Impact of acute clinical mastitis on cow behaviour

Jutta Siivonen\textsuperscript{a,c,*}, Suvi Taponen\textsuperscript{b}, Mari Hovinen\textsuperscript{a,b}, Matti Pastell\textsuperscript{a,d}, B. Joop Lensink\textsuperscript{e}, Satu Pyörräla\textsuperscript{b}, Laura Hänninen\textsuperscript{a,b}

\textsuperscript{a} Research Centre for Animal Welfare, Faculty of Veterinary Medicine, University of Helsinki (HU), Finland
\textsuperscript{b} Department of Production Animal Medicine, Faculty of Veterinary Medicine, HU, Finland
\textsuperscript{c} MTT Agrifood Research Finland, Animal Production Research, H-building, FI-31600, Finland
\textsuperscript{d} Department of Agrotechnology, HU, Finland
\textsuperscript{e} Groupe ISA Lille, 59046 Lille cedex, France

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Abstract
Acute mastitis is one of the most common diseases of high-producing dairy cows. However, there is still lack of knowledge on which precise behaviours change first at the beginning of acute mastitis, and whether behavioural changes might serve as a tool for early detection of clinical mastitis. In addition, mastitis can cause motivational conflict in the behavioural priorities of a cow, and thus blur the classical patterns of sickness behaviour: cows' increased lying behaviour which may be uncomfortable or painful due to the sore udder. To study this, we monitored the behaviour of six cows after induction of acute endotoxin mastitis. Cows served as their own controls. Their behaviour was filmed from 1 day before the induction (control day) to 24 h after the induction (induction day). To follow-up on the inflammation, milk samples were taken and the cows' health status was checked at regular intervals. Mean daily durations were determined for all behaviours, except stepping and body care, for which mean daily frequencies were counted. For standing and lying behaviours, also mean bout durations were analyzed. To examine the effects of time since the induction of mastitis, data were accordingly divided in 2-h periods. We analyzed the effect of induction on overall behaviour and behavioural rhythms with linear mixed models, taking repeated observations into account. Overall cows spent less time lying on the induction day than on the day before, and less on the side of the inflamed udder quarter. Cows also spent longer time overall for eating silage during the induction day, and they also stepped more than during the previous day. Cows spent less time lying and ruminated and drank less when the udder was severely swollen and when they had high fever. We concluded that unlike in typical sickness behaviour, cows did not increase their time spent lying, but instead stood more, and avoided lying on the side of the inflamed udder quarter. We suggest that pain experienced in the udder overrides the motivational the state of the cows' sickness behaviour.

In the future, novel mastitis detection tools should therefore be able automatically detect and combine changes on both physiological parameters (i.e. fever) and complete behavioural patterns consisting of resting, standing and eating behaviours.

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1. Introduction
Changes in behaviour of sick individuals are used by veterinarians and care-takers in the diagnosis of disease (Broom, 1987). Some elements of sickness behaviour may also be useful for detecting automatically disease outbreaks.
in adult cattle, such as changes in feeding behaviour and feed intake for predicting ketosis (Gonzales et al., 2008), bovine respiratory disease (Buhman et al., 2000) or metritis in dairy cows (Urton et al., 2005; Huzzey et al., 2007). Automatic detection of mastitis is usually based on milk analysis, but methods independent of milking are needed for example to alert cows that are too sick to voluntarily visit the milking place in automatic milking (Hovinen et al., 2009).

Sickness in animals cause changes in their behavioural motivation and leads to decreased activity in exploration, body care and sexual behaviour as well as poor appetite, a well-described behavioural pattern called sickness behaviour. The sickness behaviour is a well-organised adaptive response by the animal to enhance disease resistance and facilitate recovery from disease (Johnson, 2002). However, several factors have been found to override sickness motivated behaviours, including fear (Aubert, 1999), maternal behaviour (Aubert et al., 1997) and pain (Bolles and Fanselow, 1980). Also species, affected organ, physiological state, individual pain threshold, few to mention, may have an effect on the behavioural response to sickness. Animals’ pain threshold and physiological and behavioural responses to different diseases and pain can vary depending on how severely it distorts animals’ physiological and behavioural functioning (Kemp et al., 2008).

