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WHOLE BLOOD TRANSFUSION AS AN ADJUNCT THERAPY IN BUBALINE PHOSPHOROUS DEFICIENCY HEMOGLOBINURIA

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ABSTRACT

Phosphorus deficiency hemoglobinuria is an economically important production disease of lactating and advanced pregnant buffaloes characterized by hemoglobinuria, hemolytic anemia and mortality in severe cases of anemia. In view of the high prevalence of phosphorus deficiency hemoglobinuria in buffaloes in study region, the present study was planned with objective to evaluate the efficacy of whole blood transfusion in Phosphorus deficiency hemoglobinuria affected buffaloes suffering from severe hemolytic anemia. A total of 12 buffaloes suffering from hemoglobinuria, hemolytic anemia, hypophosphatemia and negative for any hemoprotozoan infections were randomly divided into two Treatment groups (1 and 2). Group 1 buffalo were treated with standard treatment of Phosphorus deficiency hemoglobinuria while Group 2 buffaloes were treated with standard treatment of Phosphorus deficiency hemoglobinuria along with whole blood transfusion. Clinical and hematological parameters showed faster clinical improvement in Group 2 buffaloes with recovery rate of 100% compared to slower recovery in Group 1 buffaloes with recovery rate of 83.33%. At study interval of 12 h post-treatment, Group 2 buffaloes showed increase in significantly higher values of hemoglobin and PCV compared to Group 1 buffaloes. So, it is concluded that whole blood transfusion can be used as adjunct therapy in buffaloes suffering from Phosphorus deficiency hemoglobinuria to increase the recovery rate and survival rate.

Keywords: *Bubalus bubalis*, buffaloes, phosphorus deficiency hemoglobinuria, whole blood transfusion, treatment

INTRODUCTION

Phosphorus deficiency hemoglobinuria

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(PDH) is an economically important production disease commonly affecting high yielding dairy buffaloes in tropical countries including India and is characterized by intravascular hemolysis and anemia (Sarma *et al*., 2014; Wankhede *et al*., 2021). Age, stage of pregnancy and stage of lactation were found to be important risk factors for the occurrence of PDH in buffaloes (Mahmood *et al*., 2012). Deficiency of cereals or concentrates in the diet has been attributed to phosphorus deficiency hemoglobinuria (Kumar *et al*., 2014). Feeding of dry roughages to advanced pregnant or recently calved high yielding buffaloes lead to PPH (Bhikane and Syed, 2014).

Hypophosphatemia in buffaloes prevents ATP synthesis in red blood cells leading to loss of normal deformability, increased fragility and lysis of red blood cells (Wang *et al*., 1985). Khan and Akhtar (2007) observed hemoglobinuria /coffee colored urine, tachycardia, polypnea, respiratory distress, weakness, lethargy, anorexia, pale mucous membrane, straining while defecation, suspended rumination, drop in milk yield as prominent clinical signs in buffaloes suffering from PDH. Phosphorus deficiency hemoglobinuria is characterized by severe blood loss, drop in milk yield, anemia, nephrosis and death in severe hemolytic anemia. Anemic anoxia is the important causal factor responsible for the mortality in ailing buffaloes. Timely blood transfusion can prevent anemic anoxia and shock from hypovolemia and restore the normal maturation of erythrocytes in certain idiopathic anemia. It also improves oxygen carrying capacity of blood and rectifies the clinical signs of anemia (Kumar, 2017). Despite treatment with various phosphorus salts, few buffaloes succumb to death owing to severe hemolytic anemia leading to heavy economic losses to buffalo farmers. Owing to the occurrence of mortality in spite of conventional treatment, the present study was planned to evaluate whole blood transfusion as an adjunct therapy in bubaline phosphorus deficiency hemoglobinuria.

MATERIALS AND METHODS

The present study was conducted at Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Udgir, Dist. Latur, Maharashtra. The study was approved by the Institutional Animal Ethics Committee (IAEC) vide approval number VCU/IAEC/CPCSEA/45/20 dated 16.12.2020. The buffaloes admitted to clinics with a history of advanced pregnancy or recent parturition, coffee colored urination, straining while defecation, pale or icteric pale conjunctival mucosae were screened for phosphorus deficiency hemoglobinuria. Blood samples from suspected buffaloes were subjected to complete blood count, blood smear examination to rule out any hemoprotozoan infections and serum phosphorus was estimated for confirmation of hypophosphatemia. The buffaloes showing typical signs of coffee colored urination, suffering from anemia (Hb- <5 gm/dl, PCV- <15 %), hypophosphatemia (serum P- <4 mg/dl) and negative for hemoprotozoan infections were selected for the trial study and randomly divided into two groups *viz*. Group 1 and Group 2.

