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## Post parturient hemoglobinuria in a sheep flock

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**ABSTRACT.** The syndrome of intravascular hemolysis, hemoglobinuria, and anemia has been recognized in post parturient dairy cattle and buffaloes. In this report, the occurrence of post parturient hemoglobinuria in a sheep flock was described. Six ewes were affected and 3 of them were died. The affected animals had lambed 1-2 weeks before. The clinical signs included hemoglobinuria, tachycardia, tachypnea, weakness and recumbency. Serum phosphorus concentrations and PCV values were lower than reference parameters for sheep. Phosphorus supplementation and supportive treatment were recommended. In conclusion, grazing on frozen pasture following phosphorus deficiency could be responsible for the development of post parturient hemoglobinuria in the sheep flock.

**Keywords:** post parturient hemoglobinuria, PPH, sheep flock, treatment

### INTRODUCTION

The syndrome of intravascular hemolysis, hemoglobinuria, and anemia has been recognized in post parturient dairy cattle and buffaloes (Macwilliams et al., 1982; Pirzada and Hussain, 1998). Post parturient hemoglobinuria (PPH) occurs sporadically and the incidence is relatively low. Most often high-producing multiparous cows develop clinical signs during the first month after calving (Ok et al., 2009). The pathogenesis by which the intravascular hemolysis occurs has not been fully elucidated.

The condition has been related to the marked hypophosphatemia in affected animals (Radostits et al., 2007). Severe hypophosphatemia is postulated to depress glycolysis and adenosine triphosphate (ATP) synthesis in the red blood cells (RBC). Adenosine

triphosphate deficiencies predispose RBC to loss of normal deformability and increase in fragility and hemolysis with resultant hemoglobinemia and hemoglobinuria (Smith, 2015). Gahlawat et al. (2007) proposed that reduction of glutathione in hypophosphatemia predisposes the erythrocytes to adverse effects of oxidants (Gahlawat et al., 2007). The resultant oxidative stress can cause lipid peroxidation of RBC membrane with eventual intravascular hemolysis (Kataria et al., 2013). Nevertheless, it is likely naive to suggest that hypophosphatemia alone can cause post parturient hemolysis. Rape grazing cruciferous plants has been associated with intravascular hemolysis and hemoglobinuria in hypophosphatemic sheep, cows and buffaloes (Stamp and Stewart, 1953; Radostits et al., 2007). The ingestion of cold water or exposure to extremely cold weather has

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also been associated with post parturient hemoglobinuria in cows and buffalos (Radostits et al., 2007).

The present report described a PPH following frozen pasture grazing in a sheep flock with hypophosphatemia and its therapeutic management.

### CASE DESCRIPTION

Three 4 years old ewes out of 100 cross breed sheep were presented to Veterinary Clinics and Teaching Hospital of Urmia University with the history of dark red colored urination, reduced appetite and milk yield for the past two days. The affected animals displayed signs of depression and weakness and were generally observed on sternal recumbency with S-form deviation of the neck (Fig 1). Body temperatures were within the normal range, heart rate and respiratory rates were elevated above the references ranges and mucous membranes were pale.

Anamnesis revealed that the animals had lambed 1-2 weeks before and all the affected animals showed clinical signs following frozen pasture grazing in the early morning of winter days. Three affected ewes died during the past days. Blood samples were collected from jugular vein for hematology and biochemical analyses.

Results of hematological and biochemical analyses showed a reduction of PCV, marked hypophosphatemia and hypocalcemia, relatively low magnesium, marked elevation of BUN and creatinine concentrations (Table1). Blood smears were negative for parasites.

Based on the history clinical signs and laboratory findings, the case was diagnosed as PPH due to hypophosphatemia.



**Figure 1.** Sternal recumbency with S-form deviation of the neck

### Treatment protocol

The affected animals received phosphorus supplementation and supportive treatment. Five ml Phosvit (Nasr Fariman, Iran. each ml containing 62 mg calcium hypophosphite) intravenously, and 3 ml cyanoferin (Nasr Fariman, Iran. Each ml containing 100mg Fe-dextran and 100µg cianocobalamine) intramuscularly were administered for 3 days. Thirty nutritional supplements (Supramix, Iran. each gr containing 500IU Vit A, 100IU Vit D3, 0.1mg Vit E, 180mg Ca, 90mg P, 60mg Na, 20mg Mg, 3mg Fe, 0.3mg Cu, 2mg Mn, 3mg Zn, 0.1mg Co, 0.1mg I, 0.1mg Se) mixed with food were administered, for 3 days. Affected animals were treated after two days.

