factors for this deterioration may include characteristics of the individual cow. Risk factors for becoming lame or more lame were digital dermatitis in the hind feet, a BCS lower than 2.75, early lactation (<100 DIM), late lactation (>300 DIM) and pelvic asymmetry. Risk factors for milk leakage were early lactation (<100 DIM) and distance transported (>100 km). Risk factors for wounds could not be determined in this study.
- Characteristics of the journey (<8 h) were not found to be risk factors for deterioration of the clinical condition in this study.
- Fitness for transport assessment is difficult to agree upon for different groups of professionals.
 Farmers, veterinarians, and livestock drivers agreed moderately at best when assessing lameness and fitness for transport.

Based on these conclusions, I have two suggestions for the veterinary authorities that can be implemented without requiring any further research:

Cull cows are vulnerable, and the legislation should differentiate between groups of animals so that cull cows can be covered by specific rules that take into account their condition.

Cull cows should not be transported for longer than 8 h and shorter journeys are preferable since their clinical condition can deteriorate even on short journeys.

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8. Papers

Paper I

A descriptive study of the clinical condition of cull dairy cows before transport to slaughter **Kirstin Dahl-Pedersen**, Mette S. Herskin, Hans Houe and Peter T. Thomsen *Livestock Science*, vol. 218, pp. 108-113, 2018; DOI 10.1016/j.livsci.2018.11.001

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A descriptive study of the clinical condition of cull dairy cows before transport to slaughter



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ARTICLE INFO ABSTRACT Despite its high relevance for fitness for transport, the clinical condition of cull dairy cows before they are sent to Keywords: Dairy cow slaughter has received very little scientific attention. Here, we report descriptive clinical data from cull Danish Transport dairy cows, examined on-farm in the hours before transport to slaughter. A total of 411 cows, with a median Slaughter parity of 3 (range: 1-10), from 20 Danish dairy herds, and slaughtered over a 1 year period, were included in the Clinical condition study. Almost 75% of the cows deviated from normal in at least one clinical measure, 31% were lame, 20% showed signs of mastitis and 22% had wounds, of which none were considered severe. Only few of the cows showed signs leading to direct decision of being unfit for transport, but many of them could be considered 'slightly ill or injured' as defined in the European Regulation, thus raising concern for the thresholds for fitness for transport in these animals.

1. Introduction

In modern dairy farming, the decision to cull a cow is of high economic importance and much research have focused on risk factors and reasons for culling as reviewed by Beaudeau et al. (2000) and recently by Compton et al. (2017). For decades, the most common reasons for culling have been low milk production, poor udder health, low fertility and lameness (Ahlman et al., 2011; Bascom and Young, 1998; Chiumia et al., 2013). Annual culling frequency has been reported to range from 23–36% (Chiumia et al., 2013; Nor et al., 2014; Pinedo et al., 2014; Smith et al., 2000); thus, worldwide considerable numbers of cows are culled each year. Cull dairy cows are often transported to slaughter by road, either directly or through markets (Stojkov and Fraser, 2017).

The clinical condition of cull dairy cows prior to transport has not been given much scientific attention. This is, however, highly relevant information for the required assessment of fitness for transport. The EU regulation on protection of animals during transport (Council Regulation, EC 1/2005) states that a cow can only be transported, if she is fit for transport. It is the responsibility of the animal keeper (i.e. the farmer) and the transporter to assess fitness for transport before loading. A cow is considered unfit if she is sick or injured, with the important exception that a slightly ill or injured cow may be considered fit for transport, if the transport will not cause additional suffering. It is, however, not further defined in the regulation how to assess 'slightly ill or injured' or 'additional suffering', but the EU regulation offers a list of seven pathological conditions or physiological processes that in particular makes an animal unfit for transport. Four of these concern younger animals or animals of other species, but three may be relevant for cull cows: animals that are unable to move independently without pain or walk unassisted; animals with a severe open wound or prolapse; animals in the last tenth of pregnancy or animals that have given birth in the previous week. In addition, it is stated that lactating animals transported without their offspring must be milked at intervals no longer than 12 hours. Apart from these, the EU regulation contains no specific rules regarding cull cows. However, transport of cull cows may have welfare implications as discussed by Gonzalez et al. (2012a, b); Stojkov and Fraser (2017) and Grandin (2001), who mentioned lameness and low body condition score (BCS) as major concerns.

A few studies have described aspects of the clinical condition of cull cows after transport: Nicholson et al. (2013) inspected cull dairy cows after transport and reported that 18% of the cows had a locomotion score of 3 or higher and 7% had visible foot abnormalities, 4.5% were extremely emaciated, and 24% had visible udder defects. Ahola et al. (2011) inspected dairy cows sold through markets prior to slaughter and found that 13% of the cows were emaciated or near emaciated and that 45% were lame. Strappini et al. (2012) and Rezac et al. (2014) examined cull cow carcasses at slaughter and found that 92% and 54%, respectively, had bruises. However, it is unclear

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whether the clinical conditions recorded after transport are identical to the conditions prior to transport, or whether transport as such had affected the clinical condition of the cull cows under study. Hence, knowledge about the clinical condition of cull dairy cows prior to transport is strongly needed in order to improve the understanding of potential welfare implications of transport of these animals.

The aim of this study was to describe the clinical condition of a sample of cull dairy cows prior to transport with reference to the rules regarding fitness for transport mentioned in the EU regulation (Council Regulation, EC 1/2005). The study was part of a larger study on transport of cull dairy cows (Dahl-Pedersen et al., 2018; Herskin et al., 2016).

2. Materials and methods

The study was designed as an observational study of the clinical condition of cull dairy cows before transport to slaughter. The cows originated from 20 commercial Danish dairy herds. The clinical data were collected between January 2015 and January 2016.

2.1. Participating dairy farmers

Participants were recruited among dairy farmers sending animals to one specific slaughterhouse using a hierarchical sampling procedure. The slaughterhouse, Danish Crown Beef Holsted (Danish Crown Beef, DK-6670 Holsted, Denmark), was chosen because it is the largest cattle slaughterhouse in Denmark. The first step was to select hauliers, and then farmers using these hauliers. The two Danish transport organizations DTL (Dansk Transport & Logistik, DK-1019 Copenhagen, Denmark) and ITD (International Transport Danmark, DK-6330 Padborg, Denmark) helped establish contact to hauliers transporting cull dairy cows to the slaughterhouse. The selection of the hauliers secured that they were operating in different geographical areas of Denmark. The hauliers provided their client lists, and all dairy farmers on these lists received a letter describing the study. Farmers were then listed in a random order, contacted by telephone and invited to participate, starting from the top of the list, until 25 farmers had accepted. This number of participating farmers was estimated to provide a sample of approximately 400 cows within the available data collection period of approximately one year. However, for different reasons, including selling of the farm, the final number of participating farmers was 20. All farmers, hauliers and livestock drivers were offered full anonymity.

2.2. Selection of cows

The participating farmers decided which cows to cull and were instructed to continue their normal culling routines. They were asked to call the project veterinarian whenever they had decided to cull cows and made transport arrangements. The cows were then included in the project if convenient regarding the overall logistic planning of the study, and if they met the inclusion criteria: cow, dairy breed, not unfit for transport according to the EU regulation (Council Regulation, EC 1/ 2005). Exclusion criteria were: heifer, beef cattle, last tenth of pregnancy or within two weeks after calving, housing in tie stalls and cows unfit for transport. Any culling reason, age or lactation stage (except last tenth of pregnancy or first two weeks after calving) was allowed and cows from conventional as well as organic farms could be included.

2.3. Clinical examination

Each cow was given a clinical examination at the farm before loading. The clinical examinations were done while the cows were restrained in head locks, and all examinations were done by one observer: an experienced veterinarian. No formal evaluation of intra-observer agreement was done prior to the study. The examinations included assessment of general condition; body condition scoring; inspection and

palpation of hair coat and skin; assessment of hydration level; inspection and palpation of body and limbs; inspection and palpation of udder; assessment of peripheral blood circulation; measuring of rectal temperature, respiratory rate and heart rate; auscultation of lungs, heart and rumen; and locomotion scoring. All variables were scored 0 if normal and 1 if abnormal, except for five variables: respiratory rate was recorded as breaths/min; rectal temperature in °C; heart rate as beats/ min; BCS was scored using a scale from 1 to 5 with quarter point increments, where 1 was a very thin cow and 5 a very obese cow (Ferguson, 1994); and locomotion was scored using a scale from 1 to 5, where 1 was a normally walking cow and 5 a severely lame cow (Thomsen et al., 2008). Cows with score 1 and 2 were not considered lame, cows with score 3, 4 and 5 were considered lame. The cows were let loose for locomotion scoring and observed walking a short distance of approximately 5-10 m. The majority of cows were scored on slatted or solid concrete floors, and 8% were scored while walking in deep straw bedding. As discussed below, heart rate and respiratory rate were difficult to interpret and thus excluded from further analyses.

2.4. Supplementary data and analysis

Production data were obtained from the Danish Cattle Database, which include detailed information of all cattle in Denmark. Data are entered by farmers, veterinarians, dairies, hoof trimmers and slaughterhouses and the database is maintained by the Danish agricultural industry organisation SEGES (DK-8200 Aarhus N, Denmark). The supplementary data included age, breed, days in milk (DIM), milk yield, parity and prior disease treatments of each cow.

Data were analysed using the PROC MEANS and PROC FREQ functions in SAS (Version 4.9, SAS Institute Inc., Cary, NC, USA) and all results are presented descriptively.

3. Results

A total of 411 cull dairy cows were included in the study out of the 423 cows planned to be culled by the farmers. The reasons for exclusion from the study were severe lameness, spastic paresis, low BCS and fever. The cows were of the following breeds: Danish Holstein (68%), Red Danish Dairy (14%), Danish Jersey (8%) and crossbreeds (10%). Data from the Danish Cattle Database are shown in Table 1.

Disease treatments in the six month period before culling were grouped into five categories: Mastitis, Lameness, Metabolic disorders, Reproductive disorders and Other. Of the 411 cows included in the study, 191 (46%) were treated for one or more diseases in the six month period (Table 2).

