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**Ankit Kumar**

Referral Veterinary Diagnostic and Extension Centre (RVDEC), Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Uchani, Karnal, Haryana, India

**Vipul Thakur**

Department of Veterinary Medicine, Sardar Vallabh Bhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India

**Sandeep Potliya**

Referral Veterinary Diagnostic and Extension Centre (RVDEC), Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Uchani, Karnal, Haryana, India

**Harpreet Singh**

Referral Veterinary Diagnostic and Extension Centre (RVDEC), Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Uchani, Karnal, Haryana, India

**Swati Ruhil**

Referral Veterinary Diagnostic and Extension Centre (RVDEC), Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Uchani, Karnal, Haryana, India

**Anita Ganguly**

Referral Veterinary Diagnostic and Extension Centre (RVDEC), Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Uchani, Karnal, Haryana, India

**Biswa Ranjan Maharana**

Referral Veterinary Diagnostic and Extension Centre (RVDEC), Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Uchani, Karnal, Haryana, India

**RS Bisla**

Referral Veterinary Diagnostic and Extension Centre (RVDEC), Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Uchani, Karnal, Haryana, India

**Correspondence**

**Sandeep Potliya**

Referral Veterinary Diagnostic and Extension Centre (RVDEC), Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Uchani, Karnal, Haryana, India

## Study on incidence, haemato biochemical changes and therapeutic management of post parturient haemoglobinuria in Murrah buffaloes

**Ankit Kumar, Vipul Thakur, Sandeep Potliya, Harpreet Singh, Swati Ruhil, Anita Ganguly, Biswa Ranjan Maharana and RS Bisla**

### Abstract

Post parturient haemoglobinuria (PPH) is a metabolic disease most commonly affects high-yielding dairy buffaloes, characterized by development of per acute intravascular hemolysis and anemia with potentially fatal outcome. The study was conducted on thirty nine (incidence of 5.83%, 39/668) clinical cases of Post parturient haemoglobinuria in buffalo presented to the RVDEC, LUVAS, Uchani, Karnal. Diagnosis was done on the basis of clinical signs and clinical pathology. All the animals were recently parturited (average  $3.35 \pm 0.39$  weeks), on an average in their third lactation (mean  $3.25 \pm 0.3$ ) and exhibited clinical signs such as anorexia, constipation, pale mucous membrane (both Conjunctival and vaginal), reddish brown colour urine and drastic reduction in milk production. Haemato-biochemical examination showed leucocytosis with absolute neutrophilia and lymphopenia, lower total erythrocyte count, haemoglobin, packed cell volume, increased activities of ALT and AST, hyperglycemia, hypocalcaemia and hypophosphatemia. All affected buffaloes were treated by sodium acid phosphate 80g in one litre NSS intravenously, parenteral phosphorus preparation, antibiotic oxytetracycline along with supportive treatment. Owner was also advised to feed the mineral mixture regularly along with Vitamin A, D3 & H preparation. The animals responded well to the therapy within 5 days of treatment and effectively restore the normal haemato-biochemical indices. The report records incidence, changes in haematological and serum biochemical parameters and successful therapeutic management of post parturient haemoglobinuria in Murrah buffaloes.

**Keywords:** Buffalo, haemato-biochemical changes, incidence, PPH

### 1. Introduction

Post Parturient haemoglobinuria commonly known as “Lahu mutna” or “Rakth mutna” in field conditions is an important metabolic disorder of bovines and possesses a threat to the milking buffaloes of India every year (Mahmood *et al.*, 2013 and Navjot Singh Resum *et al.*, 2017) [14, 22]. PPH is a sporadic, non-infectious disease of high producing buffaloes and Cows occurs immediately after parturition, commonly seen during their third to sixth lactation (Blood *et al.*, 1989; Mahmood *et al.*, 2013; Senthil Kumar *et al.*, 2014) [2, 14]. This disease usually occurs within 30 days of calving. Disease is mainly characterized by rapid intra vascular haemolysis, haemoglobinuria, anemia, haemoglobinemia, weakness and marked decrease in milk production (Macwillims *et al.*, 1982; Mahmut *et al.*, 2009) [13].

Hypo-phosphataemia results in decreased red blood cell glycolysis and ATP synthesis which predisposes red blood cells to altered structure and function, although exact pathogenesis is not known. (Singari *et al.*, 1991) [24]. Risk factor associated with PPH include age, stage of pregnancy, lactation number, post-partum period, dietary phosphorous deficiency, ingestion of cruciferous plants and saponin from berseem, copper and selenium deficiency in serum, excess of molybdenum in soil and fodder (Neto *et al.*, 2007; Radostits *et al.*, 2007 and Brechbuhl *et al.*, 2008) [18, 21, 3].