### DISCUSSION

Post parturient hemoglobinuria is a sporadic metabolic disease that most commonly affects high-producing dairy cattle and buffalos at the onset of lactation. It is characterized by development of intravascular hemolysis, hemoglobinemia and hemoglobinuria with severe anemia which is potentially fatal. The exact cause is unknown, however, marked hypophosphatemia has been incriminated as a biochemical finding in the most affected animals (Pirzada and Hussain, 1998; Alidadi et al., 2005; Macwilliams et al., 1982; Ok et al., 2009; Senthil Kumar et al., 2014). Many cases of severe hypophosphatemia are not associated with intravascular hemolysis. This can suggest that hypophosphatemia is not the solely responsible for PPH. Grunberg et al. (2015) showed that experimental hypophosphatemia alone was not associated with intravascular hemolysis in cattle (Grunberg et al., 2015). In this study, PPH occurred in hypophosphatemic ewes following frozen pasture grazing. Hypophosphatemia in affected animals could be due to poor diet with low phosphorus for a long time during cold months of the year in this region. The ingestion of cold water or exposure to extremely cold weather may precipitate an episode of hemoglobinuria in hypophosphatemic animals (Radostits et al., 2007). A similar condition accompanied by hypophosphatemia has been observed in late pregnancy in Egyptian buffalo and in the post parturient period in Indian buffalo (Radostits et al., 2007). Stamp and Stewart (1953) reported an intravascular hemolysis and hemoglobinuria in hypophosphatemic sheep following kale and rape grazing unrelated to parturition (Stamp and Stewart, 1953). Cruciferous

plants such as turnips or beet pulp has been associated with intravascular hemolysis in multiparous cows and buffalos following parturition (Radostits et al., 2007; Mahmood et al., 2012). Berseem feeding during winter season was associated with PPH in buffaloes (Aktar et al., 2006). Simultaneous serum copper, selenium and molybdenum deficiency with hypophosphatemia have been observed in buffalos with PPH (Akhtar et al., 2007b; Mahmood et al., 2013). Circulating oxidants have also caused erythrocyte damage in hypophosphatemic dairy cows (Jubb et al., 1990).

Almost all cows develop hemoglobinuria two to four weeks after calving (Radostits et al. 2007), whereas buffalos develop hemoglobinuria before or after parturition (Pirzada and Hussain, 1998; Reddy et al., 2014). In the current report, hemoglobinuria was detected 1-2 weeks after lambing.

The prevalence of PPH in the sheep flock studied here was 6% with the case fatality of 50%. Post parturition hemoglobinuria in cattle occurs sporadically and the incidence is relatively low (Smith, 2015). Thompson and Badger (1999), Stockdale et al. (2005) and Miller et al. (2006) reported an outbreak of 16.7%, 5.6% and 6.7% in dairy cattle, respectively (Millar et

al., 2006; Stockdale et al., 2005; Thompson and Badger, 1999). The case fatality of PPH in total cattle population ranges from 10 to 50% (Macwilliams et al., 1982; Thompson and Badger, 1999). It seems that PPH occurs in buffaloes more than cattle (Pirzada et al., 1989). Akhtar et al. (2007) reported a case fatality of 15% in affected buffalos (Akhtar et al., 2007). Regarding the findings of the present study no previous report is available on the occurrence of PPH in ewes.

In conclusion, hypophosphatemia alone cannot cause intravascular hemolysis and in this report there was no history of consumption of cruciferous plants or cold water. Therefore, grazing on frozen pasture following phosphorus deficiency could be responsible for the development of PPH in the sheep flock.

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#### CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interest. ■

Table 1. Haematological and biochemical parameters in the affected ewes with post-parturient haemoglobinuria

Parameters	(mean ± SD)	Reference range	Reference
PCV (%)	15	27-45	Thrall MA (2004)
White blood cell (cells/ $\mu$ L)	10900 $\pm$ 1400	4000-12000	Thrall MA (2004)
Neutrophils (cells/ $\mu$ L)	5100 $\pm$ 1200	700-6000	Thrall MA (2004)
Lymphocytes (cells/ $\mu$ L)	6000 $\pm$ 900	2000-9000	Thrall MA (2004)
Monocytes (cells/ $\mu$ L)	500 $\pm$ 300	0-800	Thrall MA (2004)
Plasma total protein (g/dL)	10.8 $\pm$ 0.4	6.0-7.5	Thrall MA (2004)
Fibrinogen concentration (g/dL)	0.8 $\pm$ 0.1	0.1-0.5	Thrall MA (2004)
Phosphorus (mg/dL)	1.1 $\pm$ 0.1	3.1-5.6	Kaneko JJ (2008)
Calcium (mg/dL)	4.57 $\pm$ 0.1	11.5-12.8	Kaneko JJ (2008)
Magnesium (mg/dL)	1.52 $\pm$ 0.01	2.2-2.8	Kaneko JJ (2008)
BUN (mg/dL)	55.63 $\pm$ 41.15	8-20	Kaneko JJ (2008)
Creatinine (mg/dL)	3.15 $\pm$ 0.26	1.2-1.9	Kaneko JJ (2008)
AST (U/L)	22.45 $\pm$ 3.24	60-280	Kaneko JJ (2008)
ALP (U/L)	89.97 $\pm$ 34.97	68-387	Kaneko JJ (2008)

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