In total, the clinical examination included fifty different clinical measures. For 11 of these measures, the prevalence of cows deviating from normal was 10% or more: respiratory rate, heart rate, BCS, hair coat, wounds, lymph nodes, wither pinch test, swelling, tenderness and redness of udder, hair loss, lesions and swellings of hind legs, digital dermatitis of hind feet, and locomotion score. The results of the clinical examinations are shown in Table 3.

During the clinical examination, the mean heart rate was 81 beats

Table 1

Production data obtained at the last milk yield control (typically, each cow is controlled 11 times per year) for the 411 cull dairy cows included in the study. Data were obtained from the Danish Cattle Database.

Production data	Mean	Range	Median	First quartile	Third quartile
Age, years	4.9	2.0-12.4	4.7	3.8	5.9
Parity	2.9	1-10	3.0	2.0	4.0
Days in milk	270	15-871	244	152	371
Milk yield, kg milk/ day	20.6	0.0–50.0	21.3	14.2	27.2

Table 2

The frequency of disease treatments in the six months prior to culling among the 411 dairy cows included in this study. Data were obtained from the Danish Cattle Database, and reported to the database from herd veterinarians and farmers. The 234 disease treatments are distributed among 191 cows.

Prior disease treatments	Frequency
Lameness	123
Mastitis	63
Metabolic disorders	15
Reproductive disorders	27
Other	6

per minute, ranging from 51 to 126 and the mean respiratory rate was 28 breaths per minute, ranging from 10 to 84. For both variables, a large proportion of the cows had higher values than the normal condition (Table 3). Of 38 cows with non-vesicular lung sounds, the majority had a mild expiratory rough lung sound, only one cow had clear bronchial lung sounds, both in- and expiratory.

The BCS ranged from 2.0 to 5.0 with a median of 3.0. Of the 153 cows deviating from normal, 65 cows (16%) had a BCS < 2.75, and 88 cows (21%) had a BCS > 3.75.

Locomotion score ranged from 1 to 4 with 176 cows (43%) having score 1, 109 cows (26%) score 2, 69 cows (17%) score 3, and 57 cows (14%) score 4. Cows receiving score 3 and 4 were considered lame and constituted 31% in total (Table 3). Pelvic asymmetry was found in 16 cows (4%), 11 of these were lame (score 3 or 4).

Wounds were found on 90 (22%) of the cows and more than half of these cows (57%) had more than one wound. In total, 184 wounds were recorded, primarily located on the legs (Table 4). Tail fractures were found in 22 cows (6%); all tail fractures were old and healed. Hock lesions were found on 126 (31%) of the cows in form of hairless spots, swellings and/or wounds. Hair coat quality was poor in 51 cows (12%) that had a dull, rough, shaggy hair coat.

Fig. 1 shows the distribution of cows according to the number of clinical signs where deviations from the normal condition was observed. Only clinical signs scored as present/absent is included (i.e. not body condition score, locomotion score, rectal temperature, respiratory or heart rate). It can be seen that only approximately 25% of the cows had no deviations from the normal condition.

4. Discussion

This study aimed to describe the clinical condition of cull dairy cows prior to transport to slaughter. In total, 411 cull Danish dairy cows were clinically examined on-farm before being loaded for transport. Results from the clinical examinations combined with background data from the Danish Cattle Database provide a comprehensive and valuable characterization of cull dairy cows, which has not been available before and thus may provide benchmark. Overall, the cull dairy cows constituted a diverse group of animals in terms of age, parity, milk yield, type and number of clinical findings. Almost half of the cows had been treated for some disease within half a year before culling. In relation to the transport to come, cull cows are probably not comparable to younger, healthy animals that are fed for slaughter and subsequently transported when a certain age or weight is reached, and raises concern for the thresholds for fitness for transport in these animals.

Two variables deviated relatively often from normal—heart rate (abnormal for 49% of the cows) and respiratory rate (abnormal for 26% of cows). However, this was most likely induced by the presence of the examiner and the head locking procedure, as the other circulatory and respiratory measures only showed few, mild deviations from the clinically normal. Hence, despite the relative ease with which these measures may be obtained from cull cows prior to transport, and despite

Table 3

The 411 cull dairy cows included in this study were clinically examined on-farm before transport to slaughter. All examinations were performed by the same veterinarian, while the cows were restrained in head locks (except for locomotion score, where they were released and walked freely). The table shows the number and percentage of cows, where the clinical findings deviated from the normal condition.

Clinical sign	Definition of 'not	Number (%) of cows
	clinically normal'	not clinically normal
General condition	Not bright, alert and responsive	3 (1)
Vocalization	Present	0 (0)
Ear movement	Absent	0 (0)
Cud chewing	Absent	18 (4)
Grinding of teeth	Present	1 (<1)
Nose cleaning behavior	Absent	21 (5)
Muscle tremors	Present	3 (1)
Sunken eyes	Present	0 (0)
Respiratory rate, breaths/min	Above 30 breaths/min	106 (26)
Rectal temperature, °C	Above 39.5 °C	0 (0)
Heart rate, beats/min	Above 80 beats/min	202 (49)
Body condition score	<2.75 or >3.75	153 (37)
Hair coat	Dull, rough or shaggy	51 (12)
Wounds at least 1×1 cm	Present	90 (22)
Elasticity of skin	Reduced	5(1)
Lymph nodes	Enlarged	48 (12)
Respiration, rhythm and quality	Un-rhythmical and/or superficial or abdominal	10 (2)
Coughing	Present	3 (1)
Nasal discharge	Present	0 (0)
Auscultation of lungs	Non vesicular lung sounds	38 (9)
Mucous membranes	Not moist and pink	4 (1)
Capillary refill time	Prolonged	2 (< 1)
Skin temperature	Cold	2 (< 1)
Jugular vein fill	Filled more than normal	20 (5)
Pulsation of jugular vein	Present	10 (2)
Jugular vein refill	Prolonged	3(1)
Edema Auscultation of heart	Present Heart murmur	2 (< 1) 17 (4)
Auscultation of rumen	No contractions of	3 (1)
	rumen	
Withers pinch test	Failure to flex ventrally	50 (12)
Rectal prolapse	Present	0 (0)
Vaginal discharge	Present	4 (1)
Vaginal prolapse	Present Present	0 (0) 0 (0)
Vaginal tearing Swelling, tenderness, redness	Present	83 (20)
of udder		
Milk leakage	Present Polow pormal	4 (1)
Muscle condition front legs	Below normal	0 (0)
Muscle condition hind legs Hair loss, lesions, swellings	Below normal Present	32 (8) 10 (2)
front legs Hair loss, lesions, swellings hind legs	Present	126 (31)
Bursitis, front legs	Present	8 (2)
Bursitis, hind legs	Present	20 (5)
Arthritis, front legs	Present	3 (1)
Arthritis, hind legs	Present	8 (2)
Digital dermatitis, front feet	Present	24 (6)
Digital dermatitis, hind feet	Present	77 (19)
Overgrown claws front feet	Present	7 (2)
Overgrown claws hind feet	Present	9 (2)
Pelvic asymmetry	Present	16 (4)
Locomotion score	Score 3 or higher	126 (31)

them being covered by "physiological processes" as specified in the European Council Regulation (EC 1/2005), they are probably not valid candidates as indicators of reduced fitness for transport, but merely results of the acute stress of restraint and handling (Herskin et al., 2004). Additionally, the respiratory rate may also depend on the ambient temperature with higher 'normal' rates in hot weather conditions. Hence, these two deviations from normal have been removed from the calculations of the distribution of cows according to the number of

Table 4

Localisation of 184 wounds found on 90 out of 411 cull dairy cows clinically examined on-farm before transport to slaughter.

Location of wounds ^a	Frequency	Percentage
Legs	101	55%
Body and tail	61	33%
Udder	16	9%
Head and neck	6	3%

^a Wound: lesion penetrating all layers of skin, size at least 1×1 cm.

clinical signs where deviations from the normal condition was observed.

From these calculations it is evident that only 25.5% of the cows did not present any deviation from normal, and a maximum of up to 10 deviations were found in the same animal. Even though none of these deviations as standing alone would be enough for the cows to be considered unfit for transport according to the European Regulation (EC 1/ 2005), it seems relevant to discuss whether the threshold for slightly ill or injured may be breached in some of these animals, or whether they may be considered compromised (Heuston et al., 2017), thus requiring special provisions in order to avoid undue suffering. Future studies of fitness for transport should focus on how cows clinically deviating from normal as described here, may be more vulnerable to transport stress and whether their clinical condition may worsen during transport, rendering them unfit according to the European Regulation (EC 1/2005).

As mentioned above, three of the clinical characteristics leading to a direct decision of being unfit for transport may be relevant for cull cows-being unable to move independently without pain or walk unassisted, presenting severe open wound or prolapse or being close to calving (either in the last tenth of pregnancy or within one week after giving birth). In the present dataset no cows were too close to calving. However, among the cows planned to be culled by the farmers (a total of 423 cows, of which 411 could be included in the dataset), 6 cows had a locomotion score 5 (severely lame) and therefore excluded from the study and from transport to slaughter. In addition to these cows, 31% of the cows included in the dataset were scored as lame with a locomotion score of at least 3. This prevalence is in accordance with prior studies of lameness among Danish dairy cows - and cows in many other countries (Thomsen et al., 2012a; Solano et al., 2015). Dairy cow lameness is focus of much scientific attention and is widely recognized as a painful condition leading to reduced animal welfare (Dolecheck and Bewley,

2018; Flower et al., 2008). The cows in the study were selected by the farmer for culling following normal routines and as such should be representative of cull dairy cows in Denmark. With this in mind it is reasonable to assume that transport of lame cull dairy cows is a common event. Thomsen and Sørensen (2013) reported that transport did not lead to changes in the locomotion score of non-lame dairy cows, but it is possible that this is different if the cows are already lame to some degree before being loaded for transport to slaughter. If so it may be a violation of the European Regulation (EC 1/2005), as the moderately lame cows probably belong to the category "slightly injured or ill", and taken into account that an increase in the degree of lameness upon arrival at a slaughterhouse is "causing additional suffering". Future studies, examining locomotion score before and after transport of cull dairy cows, are needed to clarify this.