Acute mastitis is one of the most commonly treated and expensive diseases of high-producing dairy cows. In bovine acute clinical mastitis, the affected mammary quarters become swollen, the appearance of the milk changes, body temperature of the cows increases and their appetite wanes (International Dairy Federation, 1999). In severe cases their rumen contractions slow down (Radostits et al., 2007). However, there is still a lack of knowledge on which precise behaviours change first at the beginning of acute mastitis, and whether behavioural changes might serve as a tool for early detection of clinical mastitis. In addition, mastitis can cause motivational conflict in the behavioural priorities of a cow, and thus blur the classical patterns of sickness behaviour: Cows’ strongly motivated lying behaviour (Haley et al., 2001; Jensen et al., 2005; Munksgaard et al., 2005), may be uncomfortable or painful due to the sore udder.

Our aim was to establish the behavioural changes that result from acute clinical mastitis. To study this, we monitored cows’ behaviour and clinical signs before and after experimental clinical mastitis induced by Escherichia coli endotoxin.

2. Materials and methods

This behavioural study was conducted as part of a larger experiment undertaken to examine the use of a thermal infrared camera for mastitis detection (Hovinen et al., 2009). The experimental procedures were approved by the Ethical Committee for the Use of Laboratory Animals at the University of Helsinki.

2.1. Animals, housing and feeding

The cows used in the experiments were of Finnish Ayrshire or Holstein-Friesian breeds. Five cows were at their first lactation and one at her second. Milk yield was 26.7 kg/d (SD 6.9 kg). They were in late lactation (190 ± 34 days in milk, range from 155 to 249 days) and were housed in a stanchion barn and fed high quality silage ad libitum, and concentrates six times a day. The cows were milked twice a day at 05:30 and 17:30. The lights were on between 05:00 and 20:00 and a dim night-light was provided in addition to the natural light coming from the windows.

Health status of the cows was examined before the experiment and all were found clinically healthy. All quarters were free from bacteria 1 week and 1 day before the challenge. All udder quarters were sampled for milk somatic cell counts (SCC) 194, 26, and 14 h before the experiment. The mean SCC of all quarters of the cows was less than 250,000 cells/mL. For the experimental quarters the SCC was less than 100,000 cells/mL.

2.2. Induction of experimental mastitis

On day −1 (control day) at 07:00, 5 ml of pyrogen-free saline was infused into the left fore quarters. On the following day (day 1, challenge day), the same quarters were infused with 10 μg of E. coli O55:B5 lipopolysaccharide (LPS) (Sigma®, Sigma-Aldrich, Inc., Missouri, USA) diluted in 5 ml of NaCl. Right fore quarters served as controls.

2.3. Sampling and follow-up of clinical signs

Milk samples were taken from the experimental and control quarters and cows examined clinically −2, 2, 4, 6, 8, 10, 12, and 24 h after LPS or saline treatment. Milk SCC, electrical conductivity and N-acetyl-β-D-glucosaminidase (NAGase) activity were determined from the milk samples.

In clinical examination cows’ rectal temperature (°C) was determined and the local signs of the udder were palpated. Local udder signs were defined with a subjective scale from normal to severe (0–2), in which udder was as normal (0), if it was not swollen; mild to moderate (1), if it was slightly swollen; and severe (2), if the udder was painful and very swollen.

Electrical conductivity was measured with a handheld meter (Lutron CD-4301). SCC was analyzed with an electronic counter DCC (DeLaval International AB, Tumba, Sweden) and milk NAGase activity was measured using a fluorometric method (Mattila, 1985).

2.4. Behaviour

Cameras were installed above the cows on the barn ceiling; two cameras filmed behaviour of two cows from the rear and two cameras from the front so that totally four cameras per two cows were installed. Twelve cameras in total were connected to a multiplexer (Multivision, Duplex, MV16i, Robot, US) and one VHS (Panasonic 6070). Cow behaviour was filmed continuously for 48 h with a 12 h mode, starting 24 h before induction of mastitis.

Cow body postures were scored either standing or lying. To establish if cows avoided lying on the affected udder quarter, the side of the cows on which they were lying was registered. In addition, we registered eating, drinking and ruminating of the cows. Behaviours were scored contin-
3. Results

All cows developed clinical mastitis showing both systemic and local signs after the LPS challenge. Rectal and udder temperatures increased and the udder quarters became swollen. The udders of cows were visibly swollen 2 to 4 h after the induction, and did not return to normal during the experimental period. Rectal temperatures from 2 to 4 h after the induction, and did not return to normal values at 6 h. Body temperatures remained above 39.2°C from 4 to 6 h post challenge (PC) and remained above 39.2°C from 6 to 10 h PC, reaching peak values at 10 h PC and milk NAGase activity at 8 h PC and remained within 12 h post challenge (Fig. 1).

