Article 113

Evaluation of Anemia in Calves up to 4 Months of Age in Holstein Dairy Herds

Ali Gholi Ramin[1], Siamak Asri-Rezaei[1], Khales Paya[2], Zohre Eftekhari[3], Mahyar Jelodary[2], Hamid Akbari*[4] and Sina Ramin[5]

Occurrence of anemia in suckling dairy calves up to 4 months old with regard to age and sex susceptibility was investigated in Urmia, Iran. From 25 dairy herds, 164 calves including 97 females and 67 males were selected. Five ml jugular vein blood was collected from calves into 2 ml with EDTA and 3 ml without EDTA tubes. Blood parameters were evaluated for hematocrit, hemoglobin, erythrocytes, leucocytes, total plasma protein, iron, total iron banding capacity (TIBC), neutrophils, lymphocytes, MCV, MCH, and MCHC. Anemia was classified according to the hematocrit values, over 24%, 20-23.99%, 12-19.99% and less than 12% as normal, mild anemia, moderate anemia and severe anemia. The percentage of anemia in calves was 17.7% including 14% mild and 3.7% was moderate anemia ($P<0.05$). The percentage of moderate and mild anemia in female calves was greater than in male calves but the differences was not significant. Significant age differences ($P<0.05$) was observed at 3 months of age for moderate and up to 2 months old for mild anemia. Mean hemoglobin, erythrocytes, iron, lymphocytes, TIBC and MCV decrease by increasing the anemia and mean leucocytes, neutrophils, MCHC and protein increase by increasing the anemia level but it was significant ($P<0.05$) only for hematocrit, hemoglobin, erythrocytes and MCHC. In conclusion, calves reveal a mild anemia, which is not related to iron deficiency. Sex difference was not obvious in anemia while three months old is the critical age for moderate anemia outbreaks in Urmia dairy calves that prevention procedures must be considered.

[1]Clinical Sci., Vet. College, Urmia Univ.,
[2]Vet Student, Urmia Univ.,
[3]Asst. Internal Med., Vet. College, Tehran Univ.,
[3]Asst. Internal Med., Vet. College Urmia Univ., [5]Medical Student, Medical Sciences,

Tabriz University, Tabriz, Iran

KEYWORDS
Anemia, hematocrit, calves, sex, age, iron.

INTRODUCTION

Anemia is considered as a decrease in red blood cells (RBC), hemoglobin (Hb) and packed cell volume (PCV) in the blood stream (Jezek et al, 2009; Randhawa & Randhawa, 2010). It could also be related to a decline in the production of RBC or Hb, and a loss or destruction of erythrocytes (Welchman, 1988). It can be congenital (Kappe et al, 2009; Pardon et al, 2010; Bastian et al, 2011), or acquired after birth at any age following severe bleeding, hemolytic diseases, nutritional defects and bone marrow disorders (Dilov et al, 1981). Diagnosis is based on the observation of pale tissues, respiratory and cardiac disorders, and finally reduced growth rate, production, and reproduction performance (Ramin et al, 2000; Moosavian et al, 2010). The laboratory diagnosis depends on the evaluation of anemia indices such as PCV, Hb, RBC and total proteins (Mc Farlane, 1988; Mohri et al, 2010). Treatment includes specific and supportive therapies but due to multi-factorial etiology of the anemia, prevention and early diagnosis would be the best approach in relief of problem (Jezek et al,

2009; Moosavian et al, 2010; Radostits et al, 2007).

There are many reasons for calf anemia (Mitruka & Rawnshky, 1981; Okabe et al, 1996) but one of the major causes is iron deficiency because of the consumption of whole milk by neonates (Tennant et al, 1975). Suckling calves, kids, lambs, foals and piglets are more susceptible to anemia than adult animals because of the low amount of iron in milk (Radostits et al, 2007). Iron deficiency during the first weeks of life does not appear as there is enough iron deposit in the body, however, after birth, a discrepancy appears up to 3 months in the level of iron absorption from the dry matter or roughage nearly at the time of weaning. For this reason, the 4 months old calves were reported to have a higher serum iron level than the neonates up to 3 months of age. Another reason could be the rapid growth rate and expansion of blood volume in young animals, resulting in immediate utilization of iron rather than storage in the body (Radostits et al, 2007). Depletion of iron reserve or iron deficiency causes delay in sexual maturity and decreased growth rate, which can cause economic losses in farm profits.

The purpose of this study was to determine whether the frequency and percentage of anemia among suckling dairy calves has any relationship to their age effects and, ultimately, the specific type of anemia.