The second clinical characteristics leading to a direct decision of being unfit for transport according to the European Regulation (1/ 2005) is the presence of at least one severe open wound or prolapse. In the present dataset, no cows were observed with prolapses, a condition which is relatively easy to define and recognize. On the contrary, a severe open wound could be less easy to define and the regulation offers no guidelines. In order to determine the severity of a wound one might take size, including depth, age (stage of healing) and anatomical localization into consideration. Twenty-two percent of the examined cows had wounds, but these were all superficial and considered not to be severe open wounds. The majority (55%) of the wounds observed in the present study were found on the legs of the cows, an anatomical location where a lot of the observed deviations from normal were located-including for example hair loss, lesions and swellings. Based on reports of clinical examinations of lactating dairy cows, these findings were not unexpected as even younger higher-producing cows also were characterized by a relatively high prevalence of clinical findings located on the limbs (e.g. Kielland et al., 2009), emphasizing the need for future work aiming to limit and prevent the occurrence of these welfare challenging conditions throughout the productive life of dairy cows.

Among the clinical findings of the present study, that would not lead to a direct decision of unfit for transport was signs of mastitis, i.e. red, swollen and tender udders, which was found in almost one in five of the cull cows. Considering the reported high prevalence of mastitis being a reason for culling, this may not have been unexpected. However, Grandin (2001) mentioned mastitis as one major reason for cows being unfit for transport, and even relatively mild cases of mastitis have been shown to be associated with sickness behavior and possibly pain (Fogsgaard et al., 2015; Kemp et al., 2008; Medrano-Galarza et al.,

30 25 Percent of cows 20 15 10 5 0 2 3 4 5 6 7 8 9 0 1 10 Number of clinical signs with deviations from the normal condition

Fig. 1. The distribution of the 411 cull cows clinically examined on-farm before transport to slaughter, according to the number of clinical signs where deviations from the normal condition was observed. Only clinical signs scored as present/absent is included (i.e. not body condition score, locomotion score, rectal temperature, respiratory or heart rate).

2012). In that respect, these cows could very well be considered 'slightly ill or injured' and thus fit for transport only if the transport will not cause 'additional suffering'. In a study of farmers' and veterinarians' attitudes towards pain in dairy cows (Thomsen et al., 2012b), the participants scored the pain associated with mastitis on a scale from 1 to 10 where 1 = no pain and 10 = very painful. Farmers gave mastitis a mean score of 3.5 and E. coli mastitis a mean score of 8.3, veterinarians gave mastitis a mean score of 2.7 and E. coli mastitis a mean score of 8.2. So farmers and veterinarians may acknowledge mastitis as being painful, however in the current study no one raised any concerns regarding the fitness for transport of cows with clinically abnormal udders or mastitis. Hence, the question is whether the regulations offers enough protection for cows with mastitis and other udder related disorders. Future research should investigate whether transport of cows with mastitis will result in additional suffering and suggest management initiatives to relieve the condition for the animals.

A much less common and highly under-studied condition found in the present dataset was pelvic asymmetry (4% of the cows). Among these cows almost 70% (11 cows) were lame. Underlying reasons for pelvic asymmetry may be several, and may not be acute. At present, consequences of these conditions in terms of animal welfare while the cows are lactating or fitness for transport when culled are not known. In an Irish survey of emergency slaughter, Cullinane et al. (2010) reported that pelvic injuries were responsible for 9% of dairy cows killed on farm, and transported to the slaughterhouse for human consumption as a carcass. Similar numbers were reported by Pistekova et al. (2004). More recently, Koralesky and Fraser (2018) reported that hip-related injuries were underlying 20% of reported cases of emergency slaughter of dairy cows in British Columbia, Canada. Until the impact of pelvic or hip asymmetry on animal welfare and fitness for transport have been documented, use of the possibility for emergency slaughter seems beneficial in order to avoid unnecessary suffering and at the same time be able to use the meat of the cows for human consumption.

5. Conclusion

This study described the clinical condition of cull dairy cows prior to transport to slaughter and included data from 411 cull Danish dairy cows. This study is among the first to focus on the clinical condition of dairy cows before transport and provides a comprehensive and valuable characterization of these animals. Overall, the cull dairy cows constituted a diverse group of animals in terms of age, parity, milk yield, type and number of clinical findings. More than 70% of the cows showed deviations from normal, and a relatively high proportion of the cows deviated in more than one clinical measure. Only few of the cows showed symptoms leading to direct decision of being unfit for transport, but many of them could be considered 'slightly ill or injured' as mentioned in the European Regulation, thus raising concern for the thresholds for fitness for transport in these animals.

6. Declaration of interest

None.

Acknowledgments

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Paper II

Risk factors for deterioration of the clinical condition of cull dairy cows during transport to slaughter **Kirstin Dahl-Pedersen**, Mette S. Herskin, Hans Houe and Peter T. Thomsen *Frontiers in Veterinary Science*, vol. 5, article no. 297, 2018; DOI 10.3389/fvets.2018.00297





Risk Factors for Deterioration of the Clinical Condition of Cull Dairy Cows During Transport to Slaughter

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Cull dairy cows are typically transported to slaughter by road. Across different types of cattle, road transport is recognized as stressful. Cull dairy cows may have different injuries or weaknesses and may thus be more vulnerable to transport stress than other types of cattle. The aim of this study was to investigate whether the clinical condition of cull dairy cows deteriorates during transport (<8 h), and to evaluate risk factors for potential deterioration of the clinical condition. A total of 411 dairy cows were clinically examined on farm before loading and again after unloading at the slaughter plant. The clinical examination included locomotion, presence of wounds, milk leakage, and general condition. One-fifth of the cows either became lame or more lame during transport, and there was a significant increase in the proportion of lame cows after transport (41% after vs. 31% before, P < 0.0001). A significant increase in the proportion of cows with milk leakage (17% vs. 1%, P < 0.0001) and wounds (34% after vs. 22% before, P < 0.0001) after transport were also found. Low body condition score (BCS) (<2.75) (P = 0.001), early or late lactation [<100 days in milk (DIM) or >300 DIM] (P = 0.01), digital dermatitis in the hind feet (P = 0.01), and pelvic asymmetry (P = 0.001) were identified as risk factors for the deterioration in lameness during transport. Early lactation (<100 DIM) (P = 0.04) and transport distance (>100 km) (P = 0.006) were identified as risk factors for milk leakage. For wounds, no significant risk factors were found. The results demonstrate that cull dairy cows are vulnerable to the strains of transport, even journeys shorter than 8 h, to the extent that the occurrence of clinical findings were increased after transport in cows legally considered fit for transport. These results call for further research into the animal welfare implications and optimization of cattle transport.

Keywords: dairy cows, animal welfare, fitness for transport, lameness, animal transportation

INTRODUCTION

Cull dairy cows are typically transported to slaughter by road, either directly or through markets (1). Across countries, recently reported proportions of the milking herd that is culled annually range from 23 to 36% (2–4), which means that worldwide millions of cull dairy cows are transported to slaughter each year.

Dairy cows are culled for a number of reasons relating mainly to productivity and health (5–7) and in many cases more than one culling reason are reported by farmers (8). Recently, we presented data from clinical examinations of the cows involved in the present study—while they were still

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on-farm before transport (9)—showing that almost 75% of the cows deviated clinically from the normal condition. Overall, the cull dairy cows constituted a diverse group of animals in terms of age, parity, milk yield, type, and number of clinical signs.

Across different types of cattle, road transport is recognized as stressful [as reviewed by Knowles (10)]; however only few studies have included cows (11-15). Based on the knowledge that dairy cows are often culled due to clinical conditions or weaknesses, such as mastitis or lameness (5-7) as well as the relatively high proportion of cows characterized by clinical deviations from normal conditions (9), it seems justified to suggest that dairy cows are more vulnerable to transport stress than other types of cattle [as suggested by Nielsen et al. (16)]. In accordance with this, Gonzalez et al. (12) found that cull cows were at a higher risk of becoming non-ambulatory during long haul transport than fattened cattle. Brown et al. (11) investigated the incidence of dark cutting beef; a well-described meat quality indicator associated with pre-slaughter stress, and found higher incidences for cull cows than for steers, heifers, or calves. In addition, Malena et al. (13) found that dairy cows had a higher risk of dying during transport than fattened cattle or calves sent to slaughter. Although these results were not based on direct comparison of cull dairy cows with other types of cattle transported under identical conditions, they may indicate that cull dairy cows tolerate transport less well than fattened cattle.

However, only few studies have described the clinical condition of cull cows after transport. Ahola et al. (17) did a survey of cattle sold through markets immediately before slaughter and found 13% of dairy cows to be emaciated or near emaciated and 45% to be lame. Nicholson et al. (14) inspected dairy cows as they arrived at slaughter plants and found 5% to be emaciated and 49% to be lame. Rezac et al. (18) and Strappini et al. (19) examined cull cow carcasses and found 54 and 92%, respectively, to be bruised. However, in order to understand the potential changes in the clinical condition after transport, it is necessary to gather information of the clinical condition both before and after transport, which was not included in the above-mentioned studies.

The European Council Regulation on animal transport [EU Regulation, EC 1/2005 (20)] is, in general, precise, and objective for instance regarding loading densities or journey durations. One exception, however, is the rules on fitness for transport, which are vaguely defined and lack clear cut-off points [as reviewed by Grandin (21) for cull sows]. The EU regulation [EC 2005/1 (20)] clearly states that animals must be fit for the journey in order to be transported and may not be transported in a way that will injure them or cause undue suffering. However, the regulation also states that animals that are "slightly ill or injured" and will not experience additional suffering due to transport may be considered fit for transport. How to assess "slightly ill or injured" and "additional suffering" is not defined, but a few severe conditions that would define an animal as unfit for transport are listed in the regulation, e.g., inability to walk unassisted, severe open wounds, or prolapses. Cull animals are not mentioned specifically in the regulation. Hence, knowledge about potential deterioration of the clinical condition in cull dairy cows during transport will add significantly to this area.

