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An assessment of the impact of rumenocentesis on pain and stress in cattle and the effect of local anaesthesia

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ABSTRACT

Rumenocentesis is commonly used to collect rumen fluid to screen for acidosis. This study was designed to investigate whether rumenocentesis induced pain and stress and, if so, whether local anaesthesia could limit this. Twenty-four dairy cows were assigned to one of three treatments: (1) rumenocentesis with local anaesthesia (AR); (2) rumenocentesis without local anaesthesia (R); and (3) local anaesthesia only (A). Treatments were performed in a restraining cage. The cows were placed in the cage on three consecutive days and anaesthesia and/or rumenocentesis was performed on the second day. Blood samples for cortisol determination and heart rate were recorded from 0.25 h before treatment until 4 h after. Behaviour was noted while the cows were caged. Feed intake and milk production were measured the week before treatment, on the day of treatment, and the day after.

With all three treatments, cortisol concentrations and heart rate were increased while cows were in the cage. Cortisol, cardiac and behavioural responses were not significantly higher in the R and AR treatments than the A group. Cortisol concentrations and heart rate did not change between days. Feed intake and milk production were unaffected by the treatments. It was concluded that rumenocentesis does not appear more stressful than local anaesthesia or handling.

Introduction

Rumen pH is the most commonly used parameter for detecting subacute ruminal acidosis (SARA), which is a major metabolic disorder in intensive dairy farm herds. Rumenocentesis (transcutaneous puncture of one of the ruminal sacs) has become established as an efficient large-scale diagnostic test for detecting SARA in samples of more than 100 cows (Morgante et al., 2007; O'Grady et al., 2008) and avoids the sample contamination by saliva which can occur when rumen fluid is sampled via the oesophagus (Nordlund, 2003). Punctures can be made over the dorsal or below the ventral sac although in both cases the aim is to collect liquid from the ventral sac. The site of puncture may result in differences in the site of aspiration, but this is of minor importance as Martin et al. (1999) demonstrated that pH is lower in the dorsal sac than the ventral sac by only 0.15 and that such a difference is not affected by diet.

As rumenocentesis is an invasive method, it is likely to induce pain. Although Kleen et al. (2004) reported that some animals showed behavioural resistance to rumenocentesis and that this could be reduced by local anaesthesia they only made visual observations. Using behavioural and physiological measurements, the present study examined whether rumenocentesis, with or without local anaesthesia, induced stress, and compared these effects with the injection of local anaesthesia alone. Additionally, as pain experienced in specific circumstances can induce a stress response when the animals are re-exposed to those circumstances (Boissy and Bouissou, 1994), we also evaluated the impact of re-exposing the cattle to the restraining system alone.

Materials and methods

The experimental protocol was approved by the Regional Ethics Committee for Experiments on Animals (reference CE3-08).

Animals and study design

Twenty-four 4 year-old lactating Holstein dairy cows were continuously housed and tethered in stalls to allow repeated blood samplings. They received a diet based on maize silage (36%), grass silage (24%) and concentrate (40%). Three treatments were compared: (1) local anaesthesia (A); (2) rumenocentesis (R); and (3) rumenocentesis after local anaesthesia (AR). The 24 cows were divided into four batches of six. Within a batch, the three treatments were applied on the same day, and each treatment was applied on two cows. Batches were started at the rate of one per week over four consecutive weeks, and each cow was monitored for 11 days.

