



Effects of percutaneous needle liver biopsy on dairy cow behaviour

L. Mølgaard^a, B.M. Damgaard^b, V. Bjerre-Harpøth^b, M.S. Herskin^{b,*}

^a Department of Biological Sciences, Aarhus University, DK-8000 Aarhus C, Denmark

^b Department of Animal Science, Aarhus University, AU-FOULUM, DK-8830 Tjele, Denmark

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ABSTRACT

In cattle, percutaneous needle liver biopsy is used for scientific examination of liver metabolism. The impact of the biopsy procedure is, however, poorly investigated. Our aim was to examine the behaviour of dairy cows during and after liver biopsy.

Data were collected from 18 dry cows. Percutaneous needle liver biopsies (after administration of local anaesthesia (2% Procaine)) and blood samples were taken during restraining. During the control treatment, animals were restrained and blood sampled.

During the biopsy procedure, cows showed increased restlessness ($P = 0.008$), frequency of head shaking ($P = 0.016$), and decreased rumination ($P = 0.064$). After biopsies, tail pressing ($P = 0.016$) and time spent perching ($P = 0.058$) increased. Time spent upright ($P = 0.10$) and number of leg movements ($P = 0.033$) increased during the night as compared to controls. Thus, liver biopsy induced behavioural changes for up to 19 h – and particularly for behaviour previously associated with pain.

Even though the exact welfare impact of percutaneous needle liver biopsies in cows is not known, and the magnitude of the behavioural changes was limited, pain always has negative effects on animal welfare. Therefore, if the present biopsy procedure – involving several biopsy passes – is to be used, improvement of the anaesthetic protocol as well as the inclusion of analgesics should be considered.

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1. Introduction

In recent years, percutaneous needle liver biopsy has become part of the scientific examination of bovine liver metabolism (Grünberg et al., 2009; Hammon et al., 2009; van Dorland et al., 2009). A standard animal liver biopsy procedure is carried out by using a Tru-Cut biopsy needle to penetrate muscle and peritoneum before reaching the liver, from which tissue samples are collected by a specific cannula containing an obturator (Hegarty and Williams, 1984). However, consequences of the procedure in terms of pain are poorly investigated. Veenhuizen et al. (1991) described that some cows decreased their feed intake after liver biopsies, but investigations of the consequences of biopsy have not been reported. It is, therefore, unknown whether the procedure causes pain or discomfort, thereby potentially influencing the welfare of experimental cows and potentially the outcome of the scientific studies.

Liver biopsy is used for scientific examination of liver metabolism in other animals, for example horses (Pearce et al., 1997; Johns and Sweeney, 2008) and sheep (Ferreira et al., 1995). Pearce et al. (1997) reported that horses may show signs of colic after accidental sampling of gut content during the procedure. Johns and Sweeney

(2008) observed minor haemorrhage in 12% of horse liver biopsies, but did not register signs of pain. In sheep, Ferreira et al. (1995) found no effect of the liver biopsy technique on feed intake or body weight. However, to date no studies have focused on behavioural changes indicating pain in animals after liver biopsy.

Typical animal biopsy procedures involve skin (Carn, 1995) or muscle (Malau-Aduli et al., 1998). However, compared to these, liver biopsy requires deep penetration through muscles, peritoneum and viscera, thereby involving a higher risk of undesirable damage. Furthermore, liver biopsy involves the risk of injuring vital organs such as distended intestines blocking the way (Pearce et al., 1997) as well as a higher risk of bleeding from the sample area as compared to other biopsy types.

In contrast to the animal data above, human pain and possible complications after liver biopsy have been documented (e.g., McGill et al., 1990; Janes and Lindor, 1993; Eisenberg et al., 2003). In humans, the procedures and equipment used are often equivalent to those used in bovine studies (e.g., Hegarty and Williams, 1984; McGill et al., 1990; Janes and Lindor, 1993) and percutaneous needle liver biopsies are widely and routinely used (Lindor et al., 1996; Castéra et al., 1999; Eisenberg et al., 2003). Mild to severe discomfort and pain at the biopsy site and the right shoulder as well as epigastric discomfort are commonly reported by patients during and up to 24 h after biopsies – most often during the first 2–4 h, and analgesics are commonly used (Hegarty and Williams, 1984; Ferencz and Batey, 2002; Eisenberg et al., 2003).

* Corresponding author. Address: Department of Animal Science, Aarhus University, AU-FOULUM, Post Box. 50, DK-8830 Tjele, Denmark. Tel.: +45 87157945.

E-mail address: MetteS.Herskin@agrsci.dk (M.S. Herskin).

In general, the knowledge of pain and discomfort in cows is limited. The majority of the available evidence has been obtained in studies of acute somatic pain after management procedures such as dehorning or branding (Lay et al., 1992; Hesselholt et al., 1996; Faulkner and Weary, 2000). Behavioural studies have been the most common and recommended approach in order to quantify pain in cows (Sanford et al., 1986; Dobromylskyj et al., 2000; Rutherford, 2002), and examples of bovine behavioural acute pain expressions are escape behaviour, restlessness, kicking and vocalisation (Lay et al., 1992; Hesselholt et al., 1996; Herskin et al., 2003; Schwartzkopf-Genswein et al., 2005).

The existence of more persistent bovine pain has not received the same scientific attention as acute pain states. It has been suggested that persistent pain might lead to reduced responsiveness to non-painful external stimulation, decreased feed intake, changes in normal standing postures, teeth grinding, decreased grooming and increased vocalisation (Hudson et al., 2008; Siivonen et al., 2011; Rasmussen et al., 2011; Fogsgaard et al., 2012).