Transfusion trial

Twelve PDH affected buffaloes suffering from anemia (Hb- <5 gm/dl, PCV- <15 %) were divided into two groups. Six buffaloes from Group 1 were treated with standard treatment for PHD comprising of Inj. Buffered phosphorous 50 ml IV SID for 4 days, Inj. D20% with electrolytes

(Sodium, Potassium and Calcium Chloride, Sodium Lactate) 1 liter IV SID x 4 days, Inj. Vitamin B complex (B_1, B_6 and B_{12}) 10 ml IM SID x 7 days, Hematinics (Calcium gluconate 4.52 gm, Ferric ammonium citrate 360 mg, Copper sulphate 300 mg, Cobalt chloride 1.5 mg, Cholecalciferol 3600 IU, Niacinamide 45 mg, Biotin 75 µg, Folic acid 1.5 mg, Cyanocobalamin 15 µg per 21 gm) 25 gm PO BID x 15 days. Group 1 buffaloes were treated with same treatment given to Group 2 and additionally compatible whole blood transfusion was done 3 ml/kg IV once.

Donor selection and blood collection

Apparently healthy, adult, non-pregnant buffaloes of Livestock Farm Complex of College, free from any ectoparasitic infestation were tentatively selected as blood donor. On hematological analysis, buffaloes negative for hemoprotozoan infections and having Hb >9 gm/dl and PCV >27% and shown compatibility with recipient buffalo blood were selected as blood donor. The blood was collected from donor buffaloes by jugular venipuncture in ready to use blood collection bags of 350 ml capacity (49 ml Citrate Phosphate Dextrose Adenine).

Blood transfusion

The dose of 3 ml/kg for whole blood transfusion in PDH affected buffaloes was finalized as per the Shah *et al.* (2021). Standard treatment given in Group 1 was also given in Group 2 buffaloes before start of blood transfusion. The whole blood was administered 3 ml/kg IV in Group 2 buffaloes with monitoring of recipient buffalo till completion of transfusion for any adverse reactions. Response to the treatment was adjudged based on clinical, hematological and biochemical parameters as well as survival rate in treated buffaloes.

Clinical signs

All 12 PDH affected buffaloes under trial were observed for clinical parameters like resumption of feed and water intake, rumination, body temperature, heart rate, respiration rate, rumen motility, conjunctival mucous membrane, restoration of urine colour, defecation etc. from the start of treatment.

Hematology

The blood samples collected from trial animals before start of treatment, 12 h, 7 day and 21 days post-treatment and analyzed for complete blood count on automated hematology analyzer (Abacus Junior Vet, Diatron GMBH, Austria).

Biochemical analysis

About 4 ml of whole blood was collected from trial animals before start of treatment and on day 21 of the study period and subjected to centrifugation 3000 rpm for 5 minutes to harvest serum. The serum samples were subjected to estimation of total, direct and indirect bilirubin, blood urea nitrogen, creatinine, phosphorus and calcium before treatment and on day 21 on biochemical analyser (UV-Visible Double Beam Spectrophotometer- SPECORD 200 PLUS-Analytikena, Endress and Hauser Company, China) using standard diagnostic kits manufactured by Agappe Diagnostics Ltd., Kerala, India.

Statistical analysis

The data pertaining to vital, hematological and biochemical parameters between buffaloes of Group 1 and 2 was compared using paired 't' test for equal number of observations before treatment and after treatment at 99% and 95% level of significance.

RESULTS

The response to the treatment was assessed based on resolution of clinical signs, restoration of feed and water intake as well as milk yield in lactating buffaloes, improvement in hematological and biochemical parameters and overall clinical recovery rate. The vital, hematological and biochemical parameters in study buffaloes of both groups have been depicted in table No. 1.

Group 1 buffalo showed resumption of food and water intake along with initiation of rumination, normalization of feed and water intake on 6 to 8th day of treatment. Straining while defecation persisted till 4 days while subsided completely on 6 to 7th day. Restoration of urine colour to clear straw from coffee colour was observed from 4 days onwards in Group 1 buffaloes. Highly significant ($P<0.01$) decrease in heart rate and respiration rate while increase in ruminal motility was observed in recovered buffaloes on day 21 of the trial. Similarly, significant ($P<0.05$) reduction in total leukocyte count, highly significant ($P<0.01$) increase in total erythrocyte count, hemoglobin and packed cell volume was observed on day 21 of trial in Group 1 buffaloes treated with standard treatment without blood transfusion. Highly significant ($P<0.01$) decrease in the values of biochemical parameters like blood urea nitrogen, creatinine, total bilirubin, direct bilirubin, indirect bilirubin was observed while highly significant ($P<0.01$) increase in values of serum inorganic phosphorus was observed in Group 1 buffalo.