Cows were allowed to become accustomed to the experimental conditions for the first 8 days and measurements were performed for the next 3 days, i.e. D0, D+1 and D+2 (Fig. 1). On D−4 a catheter was inserted into the jugular vein, on

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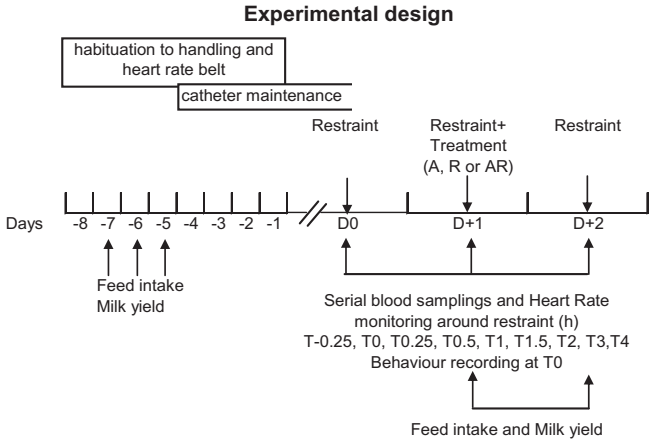

Fig. 1. Experimental protocol for investigating the effects of handling and restraint on D0, of treatment on D+1 and of memories of treatment on D+2.

D−3 to D−1 the catheter was flushed daily with 1 mL of heparin. On D0, D+1 and D+2, the cows were individually led to a restraining cage where they were held for 5 min in the presence of two technicians and one observer before they were led back to their stalls. The treatments were applied on D+1 with the cows in the restraining cage. The cows were always led to the restraining cage in the same order.

Treatments

The site for puncture was the left sub-lumbar fossa over the dorsal sac of the rumen. The site was shaved and disinfected with an iodine solution (Alcyon). For treatments A and AR, 5 mL of 2% lidocaine (Alcyon) were injected subcutaneously around the rumenocentesis site. The effectiveness of anaesthesia was established by light tapping around the site 80 s after the injection of the local anaesthetic. Rumenocentesis was then undertaken in AR cows by inserting a 4 mm diameter needle into the dorsal ruminal sac prior to liquid aspiration. For cows in treatment R, light tapping around the site was used prior to rumenocentesis to help prevent cows from being surprised by the procedure.

Blood sampling and cortisol assay

Blood sampling was undertaken remotely using a 3 m polypropylene tube (Folioplast) connected to the jugular catheter. On D0, D+1 and D+2, 5 mL of blood were taken into EDTA tubes (Sarstedt). On D+1 a pre-treatment sample was taken 15 min before the cow was restrained and a second sample taken on arrival in the cage (T0), with further samples collected when the animal was back in the stall 0.25, 0.5, 1, 1.5, 2, 3 and 4 h later. The samples were centrifuged at 3000 *g* for 5 min and at 4 °C, and the plasma stored at −20 °C until analysis.

Plasma cortisol concentrations were determined by radioimmunoassay (Boissy and Bouissou, 1994) with an antibody produced in rabbits by Cognié and Poulin (INRA Tours). The detection limit was 0.02 ng/mL. Intra- and inter-assay coefficients of variation (CV) were 19.6% and 8.1% for low (2 ng/mL) and 4.3% and 8.2% for high (32 ng/mL) controls, respectively.

Integrated cortisol response was calculated as the area under the cortisol curve during the period after treatment (or handling) when cortisol concentrations were greater than the pre-treatment (or pre-handling) value (Mellor and Murray, 1989). This period was set at 1 h for this analysis as by then plasma cortisol concentrations had recovered to initial levels in most animals.

Heart rate recordings

Heart rate was recorded using a heart rate monitoring system (Polar) attached by a 7 cm-wide thoracic belt. On D0, D+1 and D+2, the heart rate monitoring system was attached to all cows from 1 h before the first cow was sampled until 4 h after treatment on D+1 or the equivalent time on D0 and D+2. Heart rate was monitored continuously and recorded every 5 s. The mean heart rate was calculated for the pre-treatment period (30 min after belt attachment and the first blood sample on the first cow, when no staff were present in the barn), while the cow was in the restraining cage, and for each subsequent interval between successive blood samplings.