MATERIALS AND METHODS

1. Animals

A total of 164 apparently healthy suckling calves, 67 male and 97 female, aged between 1to 4 months were randomly selected from 25 Holstein dairy farms in Urmia, Iran. Number of calves from age group of 1, 2, 3 and 4 months was 22, 22, 23 and 30 and for males and 21, 17, 14 and 15 for female calves, respectively. Calves were fed whole milk two times per day until 3 months of age and at 4 months of age, they were fed milk once per day, grass and hay ad libitum.

Five ml jugular vein blood was collected from each calve into 2 ml with EDTA and 3 ml without EDTA tubes for evaluation of various blood parameters, using microhematocrit method for determining Packed cell volume; hemocytometer method for Leukocytes and Erythrocytes counts and spectrophotometer method using a commercial kit (Pars Azmon, Iran) for Total protein. Hemoglobin was determined by cyan methemoglobin (gr/dl) method. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined by automated cell counter. Sera samples were analyzed for iron (Pars Azmon, Iran) and total iron binding capacity (TIBC) with an autoanalyzer (RA-1000, USA).

Anemia in calves under study was categorized as indicated by Radostits et al, (2007) and Ramin et al (2011), on the basis of Hematocrit values. Calves having Hematocrit values of over 24%, 20-23.99%, 12-19.99% and less than11.99 % were rated as free from anemia, mildly anemic, moderately anemic and as severely anemic respectively.

2. Statistical analysis

Data were analyzed by SPSS13 statistical program, and Means±SEM were determined for the parameters under study. Case Summaries, student t-test, and Chi-Square tests were carried out to find the differences between parameters.

RESULTS

The percentage of anemia in calves under study was 17.7% (29 out of 164) including 14% mild and 3.7 % moderate anemia. The mean blood hematological parameters in male and female calves with moderate, mild anemia and without anemia are presented at Table 1. In female and male calves, moderate anemia was 0.74% and 2.96% and mild anemia was 7 % and 7 %, respectively. Student t-test showed no significant sex differences among blood parameters in different groups of anemia. Thus, after pooling the data the mean blood parameters in pooled calves with moderate, mild, without anemia and overall were also shown in Table 1.

The mean concentrations of hemoglobin, erythrocytes, iron, lymphocytes, TIBC and MCV

reduced with anemia whereas the mean leukocyte, neutrophils, MCHC and plasma protein increased (Table 1). The comparison of the mean blood parameters (t-test) in the moderate, mild and without anemia showed significant differences in PCV, Hb, RBC and MCHC which in mild anemia was statistically confirmed (X=12.5, df=1, P<0.01). The difference in plasma protein concentration was P <0.058.

Type of anemia and age distribution is indicated in Table 2. Most cases of moderate anemia were observed in calves with 3 months of age and mild anemia was observed in the first two months of age. Chi-Square test showed significant difference in the anemia of calves at different ages (X=5.76, df=3, P<0.01).

DISCUSSION

The diagnostic criterion for anemia involves recording PCV, Hb, RBC, iron and protein. PCV has a special priority among these parameters (Maach et al, 1991; Okabe et al, 1996; Völker & Rotermund, 2000). During present study 3.1% moderate anemia and 14% mild anemia were confirmed but there was no severe anemia. The reason for slight anemia could be related to insufficient iron in milk of suckling calves (Randhawa, 2010); therefore, a mild or moderate form of anemia can be expected (Maach et al, 1991). From the clinical point of view the presence of 1.3% moderate anemia together with 14% mild anemia in dairy animal breeding will certainly have an effect on the growth rate of calves, and should therefore be seriously considered (Völker & Rotermund, 2000).

The presence of moderate anemia in the 3 months old calves indicates that age has an effect in the incidence of anemia in calves (Rengifo et al, 2010). Our finding is in line with the observations of (Völker & Rotermund, 2000; Radostits et al, 2007) who reported anemia at the age of 3 to 4 months due to nutritional disorders This interpretation is suggestive of the fact that incidence of anemia in Urmia dairy calves starts at 1 or 2 months of age (Rengifo et al, 2010) and is exacerbated by the continuous use of milk with poor iron and lack of access to forage and concentrate up to weaning time at 3 months of age (Dilov et al, 1981). The anemia in this study was the subclinical form and clinical signs such as pale mucous, anorexia, weakness, respiratory and cardiac disorders, petechiae, ecchymosis, melena, jaundice and hemoglobinuria (Jezek et al, 2009; Mohri et al, 2010) were not observed.