The objectives of this study were to (1) investigate whether the clinical condition of cull dairy cows changes during transport to slaughter; and (2) evaluate risk factors for potential deterioration of the clinical condition. In order to do so, we performed clinical examinations of 411 cull dairy cows before and after transport to a slaughter plant with special focus on locomotion, presence of wounds, milk leakage, and general condition. We hypothesized that a potential deterioration of the clinical condition would be associated with (1) distance and duration of transport; (2) clinical weaknesses already present before transport e.g., lameness or mastitis; (3) production related factors e.g., parity or days in milk (DIM).

MATERIALS AND METHODS

Study Design

This study was an observational cross-sectional study of 411 cull dairy cows transported to slaughter by truck in Denmark between January 2015 and 2016. All cows were examined clinically on farm before being loaded onto commercial trucks. After unloading at the slaughter plant, the clinical condition of the cows was re-examined. All clinical examinations before and after transport were done by the same veterinarian. Loading, transport, and unloading took place under conditions typical for commercial Danish cow transport. Transport distance, duration, and stops underway were recorded. One slaughter plant, four hauliers (with a total of five different trucks), and 20 dairy farmers participated in the study.

Recruitment of Participants

Dairy farmers were recruited via four hauliers transporting cull cows to the slaughter plant Danish Crown Beef (6670 Holsted, Denmark). The hauliers were recruited with help from two large Danish transport organizations, ITD (Dansk Transport & Logistik, 1019 Copenhagen, Denmark) and DTL (International Transport Danmark, 6330 Padborg, Denmark). The recruitment procedure is described in detail by Dahl-Pedersen et al. (9).

Selection of Cows

The participating farmers decided which cows to cull and followed their normal culling routines. The study did not include cows clearly unfit for transport. However, in order to include animals that were maybe fit for transport (in addition to cows fit for transport) in the study, an ethical permit was issued from the Animal Experiments Inspectorate (permit no. 2015-15-0201-00716), allowing the inclusion of animals of varying levels of fitness, as long as they did not classify as unfit with respect to the specific requirements of the EU regulation [EC 1/2005 (20)]. Following normal routines, the participating farmers contacted the slaughter plant directly in order to make arrangements for transport. The project veterinarian, in collaboration with either the farmer or the haulier, then decided if a transport could be included in the project with regards to the overall logistics and time schedule.

Clinical Examination On-Farm

The clinical examination focused on locomotion, presence of wounds, milk leakage, and general condition (Table 1). Additionally, other aspects of the clinical condition of the cows were evaluated to identify cows unfit for transport and to gain information about possible risk factors for deterioration of the clinical condition during transport. These included e.g., rectal temperature, heart rate and respiratory frequency, body condition score (BCS), inspection/palpation of skin, hair coat, limbs, body, and udder, evaluation of peripheral and central circulatory system (including auscultation of heart), and evaluation of respiratory system (including auscultation of lungs) [details described in Dahl-Pedersen et al. (9)]. Pelvic asymmetry and digital dermatitis were scored as present or absent based on a visual inspection of the cows. Only active stages of digital dermatitis were recorded as "present" whereas healed lesions were not recorded as "present." The cows were restrained in headlocks while examined, but let loose for locomotion scoring and observed walking a short distance of 5-10 m (22). The majority of cows were scored on slatted or solid concrete floors, and 8% were scored while walking in deep straw bedding. The clinical examination lasted \sim 5 min per cow. If the cows were to be loaded in the morning, they were examined clinically at the farm the same day. If the cows were to be loaded during the night, they were examined clinically the evening before. Until loading, the cows would either wait in a small pen inside the barn (17 farms) or wait in a small pen outside the barn (three farms). Depending on the routines of the farmers, the cows would wait in the pens from <30 min up to 8 h. No recordings were made during this period.

Transport to Slaughter and Associated Recordings

All trucks used in the study were approved for transport of cows and all livestock drivers were authorized to transport cows according to Danish legislation. Four of the five trucks used in the study were single deck with trailer; one was double deck and no trailer (**Figure 1**). Single deck trucks with a trailer could carry a maximum of 25 cows, the double deck truck carried a maximum of 35 cows on the lower deck; cows were never on the upper deck in the present study. Current rules regarding space allowances during transport state that a cow with an approximate live weight of 325 kg must have $0.95-1.3 \text{ m}^2$, a cow with an approximate live weight of 550 kg must have $1.3-1.6 \text{ m}^2$, and cows with a live weight >700 kg must have >1.6 m² [EC 1/2005 (20)]. These

TABLE 1 | Definitions for categorizing the clinical variables of the cull dairy cows

 before and after transport to the slaughter plant as normal or deviating from

 normal.

Clinical condition	Definition of deviating from normal
General condition	Not bright, alert, and responsive
Lameness	Locomotion score 3 or higher (22)
Wound	Lesion penetrating all layers of skin, size at least 1 \times 1 cm
Milk leakage	Milk continuously dripping or flowing from one or more teats

rules were complied with at all times. In all trucks, the floors were rubber-coated and sawdust was used as bedding.

The ramps were coated with rubber, fitted with foot battens and the slope could be adjusted to fit the surroundings when loading and unloading cows, but was never steeper than ${\sim}26^{\circ}$ cf. the EU regulation [EC 1/2005 (20)]. The ramps were provided with side protections with an approximate height of 130-140 cm to avoid cows escaping or falling off. The trucks were passively ventilated through openings in the upper part of the side walls in order to ensure adequate ventilation above the cows when standing, cf. the EU regulation [EC 1/2005 (20)]. The ventilation openings were rectangular, with an approximate height of 20-25 cm and of varying length, but in total constituting <10% of the wall area. All trucks had full air suspension. When loading, the truck drove up close to the barn and the cows were loaded directly from the small pen inside the barn or from the outside pen. The driver would walk behind the cows, hold a lightweight plastic board in front of him and gently drive the cows up the ramp and onto the truck. Sticks or electric pods were never used. At two farms, the cows were led by halter one at a time. The loading time did not exceed ~ 5 min.

For all journeys, the distance, duration, number of stops, and the duration of stops were recorded. A stop was defined as the truck not moving (irrespective of the reason) for at least 5 min. In Denmark, cull dairy cows are seldom shipped from one farm in great enough numbers to fill a whole truck, and hence, it is normal practice for hauliers to pass by other farms on the way to the slaughter plant and collect cows there. During journeys, drivers might shift the partitions between the cows inside the truck and move cows around as more cows are loaded onto the vehicle. Hence, it was not possible to register the precise stocking density or the occurrence of mixing of cows after

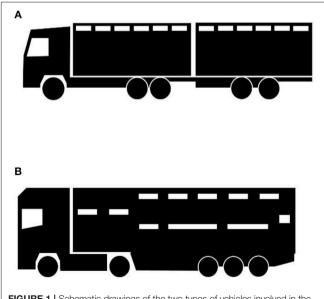


FIGURE 1 | Schematic drawings of the two types of vehicles involved in the study: single deck truck with trailer (A), and double deck truck (B). During the study, cows were never transported on the upper deck. The white rectangles mark openings in the walls of the truck for ventilation.

loading. If a farmer had ordered transport with special provisions i.e., segregating a cow in order to protect her from the rest of the animals, this was maintained throughout the journey and the use of segregation was recorded.

Clinical Examination at the Slaughter Plant

Upon arrival at the slaughter plant, the waiting time until unloading was typically short, but delays inside the slaughter plant prolonged the waiting time on a few occasions (median $5 \min$, range 0– $65 \min$). As the truck was opened and the partitions removed, the driver would enter the truck and gently move the cows, at their own pace, down the ramp from the truck. Unloading took <5 min. The cows were then separated into a holding pen, where they could move freely. The second clinical examination took place while the cows were in the holding pen and included a visual inspection of general condition, locomotion, wounds, and milk leakage. Definitions of clinical conditions are shown in **Table 1**.

Additional Data From the Danish Cattle Database

As a supplement to data from the clinical examinations, data on age, parity, DIM, milk yield, and veterinary treatments during the past 6 months before culling were obtained from the Danish Cattle Database. The database is run by SEGES, a large Danish agricultural industry organization (SEGES, 8200 Aarhus N, Denmark), and holds detailed information entered by farmers, veterinarians, hoof trimmers, dairies, slaughter plants, and others involved in dairying in Denmark.

Data Analysis

Data were analyzed in SAS (version 9.4, SAS Institute Inc., Cary, NC). McNemar's test (PROC FREQ) was used to evaluate differences in the proportion of cows with a given clinical finding before and after transport. Deterioration of locomotion was defined as any increase in locomotion score, except for an increase from 1 to 2 (as both score 1 and 2 are considered not lame). For the other clinical findings evaluated before and after transport (wounds, milk leakage, and general condition), deterioration was defined as changes from absent to present. Cows with a clinical sign (lameness, a wound, milk leakage, or abnormal general condition) both before and after transport were thus recorded as having no deterioration.

Three groups of explanatory variables were included in an analysis of risk factors for deterioration of clinical findings. The first group focused on the clinical condition of the cows before transport. Factors related to the journey such as number of stops or duration of transport were treated as a second group. The third group of variables were related to the production of the cows and consisted of data such as parity, DIM, and milk yield retrieved from the Danish Cattle Database. No cows had a clinically abnormal general condition, neither before nor after transport. Risk factors for a deterioration of clinical findings were identified for each of the remaining outcomes: locomotion, wounds and milk leakage. The following step-wise procedure was used: In step 1, the association between the outcomes and explanatory variables from all three groups of explanatory variables were

then screened one by one using a univariable logistic regression model (PROC GLIMMIX). Only variables with a P-value < 0.25 were further analyzed in step 2, where the association between each of the outcomes and variables from step 1 with P \leq 0.25 was evaluated using a binary logistic regression model (PROC GLIMMIX). The model was reduced using backwards elimination. All possible two-way interactions were included in the model. Confounding was evaluated comparing odds ratios with and without the possible confounder included in the model. If the difference was larger than 20%, important confounding was considered to be present. To evaluate the possible effect of cows at the same journey being more equal than cows at different journeys, journey was included as a random effect in the models. However, this only affected the results very little. The overall fit of the model was tested by evaluating the dispersion parameter which should be close to 1.