Measures of behaviour feed intake and milk yield

Cow behaviour was monitored by direct observation on caged cows, recording three head positions combined with three ear positions, namely, head horizontal, head held low, and head diagonal (i.e. an intermediate position between horizontal and low); ears backward, ears forward, and ears in an intermediate position. Time spent in each head-plus-ears combination and the numbers of vocalizations were recorded. Individual feed intake and milk yield were measured on three consecutive days before treatment (D−7, D−6, D−5), then on D+1 and D+2. Dry matter content of the diet was determined by oven drying at 103 °C for 24 h. The rumenocentesis site and health status of all cows were carefully observed following treatment.

Statistical analysis

Statistical analyses were performed using the PROC MIXED procedure for repeated measures of the SAS software suite (Littell et al., 1998), with animal as a random effect. Effect of batch was not found to be significant ($P > 0.05$) so was removed from the models. The effect of handling on cortisol concentrations and heart rate and their change following treatment were analysed with treatment, time, and day as fixed effects, with pre-treatment measures as a covariate for the change after treatment. For these measures during restraint, fixed effects were treatment and day, with pre-treatment measures as a covariate. This was also the case for integrated cortisol response, behavioural variables, dry matter intake and milk yield. When significant differences were detected ($P < 0.05$), differences between means were tested using the Tukey–Kramer multiple comparison test.

Results

Cortisol, heart rate and behavioural responses to handling and in the restraining cage

Mean cortisol concentrations were higher in restrained cows than during the pre-treatment time; at T0 and T0.25 mean cortisol was 10.5 ± 1.2 and 14.5 ± 1.2 ng/mL, respectively while pre-treatment mean cortisol was 7.1 ± 1.2 ng/mL ($P < 0.0001$). There was also a significant increase in heart rate from 78.7 beats per minute (bpm) at T-0.25 to 94.7 bpm at T0 ($P < 0.0001$). In restrained cows, there was no effect of day or treatment on cortisol concentration or heart rate ($P = 0.41$ and $P = 0.92$ for day and treatment on cortisol concentration, respectively, and $P = 0.16$ and $P = 0.60$ for day and treatment on heart rate, respectively, Table 1). Cows with higher pre-treatment heart rates had higher post treatment heart rates ($P < 0.001$).

Only five cows vocalized in the cage, and there was no significant effect of treatment ($P = 0.45$) or day ($P = 0.86$). Treatment had no effect on head–ear position ($P > 0.05$), but there was a day effect. Cows spent more time with head down and ears backward and less time with head horizontal and ears in the intermediate or forward position on D0 than on D+1 or D+2, while cows spent more time with head diagonal and ears backward on D+2 than on D0 and D+1 (Table 2).

Cortisol and heart rate responses after the in-cage restraint

There was a significant interaction ($P = 0.012$) between treatment and day on cortisol response (Table 1), with the same treatment ranking on D0 and D+1 (A < R < AR) but a different ranking on D+2 (R < AR < A). In the AR treatment (Fig. 2), plasma cortisol increased (but not significantly) between D0 and D+1 (8.5 ± 1.3 ng/mL vs. 12.7 ± 1.3 ng/mL, $P = 0.09$) then decreased from D+1 to D+2 (12.7 ng/mL vs. 7.6 ng/mL, $P = 0.021$). Cortisol concentrations following treatment showed no relationship to pre-treatment values (data not shown) but were related to the time of sampling during the day ($P < 0.0001$). All days showed two peaks of plasma cortisol concentration, a first peak 15 min after restraint (T0.25, 14.5 ± 1.5 ng/mL) and a second peak 2 h later (T120, 13.2 ± 2.3 ng/mL). Integrated cortisol response showed no significant difference between treatments and days ($P = 0.89$ and $P = 0.41$, respectively).

Heart rate varied with pre-treatment levels and between days (Table 1). Heart rate was lower on D+1 (78.1 ± 0.9 bpm) and D+2 (78.6 ± 0.9 bpm) than on D0 (80.7 ± 0.9 bpm, $P < 0.0005$). The day effect varied with treatment ($P = 0.006$). In treatment A, heart rate was not different between D0 and D+1 but decreased significantly