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Fluctuation of neutrophil counts around parturition in Holstein dairy cows with and without retained placenta

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ABSTRACT

Retained placenta (RP) is often diagnosed in high-yielding dairy cows and can negatively affect reproductive performances. The objective of the present study was to investigate the hematological and biochemical profile of cows with RP before and immediately after parturition, with particular emphasis on neutrophil counts, since a previous study demonstrated the presence of peripheral neutropenia in dairy cows with RP sampled a few days after parturition. Results from 12 Holstein cows affected by RP and from 17 clinically healthy controls sampled one week pre-partum, within 12 h after calving and between 48 and 72 h after parturition were compared between groups and over time. Compared with controls, cows with RP had lower lymphocyte counts before parturition, lower leukocyte and neutrophil counts at parturition, lower monocyte counts at all times, and higher β-hydroxybutyrate before and after parturition. Erythroid and biochemical parameters were similar over time in both groups, whereas RP cows did not show the increase of neutrophil counts that occurs in controls at parturition. Hence, the finding of a lower neutrophil count in a routinely hemogram performed at parturition could be used as an alarm signal suggesting to monitor the affected animals. Moreover, although the underlying pathogenetic mechanism should be better investigated, the present study describes for the first time the association between altered blood leukocyte concentrations at parturition in RP compared to control cows.

1. Introduction

The normal release of the placenta in cows is a multifactorial process due to a combination of hormonal, metabolic and immunological factors (Beagley et al., 2010). In particular, the activation of an innate immune response in the endometrium, mediated by the release of pro-inflammatory cytokines and chemokines, seems to contribute to the dissolution of the collagen link at the cotyledon-caruncle interface (Beagley et al., 2010; Davies et al., 2004). The alteration of anyone of the factors involved in the normal release may interfere with the whole process, leading to the occurrence of retained placenta (RP), a condition frequently observed in high-yielding dairy cows, that has been proven to cause negative effects on productive and reproductive performances (Dubuc et al., 2010; Kelton et al., 1998; Laven and Peters, 1996). In dairy cows, the negative energy balance (NEB) experienced during the transition period induces the activation of lipolytic metabolic pathways that are reflected by the increase of non-esterified fatty acids (NEFA) and ketone bodies in blood (Esposito et al., 2014). These molecules are responsible for direct and indirect effects on liver and immune cell functions that may determine on one side an inflammatory state and, on the other side, a suppression of immune responsiveness. Both these factors have been finally proven to increase the predisposition to production diseases as milk fever, endometritis, ketosis, displaced abomasum and retained placenta (Drackley, 1999; Sordillo and Mavangira, 2014). Several studies have shown that the decrease of neutrophil functions that characterizes the transition period is associated with or may predispose to the occurrence of RP (Gunnink, 1984a, 1984b; Kimura et al., 2002). Moreover, in a previous study we have found that cows with RP and without evidence of metabolic abnormalities and inflammatory conditions have lower circulating neutrophils counts soon after parturition compared with cows that do not experienced RP (Moretti et al., 2015a). However, in the cited study, hematological analyses were performed 3 ± 1 days after parturition, when RP had just occurred, and it was thus not possible to clearly determine if the neutropenia was an early consequence of RP or a predisposing factor for its development. The objective of the present study was to examine, through sequential blood samplings collected before and after parturition, the temporal dynamics of hematological

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and biochemical parameters around parturition in cows with and without RP, in order to better clarify the possible relationship between hematological and biochemical changes and the occurrence of this disease.

2. Material and methods

2.1. Study design, herds and groups

A prospective study was carried out on 4 intensive Holstein dairy farms located in the Po valley (Italy) from November 2013 to December 2014. The herds were composed of 270, 300, 700, and 300 animals, respectively, with 150, 130, 250 and 150 milking cows each. Herds were also characterized by mean days in milk (DIM) of 320, 180, 297 and 220 days, respectively, and a normalized production (average production adjusted for 305 days) of 9500, 10,500, 11,360 and 8900 kg, respectively. All the cows were fed with a TMR (total mixed ration). Milking was performed twice a day, at 12 h intervals. A cow was judged to have a RP when the placental membranes had been retained for at least 24 h after parturition, in agreement with Fourichon et al. (2000), in order to have the certainty to exclude doubtful cases. All cows were monitored for the occurrence of vaginal discharge in the following 30 days. The electronic database of each farm was searched in order to retrieve information concerning health and management (clinical diseases, treatments, production, and days in milk) covering the study period.

Cows with RP and without other pathological conditions within the following 30 DIM were assigned to the RP group (n = 12) whereas 17 cows, randomly selected within the cows with a normal parturition course, with fetal membranes released within 12 h (Peter, 2013) and without other pathological conditions in the following 30 DIM, were assigned to the control group (CTRL).

All the analyses performed in the present study were part of the routine laboratory panel for peri-partum monitoring so, according to the guidelines of our Institution, a formal approval from the Ethic Committee was not required.