RESULTS

Status of Included Cows

The study included a total of 411 cull dairy cows. Twelve cows, assigned for culling by the farmers, could not be included in the study as they were assessed as unfit for transport during the clinical examination at the farm. The cows were unfit due to severe lameness (score 5), fever, spastic paresis, and BCS <2. These cows were left on farm and not included in this study.

The cows were culled after a mean of 2.9 lactations (range 1–10 lactations) and a mean of 270 DIM (range 15–871 days). The cows had a mean BCS of 3.25 (range 2.0–5.0) and were of the following breeds: Danish Holstein (68%), Red Danish Dairy (14%), Danish Jersey (8%), and crossbreeds (10%). The farmers stated the primary reason for culling: 28% of the cows were culled due to reproductive failure, 26% due to low milk yield, 15% due to udder health, and 13% due to lameness. The remaining 18% of the cows were culled due to a variety of other reasons.

The Transport to the Slaughter Plant

A total of 49 journeys were included. The journeys covered a mean distance of 129 km (range 20-339 km). Thirty-eight per cent (156/411) of the cows were transported <101 km, 42% (173/411) were transported between 101 and 200 km, and 20% (82/411) were transported more than 200 km. The mean duration of journeys was 187 min (range 32-510 min). The study was designed to include journeys of up to 8 h (480 min), which is the legal maximum for transport of cull cows in Denmark, but due to a delay at unloading at the slaughter plant, six cows experienced a journey of 8.5 h (510 min). Forty percent of the cows (164/411) were transported for <121 min, 35% (144/411) were transported between 121 and 240 min, and 25% (103/411) were transported for more than 240 min, including the six above-mentioned cows. The median number of stops underway was 2 (range 0-6 stops) and the median total duration of stops (including journeys with no stops) was 48 min (range 0-155 min). In five cases, a cow was transported with special provisions i.e., segregated from the other cows for protection.

Fitness for Transport

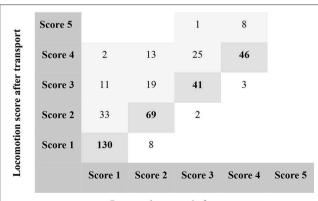
Nine cows arrived at the slaughter plant in a condition, where they, according to the current EU regulation [EC 1/2005 (20)], may have been judged as unfit for transport. They had all been lame (score 3 and 4) when leaving the farm and were severely lame (score 5) upon arrival.

Clinical Variables

Three variables changed significantly during transport: locomotion score, milk leakage, and wounds. Significantly more cows were lame after transport than before (41% after vs. 31% before, P < 0.0001). Overall, 19% of the cows became lame or more lame during transport. Among cows that were not lame before loading (score 1-2) 15.8% became lame. Among cows that were lame before loading (score 3-4), 26.6% became more lame. Locomotion scores before and after transport is presented in Figure 2. Results from the logistic regression showed no association between factors related to the journey itself, i.e., distance, duration, number of stops underway, and duration of stops underway, and deterioration of locomotion scores during the transport. Deterioration of locomotion was significantly associated with production related factors and the clinical condition of the cow: early lactation (<100 DIM) and late lactation (>300 DIM), low BCS (<2.75), digital dermatitis at the hind feet, and pelvic asymmetry (Table 2).

Significantly more cows had milk leakage after transport than before (17% after vs. 1% before, P < 0.0001). Milk leakage was significantly associated with lactation stage and transport distance. Cows that were transported more than 100 km and cows in early lactation (<100 DIM) had significantly higher odds of milk leakage (**Table 3**).

Significantly more cows had wounds after transport than before (34% after vs. 22% before, P < 0.0001). A total of 103 new, bleeding wounds were recorded together with nine older wounds



Locomotion score before transport

FIGURE 2 | Number of cull dairy cows with a given locomotion score before (x-axis) and after transport (y-axis) to slaughter, N = 411. The diagonal line of numbers in bold represents cows that scored the same before and after transport. Numbers above the diagonal represent cows that scored higher after transport; numbers below the diagonal represent cows that scored lower after transport.

where the scab covering the wound had been torn off during transport. The new wounds were primarily found on the fetlock, hock, and hips. None of the risk factors included in the analysis were significantly associated with the increase in proportion of cows with wounds.

DISCUSSION

This observational study is among the first to compare selected clinical signs of cull dairy cows before and after transport to slaughter. The objectives of the study were to investigate whether the clinical condition of the cows deteriorated during commercial transport of up to 8 h in duration as well as to evaluate possible risk factors for such a deterioration. In our dataset, including only cull dairy cows which were not unfit for transport while onfarm according to the EU regulation [EC 1/2005 (20)], one-fifth of the cows became either lame or more lame during transport and a significant increase in the proportion of cows with milk

 TABLE 2 | Results from a logistic regression evaluating risk factors for

 deterioration of locomotion score during transport of 411 cull dairy cows to

 slaughter.

Risk factor	Odds ratio	95% Confidence interval	P-value
Lactation stage			0.01
Early lactation (<100 DIM)	1.9	0.9–4.1	
Mid lactation (100–300 DIM)	1		
Late lactation (>300 DIM)	2.6	1.3–5.1	
Body condition score			0.001
≤2.50	3.7	1.7-7.9	
2.75–3.75	1		
≥4.00	0.7	0.3-1.4	
Digital dermatitis at the hind feet			0.01
No	1		
Yes	3.0	1.6-5.7	
Pelvic asymmetry			0.001
No	1		
Yes	4.9	1.4–16.3	

TABLE 3 | Results from a logistic regression evaluating risk factors for deterioration of milk leakage during transport of 411 cull dairy cows sent to slaughter.

Risk factor	Odds ratio	95% Confidence interval	P-value
Lactation stage			0.04
Early lactation (<100 DIM)	2.9	1.3-6.6	
Mid lactation (100–300 DIM)	1.3	0.7-2.6	
Late lactation (>300 DIM)	1		
Distance of journey			0.006
>200 km	10.2	2.2-46.9	
101–200 km	8.6	2.1-34.4	
<101 km	1		

leakage and wounds were found after transport. A number of risk factors for lameness and milk leakage was identified, whereas no risk factors for wounds could be identified. The results provide a basis for a deeper understanding of the consequences of transport for cull dairy cows, showing increased occurrence of injuries after transport in cull cows legally considered fit for transport. These results call for further research into the animal welfare implications and optimization of cattle transport, possibly also including intervention studies where effects of single factors can be investigated.

The identified risk factors were primarily related to the individual cow (her clinical condition and production related factors), but surprisingly only to a smaller extent to characteristics of the journeys such as duration or number of stops. In the current EU regulation [EC 1/2005 (20)], especially duration of transport is an important parameter, used to define what requirements should be met e.g., in terms of stocking density, feeding, and resting during transport. Legally, journeys are split into short (<8 h) and long journeys and regulated accordingly. The present study focused on journeys of up to 8 h. Studies of journeys of a longer duration than 8 h have shown that duration can have adverse effects on the clinical condition of cattle. Knowles et al. (23) investigated journeys of fattening cattle of different durations between 14 and 31 h and found that levels of cortisol increased with transport time and that some of the animals showed signs of fatigue and lay down after 20 h. Somewhat similar, Gonzalez et al. (12) found that on long haul journeys, the longer the cows were on the truck, the greater the risk of becoming lame, non-ambulatory, or dying. However, the present results suggest that in the case of shorter journeys of cull dairy cows, the duration is not a major risk factor for changes in the clinical condition of the animals. Further research is needed in order to examine effects of journey duration on cull dairy cows when transported more than 8 h, as is practice in many countries other than Denmark (1).

As the present study did not include any comparison between cull dairy cows and other types of cattle, it cannot be confirmed directly that cull dairy cows are more vulnerable to the stress of transport than other types of cattle as previously has been suggested (10, 13, 16). However, the present results do show that transport (even shorter than 8 h) is a straining experience for cull dairy cows. A considerable proportion of the cows were injured in terms of lameness or wounds, which is a violation of the EU regulation [EC 1/2005 (20)], clearly stating that cows must not get injured during transport. Yet, these cows would not be considered unfit according to the same EU regulation [EC 1/2005 (20)], thus the legal implications of the injuries that occurred during transport remain unclear.

The large proportion of cows that became lame or more lame during transport in the present study is in contrast to results from a study by Thomsen and Sørensen (15), showing that short distance transport of dairy cows (mean 84 km, 115 min) did not result in changes in locomotion score. However, the cows in the study by Thomsen and Sørensen (15) were all non-lame lactating dairy cows not due to be culled. Hence, despite the lack of a direct comparison, and differences in study design (experimental vs. observational) and distance driven (mean 84 vs. 129 km), the results from the present study may indicate that cull dairy cows are more vulnerable to the stress of transport than dairy cows not due to be culled. However, studies involving direct comparisons are needed to clarify this. In the present study, a few cows (13 out of 411) had a lower locomotion score after transport than before transport. This is likely due to an effect of the observer rather than a truly better locomotion after transport (22).

A small proportion of the cows (8%) were locomotion scored before transport in holding pens with deep straw bedding. Different floor types may influence the locomotion score of cows and softer surfaces can have a positive impact on the gait (24, 25). This may have meant that cows scored on deep straw bedding would have had a systematically lower locomotion score before transport compared to cows scored on concrete. However, cows scored on straw before transport did not become more lame when scored on concrete at the slaughter plant compared to cows scored on concrete both before and after transport (data not shown). The reason for this is perhaps that the cows had not been housed permanently on deep straw bedding prior to transport and therefore they were used to walking on hard surfaces.

