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Hematology reference intervals for neonatal Holstein calves

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ABSTRACT

Data regarding hematologic reference intervals (RI) for neonatal calves have not been published yet. The aims of this study were: a) to establish hematology RIs for neonatal Holstein calves, b) to compare them with the RIs for lactating cows, and c) to investigate the relationship of age and gender with the hematologic profile of calves. Two-hundred and fifty-four clinically healthy Holstein calves (1–9 days old, from 30 farms) and 82 healthy Holstein cows (between 30 and 150 days in milk, from 10 farms) were blood sampled once for a complete blood count evaluation, using the ADVIA 120 hematology analyzer. An additional blood sample was collected from each calf for serum total protein concentration measurement. RIs and age-related RIs were calculated with the Reference Value Advisor freeware. Comparisons between calves and cows and between male and female calves were performed with *t*-test or Mann-Whitney test. Red blood cell count (RBC), white blood cell count (WBC), neutrophil, lymphocyte and platelet counts in calves were higher, while mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were lower than in cows. Lymphocyte and platelets showed a notable increase through age. Finally, female calves had higher RBC, hematocrit and hemoglobin concentration than males. Age-specific RIs should be used for the interpretation of the complete blood count in Holstein calves.

1. Introduction

The evaluation of the complete blood count (CBC) is, usually, the first ancillary aid for a practitioner, following clinical examination, to establish a diagnosis or to estimate a prognosis for a patient (Jones and Alison, 2007). Determining whether a parameter or a profile is within normality requires a comparison with reference intervals (RI) from a similar population of clinically healthy individuals. Therefore, RIs should be established considering differences in genotypes, age, gender, management and of course, analytical procedures (Friedrichs et al., 2012).

The hematologic profile of calves has been investigated in the past, mostly in beef breeds (Raleigh and Wallace, 1962; Adams et al., 1992; Egli and Blum, 1998). Data for dairy breeds, and especially for Holstein calves, is limited. Tennant et al. (1974) reported hematologic values in 61 Jersey and 110 Holstein-Friesian calves from birth up to 6 months of age, using older analytical techniques. Mohri et al. (2007) reported age-related changes in 32 Iranian Holstein calves, repeatedly sampled every other week from birth up to 12 weeks of age, with an automated hematology analyzer (Nihon Kohden, Cell Tac a, MEK 6108, Tokyo, Japan). Hematologic values for calves determined with the ADVIA 120 hematology analyzer, which is widely used nowadays, have been reported only for 15 Norwegian Red calves with repeated measurements from 1 week up to 6 months (weekly up to week 5 and thereafter monthly) (Brun-Hansen et al., 2006).

None of the above studies was designed to focus specifically on the hematologic profile of Holstein calves during the crucial neonatal period, which is characterized by high morbidity and mortality due to congenital conditions, complications at parturition and, mainly, infections (Mee, 2008).

Published studies that reported comparisons between female and male calves are also limited. Raleigh and Wallace (1962) observed higher HGB and packed cell volume (PCV) values for Hereford female compared to male calves, while no differences between genders were found in Jersey and Holstein-Friesian calves (Tennant et al., 1974).

The aims of this study were to: a) establish RIs for hematologic parameters of neonatal Holstein calves with the ADVIA 120 hematology analyzer, b) compare them with the RIs of adult Holstein cows, and c) determine the association of age and gender with the hematologic profile of calves.

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2. Materials and methods

2.1. Animals and inclusion criteria

The study was conducted with the approval of the review board of the Faculty of Veterinary Medicine, Aristotle University of Thessaloniki. The farmers gave informed consent for the animals to be included in the study and the testing procedures.

Two-hundred and fifty-four Holstein calves from 30 dairy farms were enrolled in the study. Calves were 1–9 days old (day 1, $n = 32$; day 2, $n = 23$; day 3, $n = 45$; day 4, $n = 37$; day 5, $n = 31$; day 6, $n = 21$; day 7, n = 31; day 8, n = 23 and day 9, $n = 11$), 109 males and 145 females. All calves were separated from their dams within 6 h after calving, housed in individual pens and fed 3–4 L of colostrum within 6 h from birth. At the age of 3 or 4 days old, all calves were transitioned from colostrum to milk replacer feeding.

Eighty-two Holstein cows from 10 of the above farms were used to establish the relevant adult cow RIs. All cows were between 30 and 150 days in milk (DIM) and about half of them ($n = 44$) were at first lactation. Cows were housed indoors, in typical two- or three-row free-stall barns and were fed total mixed rations designed to meet production requirements.

Each calf and cow were clinically examined prior to sampling to estimate whether they were healthy and therefore, eligible for inclusion in the study. Inclusion criteria for calves were alertness and vigor, normal rectal temperature (> 38 and < 39.5 °C) and absence of dehydration, diarrhea, cough and nasal or ocular discharge. Cows with clinical signs of any disease, lameness, estrous activity, current antibiotic or anti-inflammatory therapy or with a notable milk drop during the last week were excluded from the study.