3.1. Clinical signs

All the statistical analyses were conducted with the PASW Statistics 18.0.1 (IBM Acquires SPSS Inc. 2009). A paired t-test was used to study the effects of LPS challenge on mean daily durations, bout durations, and frequencies expressed as percentages from total durations were determined for all behaviours. Also mean durations were counted similarly. To examine the effects of time since induction, the 2-h means were analyzed using the model incorporated the 2-h time periods as fixed effects. Cow was a random factor and no covariates were used.

2.5. Statistics

Detailed descriptions of the observed behaviours are shown in Table 1. All cows developed clinical mastitis showing both systemic and local signs after the LPS challenge. Rectal and udder temperatures increased and the udder quarters became swollen. The udders of cows were visibly swollen 2 to 4 h after the induction, and did not return to normal during the experimental period. Rectal temperatures from 2 to 4 h after the induction, and did not return to normal values at 6 h. Body temperatures remained above 39.2°C from 4 to 6 h post challenge (PC) and remained above 39.2°C from 6 to 10 h PC, reaching peak values at 10 h PC and milk NAGase activity at 8 h PC and remained within 12 h post challenge (Fig. 1).

Table 1

<table>
<thead>
<tr>
<th>Behavioural variables</th>
<th>Total daily frequency (N:o)</th>
<th>Total daily duration (min)</th>
<th>Total daily duration (% from total observations)</th>
<th>Total daily bout duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control day</td>
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</tr>
<tr>
<td>Standing</td>
<td>23.33 ± 1.33</td>
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<td>638.61 ± 39.82</td>
<td>759.59 ± 56.52</td>
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<td>Lying down</td>
<td>23.33 ± 1.33</td>
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<td>800.40 ± 39.79</td>
<td>670.30 ± 56.51</td>
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<td>Lying down on the control quarter</td>
<td>10.33 ± 1.15</td>
<td>8.5 ± 1.54</td>
<td>381.30 ± 42.36</td>
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<td>Lying down on the induced quarter</td>
<td>11.17 ± 0.87</td>
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<tr>
<td>Eating silage</td>
<td>73.33 ± 4.01</td>
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<td>221.57 ± 9.34</td>
<td>252.23 ± 9.82</td>
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<td>Eating concentrate</td>
<td>25.50 ± 2.78</td>
<td>19.17 ± 1.99</td>
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<tr>
<td>Drinking</td>
<td>20.50 ± 2.17</td>
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<td>13.92 ± 1.58</td>
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<td>Ruminating</td>
<td>20.83 ± 0.95</td>
<td>18.00 ± 0.86</td>
<td>415.55 ± 30.00</td>
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<tr>
<td>Body care</td>
<td>38.33 ± 12.34</td>
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<td>1160 ± 86</td>
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Table 2

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higher than in the control quarters during the following 24 h. Clinical and milk-related changes of the cows have been reported earlier in detail by Hovinen et al. (2008).

3.2. Behaviour

On the induction day cows exhibited more stepping behaviour ($p = 0.02$), tended to spend less time lying than on the control day before induction, and less time lying on the side of the affected udder quarter ($p < 0.07$, for both). Cows also tended to stand longer on the induction day than on the control day and the standing bouts were longer ($p < 0.07$). Cows spent longer time eating silage during the induction day ($p < 0.05$) and they also elevated their stepping frequency. The mean daily behaviours are shown in Table 2.

3.3. Daily rhythm

Statistically significant ($p < 0.05$) interactions between the time of the day and day of the experiment were found for the mean hourly durations of lying, ruminating and drinking water (Figs. 2–4). Mastitis had a strong effect on the lying rhythm. The lying time returned to normal 20 h PC. The mean time spent ruminating decreased between 4 and 8 h PC (at 12:00 and 16:00 PM) compared with the control day. Cows also tended to reduce their drinking between 4 and 6 h PC (from 10:00 to 12:00 AM) compared with the control day. Furthermore, cows increased their drinking at 10 h PC (at 16:00 PM).

