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Hematology and serum biochemistry of Holstein dairy calves: Age related changes and comparison with blood composition in adults

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Abstract

Specific reference intervals are needed for each animal species for appropriate interpretation of hematological and serum biochemical results. The aim of the present study was to investigate the blood composition of growing calves in order to evaluate the need for defining reference values for different age groups. Thirty two Holstein calves (18 male and 14 female) were blood sampled. A blood sample was taken within 24–48 h following birth and at 14, 28, 42, 56, 70 and 84 days of age. CBC determination and the measurements of some blood serum metabolites, enzymes, electrolytes and minerals were performed. There were significant age related changes for most hematological and biochemical parameters ($p < 0.05$) except for the numbers of band neutrophils and monocytes and the amounts of sodium, potassium, chloride and BUN. The results of the present study showed that for some hematological and biochemical parameters such as hemoglobin, MCV, MCH, MCHC, inorganic phosphorus, serum total protein, globulin, AST and ALP at the first three months of life and also, neutrophil numbers and glucose levels at the 24–48 h of life, the age specific reference values must be considered for precise interpretation of laboratory results.

Keywords: Dairy calf; Hematology; Serum biochemistry; Reference value

1. Introduction

The ability to interpret laboratory data is based on knowledge regarding the normal physiologic mechanisms underlying each laboratory test and recognition of the effects of diseases on these normal physiologic mechanisms and therefore, on the test results themselves. If performed properly, laboratory testing and interpretation of laboratory data can provide significant insights regarding diseases and therapeutic approaches (Thrall, 2004). Specific reference intervals are needed for each animal species for appropriate interpretation of hematological and serum biochemical results. Less often, a distinct reference value is needed for an analyte from a specific age or breed of animal. Many values vary with the age of the animal, with major changes occurring before puberty. Consequently, some analyte require different reference intervals for different age groups (Meyer and Harvey, 2004).

Several statistical methods exist for establishing reference ranges. The choice of method to be used partially depends on the distribution of values obtained from population sampled. With Gaussian distribution, parametric tests are appropriate for determining the reference range. Conversely, if the distribution does not form Gaussian distribution, the data are either analyzed by non-parametric statistical methods or transformed to produce a more normal distribution (Thrall, 2004).

Diseases of the newborn and neonatal mortality are a major cause of economic loss in livestock production. Thus, specific hematological and serum biochemical reference ranges could help and promote the ability of clinicians to more accurate interpretation of clinical pathology data and diagnosis of neonatal diseases. There are reports concerning the changes of hematological and serum biochemical values of dairy calves but the number of calves used in

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those studies were too low and/or different breeds and age groups of calves were used (Tennant et al., 1974; Dubreuil and Lapierre, 1997; Hugi and Blum, 1997; Egli and Blum, 1998; Knowles et al., 2000); thus the aim of the present study was to investigate the blood composition of growing calves in order to evaluate the need for defining reference values for different age-groups.

2. Materials and methods

Forty Holstein calves (20 males and 20 females) were blood sampled from birth to 84 days of age. A blood sample was taken within 24–48 h (1 = first sampling) and at 14 (2 = second sampling), 28 (3 = third sampling), 42 (4 = forth sampling), 56 (5 = fifth sampling), 70 (6 = sixth sampling), and 84 (7 = seventh sampling) days of age. The calves were kept on the three dairy farms with a similar conventional calf rearing program. All farms were under health monitoring by veterinary school of Ferdowsi university of Mashhad. The calves were observed two times daily by a veterinary technician; temperature was measured rectally and any abnormalities such as fever, anorexia, depression and soft feces were reported to the farm's veterinarian for proper handling and treatment. At the end of sampling, eight calves were removed from final statistical analysis because of fever, pneumonia, and diarrhea at one of the sampling time (±1 day). These conditions can alter leucogram and the amounts of some serum biochemical parameters such as glucose due to inflammation or stress. All remained 32 calves (18 males and 14 females; 19, 8 and 5 calves from each herd) were healthy without any abnormality during the time of study.