The aim of the present study was to assess the effects of liver biopsy on dairy cow behaviour during the procedure and in the subsequent 22 h. The behaviour of dairy cows subjected to liver biopsy and blood sampling was compared to the behaviour of blood sampled control cows. All animals received a local anaesthetic prior to biopsy. It was hypothesised that, despite the nerve block, the percutaneous needle liver biopsies led to acute pain during the procedure, and that persistent pain would be present after the effects of the nerve block had abated.

2. Materials and methods

2.1. Animals, housing and management

This study involved 18 Danish Holstein dry cows from the resident herd at Aarhus University, Department of Animal Science, AU-FOULUM, Denmark. The study was performed from August 2009 to February 2010. All cows were pregnant and 30 ± 1 days before calving (range: 22–40). Eleven of the cows had completed their first lactation, three had completed their second, and four animals had completed their third or later lactation (total range: 1–6).

The cows were maintained in groups in loose-housing pens. A few days before expected calving, cows were removed from the group and replaced by new dry cows, thereby ensuring a stable number of 24 resident cows per pen. Each pen was approximately 232 m² and equipped with concrete flooring and cubicles measuring 1.3×2.7 m with rubber mats and sawdust bedding (1 cubicle per cow). Each pen contained 24 feed bins and four water bins. The cows were fed and watered using the Insentec feeding system (Insentec, Mercknesse, The Netherlands), validated previously by Chapinal et al. (2007). The water bins were refilled automatically after each visit, and the feed bins were refilled by an automatic feeding wagon (Robot Multifeder, Cormall A/S, Sønderborg, Denmark) between 05.00 and 23.00 h. In this manner, adequate feed was provided to allow *ad libitum* intake. Artificial light was provided between 05.00 and 23.00 h and lowered between 23.00 and 05.00 h.

The cows were fed a TMR based on grass silage, maize silage and barley plus a compound feed. All procedures involving animals were approved by the Danish Animal Experiments Inspectorate in accordance with Danish Ministry of Justice Act No. 1306 (November 23, 2007).

2.2. Experimental design

The cows were divided into blocks of 2–3 based on expected calving dates and included in the experiment with intervals from 1 to 8 weeks. Within blocks, behaviour of each cow was observed

on Tuesday and Wednesday 4 weeks prior to expected calving (T4 and W4) and on Tuesday and Wednesday 3 weeks prior to expected calving (T3 and W3). On Wednesdays, the cows were restrained (head-locked) from approximately 09.30 to 10.00 h. On W3, they were subjected to percutaneous needle liver biopsy and blood sampling. On W4, the animals were blood sampled, and this treatment acted as control. The data from Tuesdays (T4 and T3) were used to examine possible effects of gestation week.

2.3. Collection of blood and liver samples

2.3.1. Blood sampling

The cows were blood sampled every second week from week -20 to week -8 prior to expected calving and every week from week -7 and until the commencement of the present experiment. Sixteen millilitres of blood were obtained by puncture of a tail vein while in a standing position and restrained by head-locking. Throughout the experiment, the procedure was performed by the same veterinarian.

2.3.2. Percutaneous needle liver biopsies

On W3, all cows were subjected to a liver biopsy while restrained by head-locking. The biopsies were sampled from the area around the right 10th intercostal space, where it crosses the line from midhumerus to the tuber coxae (Fig. 1). A percussion auscultation was firstly performed to check for distended intestines. Afterwards, a 5×5 cm area of the skin was trimmed, shaved and 70% alcohol applied, and injected with 10 ml of 2% Procaine (Proca-sel-2%, Selectavet, Weyarn, Germany). After 10 min, 70% alcohol was re-applied and a stab incision made with a pointed scalpel (0.5 cm). Through this incision, the biopsy was performed with a Trucut 20 mm diameter needle (Travenol Tru-Cut, 14 gauge $\times$ 17 mm notch; Marmon/MDTech, Gainesville, FL, USA) inserted 13–32 times (mean $\pm$ SE: 17.9 ± 1.6 ; duration: 161 ± 13 s (range: 94–278)) in order to obtain the required amount of liver tissue. The incision was clipped and sprayed with wound spray (KRUUSE Wound Plast, Karex, Jørgen Kruuse A/S, Langeskov, Denmark). During the biopsy procedure, four persons were present: the observer (always the same person), an experienced veterinarian performing the biopsies, and two technicians for sample handling and assistance. Throughout the experiment, the tasks were performed by the same persons, wearing blue overalls similar to the usual stockmen.

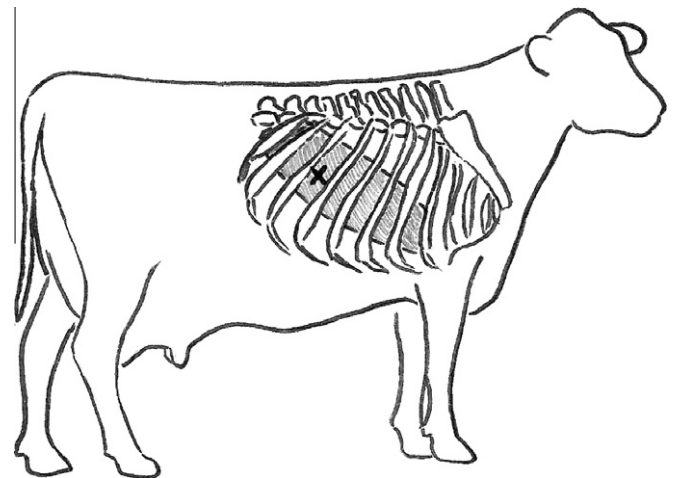


Fig. 1. Schematic drawing of bovine anatomy, showing the biopsy area. The liver biopsies were collected via the right 10th intercostal space, where it crosses the line from midhumerus to tuber coxae.

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