2.2. Sampling & analysis

Two blood samples were drawn once from each of the 254 calves by jugular venipuncture, using 21 1/4 G disposable needles, within 1 to 2 h after the morning feeding (09.00–11.00 am). The first one was collected into 3 mL sterile vacuum plastic tubes containing K_3-EDTA as anticoagulant (Vacuette®, Greiner Bio-One International GmbH, Kremsmünster, Austria) for hematology analysis and the second one into 10 mL sterile glass vacuum tubes without anticoagulant (BD Vacutainer®; Plymouth, UK), to assess serum TP concentration. Moreover, one blood sample was collected from each of the 82 clinically healthy cows into 3 mL the sterile vacuum K_3-EDTA tubes, using 18 1/2 G disposable needles, 2–4 h after the morning feeding to establish RIs for hematology parameters in Holstein cows. All samples were placed in a cooler immediately after collection, stored at 4 °C in the Diagnostic Laboratory and analyzed within 24 h.

CBC {red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cell count (WBC), differential white blood cells percentages and counts [neutrophil (NEUT), lymphocyte (LYMP), monocyte (MONO), eosinophil (EOS), basophil (BAS) and large unstained cells (LUC)], platelet count (PLT) and mean platelet volume (MPV)} was determined with the ADVIA 120 hematology analyzer (ADVIA 120 Hematology System, Siemens Healthcare GmbH, Deerfield, USA). A visual corroboration of the CBC results and evaluation of RBC, WBC and PLT morphology was performed on air-dried Giemsa-stained (Giemsa's azur eosin methylene blue solution, Merck KGaA, Darmstadt, Germany) blood smears.

After clotting, serum was harvested by low speed centrifugation (1600*g* for 15 min) and TP concentration was determined using a desktop refractometer (ATAGO T2-NE CLINICAL, Atago Ltd., Tokyo, Japan), according to manufacturers' instructions.

2.3. Calculation of reference intervals

The RIs for neonatal calves and cows were computed with the Reference Value Advisor (v.2.1) freeware (RefValAdv), a set of macroinstructions for Microsoft Excel (Geffré et al., 2011), according to the guidelines recommended by the American Society for Veterinary Clinical Pathology (Friedrichs et al., 2012). RefValAdv output displays descriptive statistics, histograms and Q/Q plots for each variable and calculates 95% RIs with 90% confidence intervals (CI), based on normality and symmetry of data distribution, outliers and sample size.

2.4. Statistical analysis

The statistical analysis was performed using the IBM SPSS Statistics V.22 software package (SPSS Inc., Chicago, IL, USA). Hematologic parameters of calves were compared with those of cows using a *t*-test or the non-parametric Mann-Whitney, when variances between groups were equal or not, respectively. Homoscedasticity was assessed with Levene's test. Comparisons for hematologic parameters among different days of age were performed with the non-parametric Kruskal-Wallis test. Within test, pairwise comparisons were automatically calculated where a decision for rejecting the null hypothesis was made. Moreover, the association of age with the hematologic profile was assessed with a univariate regression analysis, using the RefValAdv. A continuous variable can be added as a covariate in the regression model and variable-related RIs can also be obtained, depending on data distribution and sample size. In this case, the variable “age” was added as a covariate. Our calf sample size ($n = 254$) was large enough to obtain regression-based age-related reference and confidence intervals (Virtanen et al., 1998). The values of the dependent variables were Box-Cox transformed to normalize data within each age group. Additionally, a polynomial regression model was assessed, where a non-linear relationship between days of age and a hematologic parameter was suspected. Finally, comparisons between male and female calves on each parameter were evaluated with a *t*-test or the non-parametric Mann-Whitney test.

3. Results

The calculated 95% RIs with 90% CI for hematologic parameters and serum TP concentration of the 254 neonatal Holstein calves are presented in Table 1 and the hematology RIs of the 82 Holstein cows in Table 2. Comparisons between RIs in calves and cows are graphically depicted in Figs. 1 to 3.

In comparison with cows' values, calves had lower mean MCV, MCHC and MONO (13.5%, 5.2% and 56.9%, respectively; $P < 0.001$) and higher mean RDW (8.4%; $P < 0.001$). Medians for HCT, RBC, WBC, NEUT, LYMP, PLT and MPV were higher in calves compared to cows (2.7%, 18.5%, 22.1%, 29.6%, 29.6%, 35.9% and 20.6%, respectively; $P < 0.001$). Furthermore, calves had lower MCH and LUC medians than cows (16.3% and 68.2%, respectively; $P < 0.001$). No differences on HGB, Neutrophil:Lymphocyte (N:L) ratio, EOS and BAS values were observed between calves and cows.

Medians of RBC, HCT, HGB, MCHC, RDW, NEUT, LYMP, N:L ratio, MONO, LUC, PLT and MPV differed significantly among days of age ($P \leq 0.05$). RBC, HCT and HGB increased from day 1 to day 2, decreased until day 4 and increased again until day 8. MCHC showed a moderate increase across days 1 to 9 and RDW increased from day 2 up to day 9. NEUT values decreased from day 1 up to day 4 and increased thereafter. LYMP showed an increase over time, with the exception of day 4. Mean N:L ratio at day 1 was 3.35 and decreased to 1.00 at 9 days of age; MONO and LUC levels reached a plateau at day 3. PLT count increased significantly from day 1 up to day 9 and MPV decreased throughout the sampling period. Age had a non-significant effect on HCT, N:L ratio, MONO, EOS, BAS and LUC variances.

The regression analysis showed a significant linear relationship