Moderate anemia in female calves may cause delay in the growth and sexual maturity while mild anemia in male calves may not produce any significant effect. Calves can be protected from anemia by adding iron to the diet or by injection (Völker & Rotermund, 2000; Moosavian et al, 2010) or by administration of fortified milk with iron, electrolytes and vitamins (Mohri et al, 2010). This practice is widely followed in rearing suckling calves, especially in veal calves (Mc Farlane, 1988; Miltenburg et al, 1993; Volker et al, 1996).

Results of Present study showed that blood hematological indices such as Hb, RBC, MCV, MCHC and plasma proteins significantly changed following anemia. Similar findings were reported by Gosselin et al (2011). In this study, cause of anemia in the mild and moderate forms was not related to the hemorrhagic, hemolytic, chronic diseases, bone marrow factors but nutritional aspects could still be the major criteria.

In the present study, plasma protein increased with age and for calves with mild to moderate anemia was 8 and 7.14 mg/dl, respectively.

The reason for the leukocytes, neutrophil and lymphocyte evaluation was to determine the probable infectious cause of anemia (Gosselin et al, 2011), while MCV, MCH and MCHC was to determine the type of anemia (Mohri et al, 2010). As these parameters were not statistically significant, anemia in these calves was not related to infectious diseases and only MCHC increased with the increasing age of the calves, which could be a sign of hyperchromic anemia (Parsons et al, 2006).

In conclusion, 82.3% of the experimental calves were free of anemia, 14% had mild and 3.7% had moderate anemia. The fact that female calves

were affected with moderate and males with mild anemia at 3 months of age indicates that 3 months of age is critical for calf anemia. Anemia was associated with significant changes in PCV, Hb, RBC, MCV, and MCHC. Therefore, it can be said that a slight anemia occurred in Urmia industrial dairy calves at 2 and 3 months, which was not related to sex variation and iron deficiency. It is suggested to evaluate other causes of anemia such as copper, selenium, zinc and cobalt deficiencies.

REFERENCES

1. Bastian M, Holsteg M, Hanke-Robinson H, Duchow K, Cussler K. Bovine neonatal pancytopenia: Is this alloimmune syndrome caused by vaccine-induced alloreactive antibodies. Vaccine. 2011; 29:5267-75.
2. Dilov P, Antonov S, Lalov KH. Incidence of anemia in newborn calves. Vet Med Nauki. 1981; 18: 56-64.
3. Gosselin VB, Fecteau G, Nichols S. Presumptive bovine neonatal pancytopenia in a Holstein calf in québec. Canadian Veterinary Journal. 2011; 52: 788-90.
4. Ježek J, Starič J, Nemec M, Zadnik T, Klinkon M. Relationship between blood haemoglobin and serum iron concentrations and heart girth in pre-weaned dairy calves. Italian Journal of Animal Science. 2009; 8:151-3.
5. Kappe EC, Halami M, Schade B, Bauer J, Dekant W, Buitkamp J, Boettcher J, Mueller H. Fatal aplastic anaemia with haemorrhagic disease in calves in germany. Journal of comparative pathology. 2009; 141: 293-293.
6. Maach I, Grunder HD, Faio A. Hematological and hemochemical studies in black pied, clinically healthy breedind calves in Morocco. Dtsch Tierarzti Wochenschr. 1991; 98: 94-102.
7. Mc Farlane JM. Some indicators of welfare of crated veal calves on three dietary iron regimens. Journal of Animal Science. 1988; 66: 317-25.
8. Miltenburg GA, Wensing T, Breukink HJ, Marx JJ. Mucosal uptake, mucosal transfer and retention of iron in veal calves. Vet Res Commun. 1993; 7: 209-17.
9. Mitruka B, Rawnshky H. Clinical Biochemical and hematological Reference. Values in normal Experimental Animals and Normal Humans. 2nd Edn. Masson Publishing, USA, Inc. 1981; 230-231.
10. Mohri M, Poorsina S, Sedaghat R. Effects of parenteral supply of iron on RBC parameters, performance, and health in neonatal dairy calves. Biol Trace Elem Res. 2010; 136: 33-9.
11. Moosavian HR, Mohri M, Seifi HA. Effects of parenteral over-supplementation of vitamin A and iron on hematology, iron biochemistry, weight gain, and health of neonatal dairy calves. Food and Chemical Toxicology. 2010; 48: 1316-20.
12. Okabe J, Tajima S, Yamato O, Inaba M, Hagiwara S, Maede Y. Hemoglobin types, erythrocyte membrane skeleton and plasma iron concentration in calves with poikilocytosis. Journal of Veterinary Medical Science. 1996; 58: 629-34.
13. Pardon B, Steukers L, Dierick J, Ducatelle R, Saey V, Maes S, et al. Haemorrhagic diathesis in neonatal calves: An emerging syndrome in Europe. Transboundary and Emerging Diseases. 2010; 57: 135-46.
14. Parsons SDC, Penzhorn BL, Reyers F, Steyl JCA, Becker PJ. Erythrocyte morphology and haemoglobin types of neonatal roan antelopes (*Hippotragus equinus*) with hypochromic poikilocytic anaemia. J Comp Pathol. 2006; 134: 152-60.
15. Radostits OM, Gay CC, Hinchcliff KW, Constable PD. Veterinary medicine. 10th Edn, Edinburg, London. 2007; 1725-1728.
16. Ramin AG, Asri-Rezaie S, Hemati M., Eftekhari, Z, Jeloudary M., Ramin, S. Evaluation of the erythrocytes and leucocyte alterations in cows infected with Theileria annulata. Acta Vet Beograde, On press.
17. Ramin AG, Mortaz E, Harighi N. The assessment of physiological responses of anemic cows to blood infusion. Journal of Veterinary College of Tehran University. 2000; 55: 43-47.
18. Randhawa CS. Incidence of iron deficiency in crossbred cow calves reared on pucca floor. Indian journal of animal sciences. 2010; 80: 1037-1040.
19. Rengifo SA, Silva RA, Botteon RCCM, Botteon PTL. Hemogram and auxiliary serum biochemistry in neonatal crossbred calves and disease occurrence. Arq Bras Med Vet Zootec. 2010; 62: 993-7.