### 2. Materials and Methods

#### 2.1 Ethical approval

In the present study, samples were collected from clinical cases reported at RVDEC LUVAS, Karnal. For these samples approval of Institutional Animal Ethics Committee was not required as per University rules.

## 2.2 Experimental design

The study was conducted on 20 clinical cases (screened out of 39 cases) of PPH in buffaloes reported at RVDEC LUVAS, Karnal during the period from July 2017 to June 2018. In all the cases, detailed history viz. age, lactation number, post-partum period, stage of lactation, feed intake and milk yield were recorded in all the affected animals. Clinical examination of the affected animals was also made included rectal temperature, pulse rate, respiration rate and ruminal movements. Eight apparently healthy buffaloes were kept as control group in this study. Diagnosis of the disease was made on the basis of history of recent parturition, clinical signs (haemoglobinuria, pale mucous membrane) and haemato-biochemical findings and diagnosis was further validated by ruling out other diseases causing haemoglobinuria in buffaloes.

## 2.3 Haematology and Biochemical analysis

Blood samples from all the affected and healthy animals were collected aseptically using EDTA with/without vials from jugular vein of the animals using 16G needle. Complete haematology which included hemoglobin, packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) were estimated by using blood cell counter (MS4se, HD consortium). Serum biochemical parameters which included glucose, calcium, inorganic phosphorus, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) were estimated by fully automated Clinical Chemistry Analyzer (EM Destiny 180 Erba Mannheim, Germany).

## 2.4 Statistical analysis

Data was analyzed by Independent student 't' test using SPSS computer software package to compare the significances of the differences of each parameter between the diseased animals and healthy control group.

## 3. Results

An overall incidence of 5.83% (39 cases) was recorded for PPH, out of 668 cases diagnosed with metabolic diseases. Complete history, clinical signs and haemato-biochemical parameters were recorded in 20 animals. Animals having history of recent parturition, pale mucous membrane (both Conjunctival and vaginal), reddish brown colour urine (Fig 1 and 2) and hypophosphatemia on haemato-biochemical analysis were considered as a case of PPH for this study. Most of the cases of PPH occurred in the age group of 5 years (mean  $5.25 \pm 0.3$ ) as shown in table 2. It was also seen that age-wise incidence was highest in the age group of 3 to 5 years (12 cases, 60%) followed by 6-9 years (8 cases, 40%) as shown in Table 1. Similarly, incidence of PPH were highest in buffaloes which were in 1<sup>st</sup> to 3<sup>rd</sup> lactation (12 cases, 60%) followed by 4<sup>th</sup> to 6<sup>th</sup> lactation (8 cases, 40%) as shown in Table 1. Most of the cases of PPH were recorded during 3<sup>rd</sup> to 5<sup>th</sup> week post-partum (11 cases, 55%) followed by 0 – 2<sup>nd</sup> week post-partum (7 cases, 35%) and 6<sup>th</sup> – 8<sup>th</sup> week post-partum (2 cases, 10%) as shown in table 1. So, it was concluded that the cases of PPH were utmost in their 3<sup>rd</sup> lactation ( $3.25 \pm 0.3$ ), within the first three weeks ( $3.35 \pm 0.39$ ) after parturition as shown in table 2.

In all the affected buffaloes, there was history of recent parturition and characteristic clinical sign observed were

anorexia, less water intake, constipation, reddish brown colour urine and drastic reduction in milk production ( $67.84 \pm 2.03\%$ ). Clinical examination revealed normal rectal temperature, tachycardia, dehydration and pale mucous membrane (both Conjunctival and vaginal).

The mean blood hemoglobin value ( $5.06 \pm 0.54$  g%), total erythrocyte count ( $2.4 \pm 0.25 \times 10^6/\text{mm}^3$ ), lymphocyte ( $26.6 \pm 4.1$  %) and packed cell volume ( $15.5 \pm 1.72$  %) in buffaloes with PPH was significantly lower whereas total leukocyte count ( $14.8 \pm 1.93 \times 10^3/\text{mm}^3$ ) and neutrophils ( $70.8 \pm 4.49$  %) was significantly higher as compared to healthy control animals depicting leucocytosis with absolute neutrophilia and lymphopenia as shown in table 3. The mean levels of serum calcium ( $8.7 \pm 0.43$  mg/dl) and serum inorganic phosphorus ( $2.84 \pm 0.16$  mg/dl) in all PPH buffaloes were significantly lower while those of serum glucose ( $110.94 \pm 10.93$  mg/dl), serum ALT ( $72.19 \pm 8.53$  mg/dl) and serum AST ( $256.91 \pm 25.08$  mg/dl) were significantly higher compared to healthy controls (Table 4).