2.2. Blood sampling

Peripheral blood samples from the coccygeal vein were collected in EDTA tubes (Venosafe plastic tubes for hematology, Terumo, Europe) and in plain tubes (Venosafe plastic tubes for serum, Terumo, Europe) 7 to 2 days before parturition (T0), within 12 h after calving (T1) and between 48 and 72 h after parturition (T2). Both the samples in EDTA and those in plain tubes were immediately placed at 4 °C and submitted to the Central Laboratory of the Veterinary Teaching Hospital of the University of Milan where routine hematology was immediately performed as described below. Samples in tubes without anticoagulant were allowed to clot at room temperature for 30 min and then centrifuged at 2500 g for 10 min. Harvested sera were then frozen at −80 °C for a maximum of 3 months before biochemical tests were performed.

2.3. Hematology

Routine hematology was performed upon arrival at the laboratory after careful mixing of blood into the tubes, using an automated laser hematology analyzer (ADVIA 120 with multispecies software for veterinary use, Siemens Healthcare Diagnostics, Milan, Italy). The following variables generated by the instrument were recorded: hemoglobin (Hb) concentration, hematocrit (Ht), erythrocyte (RBC) counts, total white blood cell (WBC) counts, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean cellular volume (MCV), and platelet (PLT) counts. The leukocyte differential provided by the instrument was checked microscopically on blood smears stained with a modified Romanowsky rapid stain (Dif-stain kit, Titolchimica S.P.A., Rovigo, Italy). The number of each leukocyte population was then calculated based on the total number of WBC and on the percentage of each cell population.

2.4. Clinical chemistry

In order to obtain information on biochemical analytes that may be associated with RP, routine biochemical analyses were run on serum with an automated spectrophotometer (ILAB300 plus, Instrumentation Laboratory S.P.A., Milan, Italy) using reagents provided by the manufacturer of the instrument, except when otherwise specified. The following analytes were measured: β-hydroxybutyrate (BOHB, D-3-Hydroxybutyrate dehydrogenase method, Randox Laboratories Ltd., Crumlin, Co. Antrium, UK), calcium (orthocresoftaleine method), creatinine (Jaffè method), glucose (GOD-POD method), non-esterified fatty acid (NEFA, ACS-ACOD method, Randox Laboratories Ltd., Crumlin, Co. Antrium, UK), phosphate (phosphomolibdate method), and total proteins (biuret method).

2.5. Statistical analysis

Within each sampling time (T0, T1 and T2) results from cows affected by RP and from CTRL group were compared using a non-parametric *t*-test for independent samples (Mann-Whitney *U* test) since data did not have a normal distribution, based on a Kolmogorov-Smirnov test. Within the two groups (RP and CTRL) results were compared over time with a non-parametric ANOVA for paired samples (Friedman test) followed by a Wilcoxon signed rank test, when a significant difference was found. Statistical analyses were done on an Excel (Microsoft Corp, Redmond, WA, USA) spreadsheet using the Analyse-it software (Analyse-it Software Ltd., Leeds, UK) with P value set at 0.05 for all calculations.

3. Results

3.1. Characteristics of the study population

A total of 111 cows were initially sampled during the study period (13, 46, 36 and 16 animals from herd A, B, C and D respectively). The incidence of RP among the sampled animals was 0% (0/13), 13% (6/46), 5.5% (2/36), and 25% (4/16) in herd A, B, C, and D respectively. No twins occurred. All the animals with RP were included in this study. Conversely, among the 99 cows that had normal parturition over the study period, only the 17 animals (3 form herd A and 14 from herd C) on which it was possible to collect and to properly process the complete sequence of samples (T0, T1 and T2) were included in the study. No significant differences between groups were found in terms of age (median age in the CTRL group = 3 years, min-max range = 2–7 years; median age in the RP group = 4 years; min-max range = 2–8 years) or in terms of numbers of lactation (median number in the CTRL group = 3, min-max range 1–7; median number in the RP group = 3, min-max range 1–5).

Fig. 1. Hematological parameters that were significantly different over time (T0 = 2 to 7 days before parturition; T1 = within 12 h after calving; T2 = between 48 and 72 h after parturition) or between groups (CTRL = controls; RP = cows with retained placenta). Results of CTRL groups are indicated by white boxes, while results from RP cows are indicated by the grey boxes. The boxes indicate the I–III interquartile interval, the horizontal line corresponds to the median, vertical lines are the limits of outlier distribution according to the Tukey rule. Near outliers are indicated by the symbols "x" and far outliers with asterisks outside the boxes. The shaded grey area indicates the reference interval adopted in our laboratory for dairy cows at 3 ± 1 days in milk. Bolded symbols within boxes indicated significant differences as follows: significant differences compared with T0 within the same group are expressed as *($P < 0.05$), **($P < 0.01$), and ***($P \le 0.001$); significant differences compared with T1 within the same group are expressed as †($P < 0.05$), ††($P < 0.01$), and †††($P \le 0.001$); significant differences in the RP group compared with the same time sampling of CTRL cows are expressed as ‡ ($P < 0.05$), ‡‡ ($P < 0.01$), and ‡‡‡ ($P \le 0.001$).