20. Tennant B, Harrold D, Reina-Guerra M, Kaneko JJ. Hematology of the neonatal calf. III. Frequency of congenital iron deficiency anemia. Coenell Vet. 1975; 65: 543-56.
21. Völker H, Rotermund L. The possibilities of oral iron supply in calves to maintain their health status. Deutsche Tierarztliche Wochenschrift. 2000; 107: 16-22.
22. Volker H, Rutermund L, Bauer U. Animal welfare aspects of the production of veal. Berl Munch Tierarzti Wochenschr. 1996; 109: 55-62.
23. Welchman, DD. Hematology of calves reared in different husbandry systems and the assessment of iron deficiency. Vet Rec. 1988; 123: 645-8.

*Address for correspondence:
Hamid Akbari*, Vet. College, Urmia Univ., Urmia, Iran.
e-mail address: h.akbari@urmia.ac.ir

TABLES

Table 1. Mean blood parameters in male (n=67), female (n=97), and total calves (n=164) with moderate (n=5), mild (n=24), without anemia (n=135), and overall (n=164).

Parameters	*Moderate anemia (PCV=12-19.99)*		*Total*	*Mild anemia (PCV=20-23.99)*		*Total*	*Without anemia (PCV>24)*		*Total*	*Overall*
	Female	Male		Female	Male		Female	Male		
PCV (%)	19	18.8	18.8[a]	21.6	22	21.8[a]	29.8	31.5	30.7[b]	29
Hb (g/dl)	7.4	7.5	7.5[a]	8.6	8.6	8.6[a]	10.9	11.6	11.3[b]	10.8
WBC (per/µl)	14000	14212	15307	13172	13208	13143	11795	12653	12318	12294
RBC ($\times 10^6$/µl)	3.6	3.6	3.6[a]	4.2	4.2	4.2[a]	5.6	5.8	5.7[b]	5.7
MCV (fl)	52.8	51.04	51.7[a]	51.2	51.9	51.6[a]	53.7	54.5	54.2[b]	54.2
MCH (pg)	20.6	20.6	20.6	20.4	20.2	20.3	19.7	20.3	20.1	20.1
MCHC (g/ dl)	39	40	39.8[a]	39.7	39.1	39.4[a]	36.9	36.7	36.7[b]	36.8
Fe (µg/dl)	196.7	189.4	190.9	186.9	240.1	220.5	202.7	236	221	222.2
TIBC (µg /dl)	61.9	304.8	256.2	410.7	402.6	408.8	379.6	388.9	384.8	385
Protein (g/dl)	5.9	8.7	8	7.5	7.4	7.5	6.7	7.4	6.9	6.9
Neutrophils (per/µl)	14000	6955	8364	5773	5793	5783	5164	5408	5332	5316
Lymphocytes (per/µl)	5600	7073	6778	7024	7359	7192	6585	7408	7109	7097

Table 2. The frequency of moderate and mild anemia in male and female calves.

Anemia	Calves	One month	Two months	Three months	Four months	Total	
Moderate anemia	Female	-	-	3	1	4	5
	Male	-	-	1	-	1	
Mild anemia	Female	2	2	6	2	12	24
	Male	2	2	3	5	12	
Total	Overall	4	4	13	8	29	