We found that the risk factors for becoming lame or more lame included low BCS and pelvic asymmetry. Both these clinical conditions can be recognized without having to perform a thorough clinical examination. Earlier studies including our own data from pre-transport (9) have linked BCS to lameness: (26) did an 8-years study of one dairy herd and found that low BCS predisposed cows to lameness and that the risk of lameness decreased with increasing BCS. Similarly, (27) found that cows with low BCS were more likely to be treated for lameness during the following 4 months. Thus, future recommendations for assessment of fitness for transport of cull dairy cows could include that special attention should be given to cows with low BCS.

Pelvic asymmetry can be caused by different severe pathological conditions, such as coxofemoral luxation or fractures of the pelvis which may result in varying degrees of lameness (28). Pelvic asymmetry and lameness thus may share common etiologies and the associations between the two conditions are not entirely clear: Does pelvic asymmetry cause lameness, does lameness cause pelvic asymmetry, or are the two conditions simply caused by the same underlying pathology? How cows with pelvic asymmetry are affected by transport has to our knowledge not been investigated before, but a statement from the Danish Veterinary Health Council advises against transporting cows with pelvic asymmetry and/or unspecific lameness of the hind legs as it may cause the cows undue suffering (29). However, further research is needed in order to understand the welfare consequences of pelvic asymmetry in general, and in relation to transport specifically. It needs to be clarified whether cows with pelvic asymmetry can be transported with special provisions without risk of further injury or increased strain, or if cows with pelvic asymmetry should simply not be transported.

The present study showed a significant increase in the proportion of cows with milk leakage after transport compared to before. In the current EU regulation [EC 1/2005 (20)], milk leakage is not associated with reduced fitness for transport. Yet,

overfilling of the udder has been related to discomfort or pain. A study by Bertulat et al. (30) showed higher udder pressure after sudden dry-off as well as elevated levels of fecal glucocorticoid metabolites, interpreted as an indirect indicator of stress related to pain. Similarly, Kohler et al. (31) investigated the potential effects of a prolonged milking interval (24 h) and found increased udder pressure, decreased eating time as well as changes in locomotion such as an increased abduction of the hind legs, possibly to avoid pressure on the udder. In the present study, the risk factors for milk leakage were early lactation (<100 DIM) and distance transported (>100 km). The cows that were transported for the relatively long distances typically came from farms, where the loading took place in the middle of the night. In many cases, the cows had been milked in the evening as part of the normal farm routine, and then waited for several hours to be loaded and transported. Contrarily, cows from farms closer to the slaughter plant would normally be milked in the morning right before being loaded. Thus, the effect of distance might be an effect of time since last milking. If so, it is not only the time cows spend on the truck that matters, but the entire interval from last milking to slaughter. This underlines the importance of milking the cows immediately before loading in order to reduce milk leakage and the potential pain or discomfort related to overfilling of udders. This is already addressed in the EU regulation [EC 1/2005 (20)], where is it stated that lactating animals must be milked immediately before loading and then at intervals of not more than 12 h. Additionally, the daily milk yield may also play a role for the risk of milk leakage after transport. High yielding cows may have a higher risk of milk leakage after transport. This risk may be reduced by decreasing the number of daily milking gradually a few days before transport to slaughter. Future research on milk leakage and the related discomfort or pain should include precise information of time from last milking to slaughter to elucidate this matter further.

None of the wounds recorded after transport in the present study were considered "severe," as is listed as a specific reason for a cow to be judged as unfit for transport in the current EU regulation [EC 1/2005 (20)]. However, as mentioned above, the regulation does state that cows must not be transported in a way that causes injury and in that sense the present wounds were violations of the regulation. The presence of the injuries shows that transport conditions were sub-optimal. None of the risk factors included in the study were, however, associated with the increasing proportion of wounds, and further studies are required to determine how, why and when the wounds appeared and how to avoid them. Strappini et al. (32) investigated bruising events in cull cows and found rough handling during loading and unloading, and inadequate stunning facilities to be the most important risk factors. These risk factors cannot explain the increase in the proportion of wounds found in the present study, however, as rough handling was never observed and the clinical examinations were done before the cows entered the stunning box. Very little is known about what goes on inside the trucks during the different stages of transport and more knowledge is needed. Cockram and Spence (33) used video recordings to monitor trucks transporting cattle in order to evaluate effects of driving events on the stability of the animals. They found that cattle primarily lost the balance during cornering and that losses of stability were five times more frequent on minor and main roads compared to motorways. Further studies, including collection of behavioral data during journeys, including both driving and stationary periods as well as loading and unloading are needed to provide information about where in the pre-slaughter logistic chain injuries might be inflicted and, subsequently, avoided.

All clinical observations in the present study were made by the same trained veterinarian. Even though this design removes the risk of inter-observer disagreement, the observer might remember the pre-transport scores of a particular cow and may be biased by this during the after transport scoring of the cow. However, we find that this bias is small and insignificant in the present setting.

CONCLUSION

The objectives of this observational study were to determine whether the clinical condition of cull dairy cows deteriorate during commercial transport of up to 8 h duration as well as to evaluate possible risk factors for such a deterioration. The study revealed a significant deterioration of lameness, milk leakage and number of wounds. A number of risk factors for lameness and milk leakage were identified, mainly related to the individual cow (her clinical condition and production related factors), and to a minor extent to characteristics of the journey such as the distance. No risk factors for wounds could be identified. Overall, the results indicate that future recommendations for assessment of fitness for transport of cull dairy cows could specify that special attention should be given to cows with low BCS, cows showing signs of lameness before loading, cows in early or late lactation, cows with digital dermatitis and cows with pelvic asymmetry. Lactating animals should always be milked immediately before loading. The observed increased occurrence of injuries after transport in cows legally considered fit for transport, calls for further research and development into the concept of fitness for transport as well as a consideration of the implications for animal welfare and strategies that would optimize transport of cattle.

AUTHOR CONTRIBUTIONS

PT and MH conceived the idea of the study. The experimental protocol was developed by all authors. KD-P collected all data and did the data analyses together with PT. KD-P did the literature search and drafted the manuscript, which was edited and finalized by all authors.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Paper III

Lameness scoring and assessment of fitness for transport in dairy cows: Agreement among and between farmers, veterinarians, and livestock drivers

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Lameness scoring and assessment of fitness for transport in dairy cows: Agreement among and between farmers, veterinarians and livestock drivers



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ABSTRACT

Cull dairy cows are transported to slaughter, but may be more vulnerable to transport stress than younger livestock. In order to ensure the welfare of cull cows during transport their fitness for transport must be assessed before transport. Lameness is a common reason for culling dairy cows, and assessing fitness for transport in lame dairy cows is a frequent task for farmers, veterinarians and livestock drivers. The aim of this study was to evaluate the agreement within and between these three groups of professionals in relation to lameness scoring and assessment of fitness for transport. The study used an online questionnaire consisting of 30 video recordings of walking cows. Participants were asked to score lameness for each cow and assess if the cow was fit for transport or not. Weighted and unweighted kappa were used as a measure of interrater agreement within and between their own group as well as compared to veterinarians and livestock drivers when assessing fitness for transport. In general, it raises concern that the level of agreement on fitness for transport was moderate or even lower. These results call for more focus on the assessment of fitness for transport, including research and possibly training of the different professional groups in order to ensure good animal welfare during transport.

1. Introduction

After the production period, cull dairy cows are transported to slaughter. In recent years, the development in international meat production and processing (as discussed by Miranda-De La Lama et al., 2014) has led to a decreased number of slaughterhouses in many regions of Europe and North America and thus to longer distance to the nearest slaughterhouse. This calls for an increased focus on animal welfare during transport.

Cull dairy cows are suggested to be more vulnerable to transport stress than younger livestock (Gonzalez et al., 2012; Nielsen et al., 2011) and may be characterised by diseases or other weaknesses (Beaudeau et al., 2000; Booth et al., 2004; Fetrow et al., 2006), potentially increasing the severity of transport as a stressor. This emphasises the need to be able to assess fitness for transport before loading, in order to ensure an acceptable level of animal welfare. However, to date only very few studies e.g. Vecerek et al. (2006) have included data from transport of this specific group of animals, or has included aspects of fitness for transport.

According to EU legislation EC 1/2005 (Anonymous, 2005) it is the

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duty of farmer as well as livestock driver to ensure that all cows are fit for the intended journey before loading onto the transport vehicle. Throughout EU, farmer and livestock driver share the legal responsibility for the fitness of the cows. In case of doubt, veterinary assistance must be sought. Thus, farmers, veterinarians and livestock drivers all play vital roles in relation to the assessment of fitness for transport. Based on this important role, the knowledge about, and experience with fitness for transport in dairy cows among cattle livestock drivers as a professional group was examined recently by Herskin et al. (2017).

It is generally agreed that assessment of fitness for transport in livestock is not simple (Grandin, 2016). The EU legislation EC 1/2005 (Anonymous, 2005) clearly states that cows must be fit for transport and that ill or injured cows are not considered fit for transport. Cows that are slightly ill or injured may be considered fit for transport, if the transport will not cause them additional suffering. However, the term animal suffering has no clear scientific definition (Weary, 2014), and has been suggested as for example 'a wide range of unpleasant emotional states' (Dawkins, 1980). A few studies have described the clinical condition of cull cows arriving at the slaughterhouse, but not in great detail (Gonzalez et al., 2012; Warren et al., 2010) and very little is

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known about risk factors for the development of states of suffering during transport. Thus, at present, the decision of whether a certain transport will cause additional suffering is largely a matter of subjective assessment.