All the cases were treated by sodium acid phosphate (8% solution) intravenously, parenteral phosphorus preparation, antibiotic Oxytetracycline and supportive therapy which include antihistaminic, vitamin C, vitamin B complex and oral liver tonic. Owner was also advised to feed the mineral mixture regularly twice a day along with Vit A, D3 & H preparation. The affected buffaloes responded to the given treatment and haemato-biochemical indices were restored to normal after 5 days of treatment. Milk production increases gradually with continuous use of mineral mixture.



Fig 1: Urine sample showing Haemoglobinuria

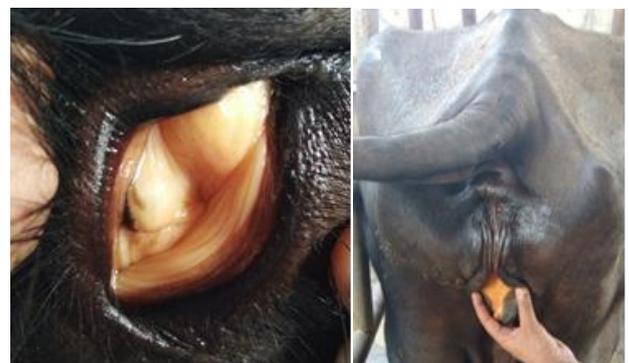


Fig 2: Pale Conjunctival and vaginal mucous membrane

**Table 1:** Incidence of PPH in buffaloes with regard to age, lactation number and lactation stage (N=20).

	Age wise (Years)		Lactation No.		Lactation stage (week)		
	3 - 5	6 - 9	1 <sup>st</sup> - 3 <sup>rd</sup>	4 <sup>th</sup> - 6 <sup>th</sup>	0-2	3- 5	6 - 8
No. of cases	12	8	12	8	7	11	2
%	60	40	60	40	35	55	10

**Table 2:** History (related to lactation) in buffaloes suffering from primary ketosis.

	Age (years)	Lactation chronology (no.)	Lactation stage (week)	Milk Yield (Litres/day)		Decrease in milk yield (%)
				Before illness	After illness	
Mean± S.E	5.25 ± 0.3	3.25 ± 0.3	3.35 ± 0.39	15.3 ± 0.62	4.9 ± 0.36	67.84 ± 2.03

**Table 3:** Haematological parameters in healthy and PPH affected buffalo

Parameters	Infected Buffalo (N=20) Mean± SEM	Healthy Buffalo (N=8) Mean± SEM
Hb (gm/dl)	5.06 ± 0.54**	11.35 ± 0.31
PCV (%)	15.5 ± 1.72**	32.7 ± 0.97
TEC(×10 <sup>6</sup> /μL)	2.4 ± 0.25**	6.29 ± 0.306
TLC (×10 <sup>3</sup> /μL)	14.8 ± 1.93**	9.25 ± 0.34
Lymphocyte (%)	26.6 ± 4.1**	38.3 ± 1.39
Neutrophil (%)	70.8 ± 4.49**	58.05 ± 1.52
Mixed	2.6 ± 0.3 <sup>NS</sup>	3.65 ± 0.44

Hb: Haemoglobin, PCV: packed cell volume, TEC: total erythrocyte count, TLC: total leukocyte count. N = Number; Significant ( $p \leq 0.05$ ) = \*; Significant ( $p \leq 0.01$ ) = \*\*

**Table 4:** Serum biochemical parameters in healthy and PPH affected buffalo

Parameters	Infected Buffalo (N=20) Mean± SEM	Healthy Buffalo (N=8) Mean± SEM
Ca(mg/dl)	8.7 ± 0.43**	11.5 ± 0.46
P(mg/dl)	2.84 ± 0.16**	5.13 ± 0.22
ALT (U/L)	72.19 ± 8.53*	38.03 ± 1.86
AST(U/L)	256.91 ± 25.08**	100.4 ± 3.92
Glucose (mg/dl)	110.94 ± 10.93**	69.62 ± 4.33