Blood samples were taken between 8.00 and 12.00 AM by jugular venipuncture using disposable syringes. Blood (2.5 ml) anticoagulated with disodium-EDTA was used for CBC and 7.5 ml transferred to plane tube for serum separation. All tubes were placed immediately on ice and were transferred to the laboratory. Anti-coagulated blood was used for CBC determination using automated veterinary hematology analyzer (Nihon Kohden, Cell Tac α, MEK 6108, Tokyo, Japan). Differential leukocyte count was performed microscopically on Giemsa stained blood film using cross sectional method (Jain, 1986). Plane tubes were centrifuged at 1800*g* for 10 min followed by removal of serum. Serum was stored at −20 °C until analyzed. The amounts of total serum protein (tp), albumin (alb), blood urea nitrogen (BUN), creatinine (cre), glucose (glu), magnesium (Mg), calcium (Ca), inorganic phosphorus (Pi), chloride (Cl), iron (Fe), total bilirubin (bil), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) were measured by commercial kits (Pars Azmoon, Tehran, Iran) using an autoanalyser (Biotecnica, Targa 3000, Rome, Italy). The amounts of sodium (Na) and potassium (K) were measured by a flame photometer (Jenway 6105 Clinical, Jenway LTD Felsted England). Control serum (Randox control sera, Antrim, UK) was used for controlling measurement accuracy. The concentration of globulin (glo) calculated as the difference between total serum protein and albumin.

Statistical analysis was conducted using SPSS for windows (release 9, SPSS Inc, Chicago, Ill). Age effect was examined using ANOVA. All analysis was corrected for repeated measurements and included age, sex and herd as fixed factors and calves as random factor. In addition, a paired T test was used for the comparison of sampling stages with first sampling time. For each parameter, age related changes were showed by a graph with upper and lower limits of previously reported reference value (Latimer et al., 2003; Radostits et al., 1994). Arrows in the graph indicate the direction of the upper or lower limit when out of graph scale.

3. Results

There were no interactions between age and sex and also age and herd of calves for the measured parameters.

Sampling time had a significant effect on PCV ($p < 0.001$, Fig. 1). Mean PCV showed a declining trend from 24 to 48 h to day 28 and also, an increasing trend up to day 84. There were significant differences between days 14, 28, 42, and 84 compared with the amount at 24–48 h ($p < 0.05$).

Sampling time had a significant effect on RBC ($p < 0.001$, Fig. 1), with an increasing trend throughout the study. Significant differences were seen between days 42, 56, 70, and 84 compared with the amount at 24–48 h ($p < 0.05$).

Concentrations of hemoglobin significantly decreased from birth to day 28 and then significantly increased up to 84 day ($p < 0.001$). Significant differences were observed between days 14, 28, 42, 70, and 84 compared with the concentration at 24–48 h ($p < 0.05$, Fig. 1).

Sampling time had a significant effect on MCV ($p < 0.001$, Fig. 2). MCV values were significantly decreased until day 42 and then remained stable up to day 84. MCV was consistently lower compared to the first sampling at all of the sampling times ($p < 0.001$).

Sampling time had a significant effect on MCH values ($p < 0.001$, Fig. 2). MCH amounts were significantly decreased from birth to day 42 and then increased to amounts at day 84. MCH values of days 14, 28, 42, and 56 were significantly different compared with the amount at 24–48 h ($p < 0.01$).

MCHC decreased significantly from birth to day 28 and, then increased up to day 84 , with significant differences between days 14, 28, 42, 70, and 84 compared with the amount at 24–48 h ($p < 0.01$, Fig. 2).

Sampling time had a significant effect on WBC ($p < 0.01$, Fig. 3). WBC showed a declining trend from birth to day 42, followed by an increasing trend that extended to day 84. There were significant differences between days 14, 28, and 42 compared with the amount at 24–48 h ($p < 0.05$).