Lameness is a major welfare problem in dairy herds worldwide (Whay et al., 2003), with a mean herd level prevalence of 25-55% (Dippel et al., 2009; Leach et al., 2010; Thomsen et al., 2012; von Keyserlingk et al., 2012). Lameness is a common reason for culling dairy cows (Ahlman et al., 2011; Booth et al., 2004; Chiumia et al., 2013; Esslemont and Kossaibati, 1997). Nevertheless, the occurrence of lameness in cull cows is not known, but probably at least at the same level as in the herds in general. Thus, assessing fitness for transport in lame dairy cows is a frequent task for farmers, veterinarians and livestock drivers. Several lameness scoring systems exist, where lameness is scored based on e.g. head bobbing, back arching and leg placement (Flower and Weary, 2006; Sprecher et al., 1997; Thomsen et al., 2008; Winckler and Willen, 2001). In general, the systems do not require technical equipment and are therefore suitable for on-farm use. However, subjective scoring can result in considerable intra- and interrater variation. A study of veterinarians scoring lameness, hock lesions and cutaneous lesions showed moderate levels of interrater agreement with higher levels of agreement in the most severe cases (Thomsen and Baadsgaard, 2006). Studies of on-farm lameness scoring have found that farmers generally only identify approximately one third of the lame cows in their herds and that they find it easier to identify severely lame cows than moderately lame cows (Alawneh et al., 2012; Espejo et al., 2006; Whay et al., 2003). Garcia et al. (2015) evaluated intra-observer agreement of lameness scorings in groups of farmers and veterinarians. However, to date no studies have evaluated lameness scorings performed by livestock drivers. Nor has there been any focus on the agreement of lameness scoring across these three groups of professionals.

The aim of this study was to evaluate the agreement within and between three groups of professionals - farmers, veterinarians and livestock drivers - in relation to lameness scoring and assessment of fitness for transport in dairy cows.

2. Materials and methods

2.1. Recruitment of participants

The study used an online questionnaire (SurveyExact, Ramboll Management Consulting, Aarhus, Denmark). An invitation to participate was sent to 400 dairy farmers randomly sampled from a mailing list of 2415 Danish dairy farmers provided by the breeding association Viking (Viking Genetics, Assentoft, Denmark) and to 34 veterinary clinics listed on the Danish Veterinary Association's homepage as having at least one veterinarian working in cattle practice. Livestock drivers were contacted via an electronic newsletter from a major Danish transport organisation, DTL (Danish Transport and Logistics Association, Copenhagen, Denmark), sent to 35 trucking companies working with animal transport. This approach, however, led to only eight responses from livestock drivers. Another nine livestock drivers were therefore recruited in person when unloading cattle at a large Danish cattle slaughterhouse. Thus, some participants were recruited through convenience sampling (Houe et al., 2004). All participants were emailed a unique link to the survey.

2.2. Collection of data

The questionnaire consisted of 30 video recordings of walking Holstein cows seen from the side at a distance where the cows would take up approximately two thirds of the picture. Each cow walked approximately 10 m and each video recording lasted between 4 and 11 s. The 30 recordings were a convenience sample of available videos and covered the spectrum from not lame to severely lame. The recordings were presented in an arbitrary order (same for all participants) with respect to degree of lameness. For each cow, the participants were asked to indicate if the cow was 'not lame', 'mildly lame' or 'lame'. Definitions of these categories were given to the participants before they saw the recordings: A 'not lame' cow was defined as a cow with a normal gait. A 'mildly lame' cow was defined as a cow that did not have a normal gait but where the affected limb could not be identified. A 'lame' cow was defined as a lame cow where the affected leg or legs could be identified. In addition, the participants were asked if the cow was fit for transport or not, based solely on its degree of lameness, given that the duration of the transport would not exceed 8 h. However, no specific threshold for lameness in relation to fitness for transport was stated. This judgement was left for the participants. Participants were reminded about the legislation in brief (slightly ill or injured animals might be transported provided that their condition do not worsen, only non-lame and mildly lame animals may be transported). Participants were instructed not to watch each video recording more than three times. Each question had to be answered in order to proceed. Total time needed to answer the survey was approximately 10 min.

2.3. Statistical analysis

Weighted and unweighted versions of Cohen's kappa were used as the measure of interrater agreement between pairs of observers. The kappa statistic measures the obtained degree of agreement above chance. For the interpretation of kappa values we used the cut-offs suggested by Landis and Koch (1977): values < 0 = no agreement; 0-0.20 = slightagreement. 0.21-0.40 = fairagreement, 0.41-0.60 = moderate agreement, 0.61-0.80 = substantial agreement and 0.81-1 = almost perfect agreement. In addition to kappa for pairs of raters, we also calculated the (unweighted) variant for multiple ratings per subject including 95% normal approximation confidence limits, see Fleiss et al. (2003). This was calculated to check that the approach described below gave reasonable results for within group agreement. Lameness was rated on an ordinal scale with categories 0 (not lame), 1 (mildly lame), and 2 (lame). For the analysis of weighted kappa, agreement was weighted by 1 (agree), 0.4 (disagreement between successive categories: 0-1 or 1-2), or 0 (disagreement between categories 0 and 2).

Two approaches were made to investigate if 1) agreement within group differed between groups, and 2) agreement between groups differed between pairs of groups (i.e. three pairwise combinations: farmers-veterinarians, farmers-livestock drivers and veterinarians-livestock drivers).

Approach 1 - agreement within groups: For each professional group (farmers, veterinarians, and livestock drivers) kappa was calculated between all pairs of individuals within that group. To compare within group agreement between groups, these kappa values were then used as response in a linear mixed model with a fixed effect of group and two random effects for subject repeatability (one for the first subject and one for the second subject within a pair). The overall effect of group was tested by a likelihood ratio chi-square test on two degrees of freedom (χ_2^2), specifically given by minus two times the logarithm of the ratio between the likelihood functions obtained by maximum likelihood estimation of the models with and without the fixed effect of group. Contrasts between groups were estimated and assessed by a standard normal distribution Wald's test (z), specifically calculated by division of the estimated difference (δ) by the corresponding standard error (se). The p-values from the three tests of contrasts were adjusted for multiple comparison to control the familywise error rate by the single-step procedure suggested by Hothorn et al. (2008) and implemented in the 'glht' function from the 'multcomp' package in R (R-Development Core Team, 2016).

Approach 2 - agreement between groups: For each pair of professional groups (farmers-veterinarians, farmers-livestock drivers, veterinarians-livestock drivers) kappa was calculated between all pairs of individuals with one from each of two groups, i.e. each farmer was compared to each veterinarian and so forth. These kappa values were then passed on to linear mixed models to estimate the mean agreement between each pair of groups and to estimate and test the three contrasts, i.e. to compare the pairs of groups while taking into account the possible correlation between kappa values obtained by comparing the same individual to multiple other subjects. This was done by use of a fixed effect corresponding to the pair of groups investigated and, again, two random effects, one for subjects from the first group and one for subjects from the second group. Using the same methods as in approach 1, an overall χ_2^2 likelihood ratio test for the fixed effect and single-step adjusted Wald's z tests of contrasts was made.

The corrections for multiple comparison were carried out separately within each of the two approaches mentioned above and separately within each measure (lameness with unweighted assessment of agreement, lameness using weighted disagreements, and fitness for transport). A significance level of 5% was applied, i.e. differences were interpreted statistically significant when the corrected *p*-value was below 0.05. For estimation of standard errors of means and calculation of corresponding 95% confidence intervals from the linear mixed models, the restricted maximum likelihood (REML) approach was used. The assumptions of normal distribution underlying linear mixed models were checked by inspection of normal quantile-quantile plots.

3. Results

The total number of participants was 55: nineteen farmers (one female) with a mean age of 46 years (standard deviation (sd) = 11), 19 veterinarians (8 females) with a mean age of 48 years (sd = 13), and 17 livestock drivers (all males) with a mean age of 51 years (sd = 10).

For approach 1 – agreement within groups – the levels of agreement for scoring of lameness, unweighted and weighted, were moderate within all three groups. For assessment of fitness for transport, the levels of agreement within the veterinarians and the livestock drivers were moderate, and the level of agreement within the farmers was fair (Table 1). The multi-rater unweighted kappa agreed well with these results; lameness: farmers $\kappa = 0.42$ (95% CI: 0.40–0.44), veterinarians $\kappa = 0.49$ (95% CI: 0.47–0.51), and livestock drivers $\kappa = 0.47$ (95% CI: 0.45–0.49); fitness for transport: farmers $\kappa = 0.35$ (95% CI: 0.33–0.38), veterinarians $\kappa = 0.52$ (95% CI: 0.49–0.55).

The professional groups were not significantly different when scoring lameness, neither with unweighted kappa ($\chi_2^2 = 4.79$; p = .09) nor weighted kappa statistics ($\chi_2^2 = 3.83$; p = .15). For assessment of fitness for transport, an overall statistically significant distinction

Table 1

Estimated agreement (shown as mean kappa) within three groups of professionals: farmers, veterinarians and livestock drivers when asked to score lameness and assess fitness for transport of dairy cows from 30 video recordings of walking cows. For lameness scoring both unweighted and weighted kappa is shown.

Group of professionals	Mean kappa	95% confidence interval of kappa
Farmers	0.42	0.37-0.47
Veterinarians	0.49	0.44-0.54
Livestock drivers	0.48	0.42-0.53
Farmers	0.50	0.45-0.55
Veterinarians	0.57	0.52-0.61
Livestock drivers	0.55	0.50-0.60
Farmers	0.35	0.25-0.44
Veterinarians	0.53	0.43-0.62
Livestock drivers	0.53	0.43-0.63
	professionals Farmers Veterinarians Livestock drivers Farmers Veterinarians Livestock drivers Farmers Veterinarians	professionalskappaFarmers0.42Veterinarians0.49Livestock drivers0.48Farmers0.50Veterinarians0.57Livestock drivers0.55Farmers0.35Veterinarians0.53

Interpretation of kappa values (Landis and Koch, 1977): < 0: No agreement; 0–0.2: slight agreement; 0.21–0.4: fair agreement; 0.41–0.6: moderate agreement; 0.61–0.8: substantial agreement; 0.81–1: almost perfect agreement.

Table 2

Estimated agreement (shown as mean kappa) between three pairs of groups: farmers-veterinarians, farmers-livestock drivers, veterinarians-livestock drivers when asked to score lameness and assess fitness for transport of dairy cows from 30 video recordings of walking cows. For lameness scoring both unweighted and weighted kappa is shown.