Ca: Calcium, P: Phosphorus, ALT: alanine aminotransferase, AST: aspartate aminotransferase N = Number; Significant ( $p \leq 0.05$ ) = \*; Significant ( $p \leq 0.01$ ) = \*\*

#### 4. Discussion

The outcome of the present investigation related to various parameters viz. age, lactation number, lactation stage showed that most of the animals were in peak lactation stage. The lactation stage in PPH affected buffaloes (range between 7 days to 8 weeks, mean  $3.35 \pm 0.39$  weeks); lactation number (range between first to sixth lactation, mean  $3.25 \pm 0.3$ ) and age group ( $5.25 \pm 0.3$  years) corresponded with the peak lactation stage. Similar findings were also reported by various authors (Chugh *et al.*, 1996 and Pandey and Misra, 1997) [5, 20]. Various authors like Durrani *et al.* (2010) [7] and Mahmood *et al.* (2012) [15] reported highest prevalence at 5th lactation while the lowest one was recorded at the 1st lactation. Milk yield of all the affected buffaloes before occurrence of disease ranged between 10 litres to 20 litres (Average 15.3 liters) which showed that the animals were high yielders and after illness, there was drastic reduction in milk yield (mean  $67.84 \pm 2.03\%$ ). Clinical signs such as anorexia, less water intake, constipation, reddish brown colour urine and pale mucous membrane (both Conjunctival and vaginal) were in agreement with the earlier reports (Chugh *et al.*, 1996; Gahlawat *et al.*, 2007; Durrani *et al.*, 2010; Sujatha Turker *et al.*, 2013; Soren *et al.*, 2014) [5, 8, 7, 26, 27]. However, some authors like Akhtar *et al.* (2008) [1] and Mahmood *et al.* (2012) [15] observed that PPH is not associated with high milk yield.

In the present investigation, there was a significant decrease in the level of haemoglobin, TEC and PCV which is mainly due to intravascular haemolysis resulting from phosphorus deficiency which results in impaired glycolytic pathway and depletion of ATP in erythrocytes. Decrease in concentration of ATP predisposes red blood cells to alter functions and

structure, loss of normal formability, and an increase in fragility, which ultimately leading to haemolysis. Similar findings were also reported earlier (Suttle, 1991; Radostitis *et al.*, 2007; Durrani *et al.* 2010; Kumar *et al.*, 2014 and Sharma *et al.*, 2014) [28, 21, 7, 11, 23]. Increase in TLC count in the present study is not of much importance, as leukocytic response to production diseases is not of much significance, however, it may be due to secondary bacterial infection affecting the animals. Finding of hypophosphatemia in the present investigation were also reported by various authors (Chugh *et al.*, 1998; Karapinar *et al.*, 2006; Dua, 2009 and Durrani *et al.*, 2010) [4, 9, 6, 7] and may be due to heavy drainage in milk after parturition in high producing animals, low phosphorous diets/rations, prolonged feeding on cruciferous and or/toxic plants particularly berseem etc. High calcium content with low phosphorus diet also causes hypophosphatemia by decreasing phosphorous absorption from the gastro-intestinal tract due to wider ratio of calcium and phosphorous. Molybdenum excess also decreases phosphorus by interfering with its absorption and increasing its elimination through urine (Khan and Akhtar, 2007 and Dua, 2009) [10, 6]. Hyperglycemia may be due to increased stress in disease condition which leads to increased cortisol level (Marik and Bellomo, 2013) [16]. Increase in the liver function profile (ALT and AST) in the present investigation is consistent findings associated with intravascular haemolysis (Meyer and Harvey, 2004 and Wakayo *et al.*, 2013) [17, 29]. Successful treatment of PPH with intravenous administration of phosphorus has also been stated and supports our findings (Singh *et al.*, 1989; Sujatha Turkar, 2013 and Senthil Kumar *et al.*, 2014) [25, 27].

## 5. Conclusion

Overall incidence of PPH 5.83 % in Murrah buffaloes, with highest age wise incidence in the age group of 3 to 5 years in to its 1<sup>st</sup> to 3<sup>rd</sup> lactation was recorded. The stage of lactation in PPH affected animals range from 1 week to 8 weeks (average approximately 3 weeks). Hematobiochemical examinations revealed leucocytosis with absolute neutrophilia, anaemia and decreased level of inorganic phosphorus. All the cases were successfully treated by sodium acid phosphate intravenously followed by other supportive treatment.

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