4. Discussion

Cows suffering from endotoxin-induced acute mastitis showed some typical features of sickness behaviour i.e. by showing poorer appetite. When the clinical signs were at the strongest, the cows were spending more time eating but less time for ruminating and drinking. In contrast to typical sickness behaviour, cows did not spend more time lying, but stood more, and avoided lying on the side of the sick quarter. Moreover, in contrast with earlier reported sickness behaviour, cows exhibited increased stepping.

The cows increased their standing time simultaneously with the local swelling of the udder quarter and the high fever. The decreased lying is in contrast to the typical finding in sick animals (Dantzer, 2001). Probably sick cows tried to cope with the discomfort or pain due to the swollen udder and may have compromised their need to lie down with their sick udder against the stall flooring. We suggest that pain experienced in the udder overrode the motivational state of animals’ sickness behaviour, as suggested by Aubert (1999). The extended standing after LPS challenge is in contrast with behaviour of calves, which tended to lie down after a LPS challenge (Borderas et al., 2008). Different experimental settings may explain this difference, as the calves were challenged intraperitoneally, while cows in the present study were infused via the teat.

Cows increased their stepping during the day they suffered from mastitis. Cows may have a need to avoid discomfort and thus represent pain avoidance behaviour as suggested by Molony and Kent (1997). An altered stance has been earlier shown in cows suffering from a spontaneous mastitis (Kemp et al., 2008). The increased standing and stepping may, however, be also sign of an increased vigilance, which helps the cows to detect a given stimulus at a given time (Dimond and Lazarus, 1974). Increased vigilance has also been observed in lactating mice during a LPS challenge (Aubert et al., 1997).

In our study, cows with acute mastitis adjusted their eating behaviour by spending more time eating, especially silage. Cows ate longer most likely due to fever and ill-health, but we did not observe the typical sickness behaviour associated with poor appetite (Dantzer, 2001). Poor appetite of a sick individual has been interpreted as a means of reducing ingestion of some micronutrients necessary for pathogen multiplication (Murray and Murray, 1979). However, cows are ruminants, and their sickness-related eating behaviour could differ from that of a single-stomach animal. Similar behaviour was recorded in previous studies of ketonemic cows, which also adjusted feeding time and feeding rate due to this metabolic dis-
order, but ate faster and consumed the same amount of feed in a shorter time (Gonzales et al., 2008). Metabolic disturbances are difficult to compare with inflammatory conditions such as mastitis, which can be considered as painful. Assessing the usability of sickness behaviours in early detection of dairy cow diseases requires understanding a broad range of physiological and psychological factors that affect an animal’s motivational hierarchy in relation to sickness behaviour.

The reduced lying time of the cows lasted until 20 h PC, i.e. during the acute phase of mastitis. Milk SCC and NAGase remained elevated after the 24-h observation period, but this was no more reflected in the observed behaviour of the cows. However, a lowered mechanical threshold to pain was reported in cows with mild mastitis (Kemp et al., 2008), thus in general more research on this is needed.

High fever probably caused reduced rumination time here, as reduced rumen contractions are typical signs of
clinical mastitis (Radostits et al., 2007). Furthermore, the feverish animals drank less. Fever has a high metabolic cost, but it plays a critical role in recovery from infections. One function of the sickness behaviour has been shown to be saving energy and minimizing thermal loss to allow full development of fever (Aubert, 1999; Dantzer, 2001). Here cows compensated their loss of water by drinking more during the first hours after recovery from fever.

The results reveal that both behavioural and clinical signs aroused rather simultaneously along the disease outbreak being most common from 4 h after the induction. In the future, novel mastitis detection tools should therefore be able automatically detect and combine changes on both physiological parameters (i.e. fever) and complete behavioural patterns consisting of resting, standing and eating behaviours.

The results provided evidence that the onset of clinical mastitis causes changes in a cow’s motivation to exhibit complete sickness behavioural patterns. Although pain was not directly assessed, the changes in behaviour could indicate that discomfort in udder caused prolonged standing. Decreased resting behaviour might have an impact on cows’ recovery process and therefore the consequences of changed behaviour for cows recovery needs to be further studied.

5. Conclusion

Cows with endotoxin-induced acute mastitis showed clinical signs and behavioural changes simultaneously along the development of the clinical symptoms. Sick cows adapted to affected and swollen udder quarter and increased body temperature by spending less time lying, ruminating and drinking, and by eating slowly. This can be an indication that sick cows change their behavioural priorities to adapt with pain or discomfort. More studies are needed on detecting changed behavioural patterns in sick individuals.

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