Measurement	Pair of groups	Mean kappa	95% confidence interval of kappa
Lameness,	Farmers-Veterinarians	0.41	0.36–0.47
unweighted	Farmers-Livestock drivers	0.44	0.39–0.50
	Veterinarians- Livestock drivers	0.45	0.40-0.50
Lameness, weighted	Farmers-Veterinarians	0.50	0.45-0.55
	Farmers-Livestock drivers	0.52	0.47–0.58
	Veterinarians- Livestock drivers	0.53	0.48–0.58
Fitness for transport	Farmers-Veterinarians	0.39	0.30-0.48
-	Farmers-Livestock drivers	0.37	0.28–0.46
	Veterinarians- Livestock drivers	0.52	0.43–0.61

Interpretation of kappa values (Landis and Koch, 1977): < 0: No agreement; 0–0.2: slight agreement; 0.21–0.4: fair agreement; 0.41–0.6: moderate agreement; 0.61–0.8: substantial agreement; 0.81–1: almost perfect agreement.

between the groups was found $(\chi_2^2 = 8.93; p = .01)$ with significant differences between farmers and veterinarians ($\delta = -0.180$; se = 0.069; z = -2.59; adjusted *p* = .03) and between farmers and livestock drivers ($\delta = -0.185$; se = 0.072; z = -2.58; adjusted *p* = .03) but not between veterinarians and livestock drivers ($\delta = -0.005$; se = 0.072; z = -0.074; adjusted *p* = .99).

For approach 2 - agreement between pairs of professional groups the levels of agreement between pairs of groups when scoring lameness, unweighted as well as weighted, were moderate for all pairs. For assessment of fitness for transport, the level of agreement between veterinarians and livestock drivers was moderate, and the levels of agreement between farmers and veterinarians and between farmers and livestock drivers were fair (Table 2).

No significant differences were found between pairs of professional groups when scoring lameness (unweighted kappa: $\chi_2^2 = 1.44$, p = .49; weighted kappa: $\chi_2^2 = 1.30$, p = .52). In assessment of fitness for transport, the fixed effect corresponding to the pair of groups was statistically significant ($\chi_2^2 = 7.93$; p = .02). This was propelled by a significant difference between farmers and livestock drivers, and veterinarians and livestock drivers ($\delta = -0.155$; se = 0.055; z = -2.82; adjusted p = .01) and a tendency between farmers and veterinarians, and veterinarians and livestock drivers ($\delta = -0.131$; se = 0.064; z = -2.06; adjusted p = .09).

Overall, disagreement between the groups were random; in most cases, no group scored lameness or fitness for transport systematically different from the other groups. However, a few noteworthy exceptions were found. In eight out of the 30 cases of evaluating lameness, a higher proportion of veterinarians scored the cow as lame compared to farmers and livestock drivers. In five out of 30 cases, a lower proportion of farmers scored the cow as unfit for transport compared to the proportion of veterinarians and livestock drivers.

4. Discussion

This study is among the first to focus on assessment of fitness for transport, which is a central part of the pre-slaughter logistic chain. By use of an online questionnaire consisting of video recordings, we compared lameness scoring and assessment of fitness for transport in dairy cows within and between three groups of professionals – farmers, veterinarians and livestock drivers. All three groups are involved in

securing the welfare of dairy cows transported to slaughter. Overall, the three groups of professionals agreed moderately at best within as well as between groups. However, some groups/combinations only agreed fairly when assessing fitness for transport. These results suggest that assessment of fitness for transport is not similar within or between the three professional groups, and if confirmed in larger studies, there is a need for improvement – by training, access to assessment tools or guidelines including new technologies in order to assure consistent assessment of fitness for transport.

Overall, the level of agreement for lameness scoring within and between the three professional groups was moderate. Previous studies have shown that farmers failed to recognise the majority of lame cows in their herds (Alawneh et al., 2012; Espeio et al., 2006; Whay et al., 2003), but findings from the present study suggest that in an experimental setting with video recordings, and the possibility of reviewing the cows more than once, the farmers did not perform worse than the veterinarians and livestock drivers. This is in accordance with a study by Garcia et al. (2015) who found that both farmers and veterinarians scored high in agreement when scoring lameness using video recordings of dairy cows. It can be speculated whether farmers fail to recognise lame cows on-farm not so much due to lack of ability to identify a cow as lame, but maybe more due to lack of opportunity (e.g. no time allocated to observation of lame cows) or due to farmers being so used to high levels of lame cows that even moderately lame cows are perceived as normal. Also, farmers might prioritise other health issues such as mastitis over lameness (Leach et al., 2010).

The present sample size was relatively small and not selected randomly. The recruiting of volunteers for the present study may have meant that people with a special interest in the study topic were overrepresented among the respondents. Future research could include a larger study of lameness scoring in an experimental setting as well as during practical conditions on-farm, and include all three groups of professionals in order to investigate and compare the ability of each group to score lameness under different circumstances. Additionally, the effect of sex could be interesting to include in future studies. Previous studies have demonstrated an association between sex and attitudes of veterinarians towards pain and the use of analgesia (Raekallio et al., 2003; Thomsen et al., 2010).

Among the video recordings used in the present study, the least lame cows resulted in the lowest levels of agreement within all three professional groups (results not shown). This was not unexpected, as farmers are known to be better to identify severely lame cows (Alawneh et al., 2012; Espejo et al., 2006; Whay et al., 2003). Furthermore, Thomsen and Baadsgaard (2006) showed that veterinarians had higher levels of agreement when scoring severe cases of lameness, hock lesions and cutaneous lesions compared to less severe cases. In addition, Houe et al. (2002) found that veterinarians assessing udder health showed substantial variation when evaluating non-pathological measures such as teat shape, while showing far less variation when assessing pathological measures such as skin lesions. To date, no studies have focused on the ability of livestock drivers to recognise clinical measures in cattle. Based on the present knowledge, the overall picture seems to be that subtle differences and less well-defined conditions are most difficult to agree upon. This is problematic with reference to the EU legislation on transport, EC 1/2005 (Anonymous, 2005), and its vague definitions of fitness for transport. Slightly ill or injured animals might be fit for transport according to the legislation, but, in light of the above, 'slightly' is probably very difficult to assess in a correct and consistent manner. If assessment is simplified, it might be easier to handle both in practice for farmers, veterinarians and livestock drivers, and in a legal context for the authorities (as discussed by Robertson (2015) for animal welfare law in general), but there is a risk that animal welfare will be compromised if too many nuances are left out. In a recent review on cull sows and boars, Grandin (2016) emphasised the need for clearer guidelines and cut-off points in the definition of fit for transport. Future research should investigate the concept of fitness for transport further in order to be able to do proper characterisation of unfit animals as well as make assessment easier without oversimplifying. Studies should include all types of professionals involved in fitness assessment within the pre-slaughter logistic chain and expand to more than just the one clinical measure, lameness, used in this study.

When assessing fitness for transport, livestock drivers and veterinarians agreed moderately within their professional group and compared to each other. Farmers, on the other hand, agreed only fairly within their group and compared to livestock drivers and veterinarians. To date, only very limited research has focused on the concept of fitness for transport and how to assess this (Grandin, 2001), but these results were not unexpected. In a recent study by Herskin et al. (2017) involving a questionnaire survey among Danish livestock drivers transporting dairy cows to slaughter, 75% of the respondents stated that they had at least once loaded a cow onto their truck even though they were not sure if the cow was fit for transport. Previous studies have shown that transportation includes complex stressors such as duration, mixing of animals, temperature, flooring and driving conditions, handling and loading practices (Goldhawk et al., 2015; Gonzalez et al., 2012; Pettiford et al., 2008; Warren et al., 2010). All these risk factors must be taken into account when assessing fitness for transport and assessing whether the intended journey will cause additional suffering for the animals. The difference between farmers' assessment of fitness and livestock drivers' and veterinarians' assessment of fitness cannot be explained based on the present study alone and should be studied further in set-ups involving larger datasets and more systematic sampling of participants.

The present study focused only on dairy cows and lameness. However, lameness is just one part of assessment of fitness for transport and other conditions might have an impact on fitness as well, such as for example body condition score. Hence, in reality assessment of fitness for transport is a much more complex task than the one given in this study, where the assessment was to be based on lameness alone. In order to get a better understanding of fitness for transport and better possibilities for assessing fitness for transport, further studies including other conditions are needed. Also, different types of cattle might not be equally fit for transport. As an example, Gonzalez et al. (2012) found that cull cattle had a higher risk of becoming non-ambulatory or dying during transport compared to beef cattle. In contrast, Thomsen and Sørensen (2013) showed that lactating dairy cows, that were not lame before transport, did not become lame from being transported a relatively short distance. Differences between the results from Gonzalez et al. (2012) and Thomsen and Sørensen (2013) may also be caused by differences in duration and distance (long vs. short). Yet, since lameness is an important reason for culling, as shown by for example Chiumia et al. (2013), cull cows might often be lame before loading.

The present study focused on short transportations (shorter than 8 h) and involved only Danish observers. Although longer duration in itself may not be a risk factor for compromised welfare, cows will be exposed to other risk factors e.g. lack of feed, water and rest for a longer time with increasing transport durations (Nielsen et al., 2011). It is therefore important that future research focus on fitness for both short and long transports. In 2011, a report on the impact of the EU transport legislation (Anonymous, 2011) showed that - among other issues fitness for transport was a problematic concept due to poor compliance and lack of common interpretations of requirements across the EU. It is conceivable that fitness for transport may be interpreted differently from country to country due to culture and tradition. With this in mind, future research should focus on international cooperation and training of all parts of the pre-slaughter logistic chain in order to secure more uniform interpretation of fitness for transport across international borders.

5. Conclusion

This study focused on lameness scoring and assessment of fitness for

transport of cull dairy cows. Assessment of fitness is a central part of the pre-slaughter logistic chain. By use of an online questionnaire consisting of video recordings, we compared lameness scoring and assessment of fitness for transport in dairy cows among three groups of professionals – farmers, veterinarians and livestock drivers. The levels of agreement within and between the three groups were at best moderate. Farmers agreed less than moderately both within their own group and compared to livestock drivers and veterinarians when assessing fitness for transport. If the present results are confirmed by larger studies involving more systematically sampled participants, such moderate levels of agreement call for further research, development and training focusing on improving the definition and assessment of fitness for transport across relevant professional groups.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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