Group 2 buffalo suffering from PDH were treated with standard treatment used in Group 1 buffalo with an additional whole blood transfusion 3 ml/kg body weight. Clinical improvement was observed in Group 2 buffaloes starting from blood transfusion immediately. Up on whole blood transfusion on day 1 of trial, treated buffaloes showed alertness, became active, recumbent buffaloes regained muscular strength and were able to stand and walk after transfusion (Figure 1 and 2). Initiation of feed and water intake was observed from 2nd day of treatment in Group 2 buffaloes with reduction in the severity of straining during defecation while complete subsidence of straining was observed on day 4 of trial. Restoration of urine colour to clear straw from coffee colour was observed from 3 days onwards in Group 2 buffaloes. Highly significant ($P<0.01$) decrease in heart rate and respiration rate while increase in rumen motility was observed in Group 2 buffaloes on day 21 of trial suggestive of clinical improvement. Hematological analysis showed highly significant ($P<0.01$) increase in values of total erythrocyte count, hemoglobin and packed cell volume while significant ($P<0.05$) decrease in values of total leukocyte count was observed on day 21 of trial in Group 2 buffaloes. Similarly, highly significant ($P<0.01$) decrease in the values of blood urea nitrogen, creatinine, total bilirubin, direct bilirubin, indirect bilirubin was observed and highly significant ($P<0.01$) increase in values of serum inorganic phosphorus was observed in Group 2 buffaloes treated with additional whole blood transfusion.

Variations in the values of hematological parameters like haemoglobin and packed cell volume was analyzed on 0 h, 12 h post-treatment, 7 and 21 days of trial to compare the efficacy of treatment protocol in PDH affected buffaloes. The decline in the values of Hb (-0.51 gm/dl) and PCV (-1.29%) was observed in Group 1 buffaloes 12 h post-treatment while increase in Hb (+0.41 gm/dl) and PCV (+1.32%) values were observed in Group 2 buffaloes at the same time interval indicating role of blood transfusion in improving Hb and PCV status

in Group 2 buffaloes. Out of 6 buffaloes treated in Group 1, 5 buffaloes recovered completely while one buffalo died in spite of standard treatment owing to severe haemolytic anaemia, with overall clinical recovery rate of 83.33%. Out of 6 PDH affected buffaloes treated with standard treatment along with whole blood transfusion, all 6 buffaloes recovered completely indicative recovery rate of 100% signifying the importance of whole blood transfusion as lifesaving modality in PHD affected buffaloes.

DISCUSSION

Phosphorus deficiency hemoglobinuria is an important production disease of buffaloes in Asian subcontinent including India (Bhikane *et al*., 2004; Sarma *et al*., 2014; Pirzada and Hussain, 1998). The important clinical signs observed in PDH affected buffaloes in the present study were voiding of dark coffee coloured to reddish urine, inappetence to anorexia, decrease in milk yield in lactating buffaloes, straining while defecation with pelleted feces, weakness, pale to icteric pale conjunctival mucous membranes, tachycardia, tachypnea and decreased rumen motility. Similar clinical signs have been previously reported in PPH affected buffaloes by Bhikane *et al.* (2004); Dhonde *et al.* (2007); Gupta *et al.* (2010). The elevated heart rate and respiration rate in PDH affected buffaloes might be attributed to severe destruction of erythrocytes with increasing severity of anemia. Hematological and biochemical alterations indicative of hemolytic anemia, jaundice and kidney damage were observed in PDH affected buffaloes are in agreement with the findings of Gautam *et al.* (1972); Digraskar *et al.* (1991); Bhikane *et al.* (2004). Decrease in erythrogram in the PDH affected buffaloes is attributed to increased erythrocyte hemolysis from impaired shape transformation in ATP deficient erythrocytes due to hypophoshpatemia (Samad and Malik, 1996). Similarly, elevated values of biochemical parameters like BUN and creatinine in PDH affected buffaloes have been attributed to kidney damage from hemoglobinuria (Digraskar *et al*., 1991; Samad *et al*., 1979). The significant elevation of indirect and total bilirubin in PDH affected buffaloes is attributed to intravascular haemolysis (Samad *et al*., 1979). Decreased serum inorganic phosphorus concentration in PDH affected buffaloes is a pathognomic biochemical finding for confirmation of role of phosphorus deficiency in occurrence of the disease.

Different blood transfusion triggers have been recommended by different authors for blood transfusion in bovines. Whole blood transfusion is indicated in animals when Hb <5 gm/dl and PCV <15% to support the life (Divers, 2005). Depending on the type of blood loss, Balcomb and Foster (2014) indicated blood transfusion in animals when haematocrit <15 to 20% in case of acute blood loss while, haematocrit <10 to 15% for cases suffering with chronic anaemia. In face of severe anaemia with Hb <5 gm/dl, myocardial oxygenation is affected culminating into deadly tissue hypoxia. Acute blood loss with PCV of 12% or less is indicative of critical anemia and hence immediate blood transfusion is indicated (Hunt and Wood, 1999). Ideally a gallon of blood transfusion is indicated in bovines in face of anemic condition or hemorrhages (Bell, 2006). Administered blood from healthy donor in adult anemic cow raises PCV by approximately 0.75% per liter of blood transfused (Soldan, 1999).

Blood transfusion provides a short-term beneficial effect through replenishment of blood

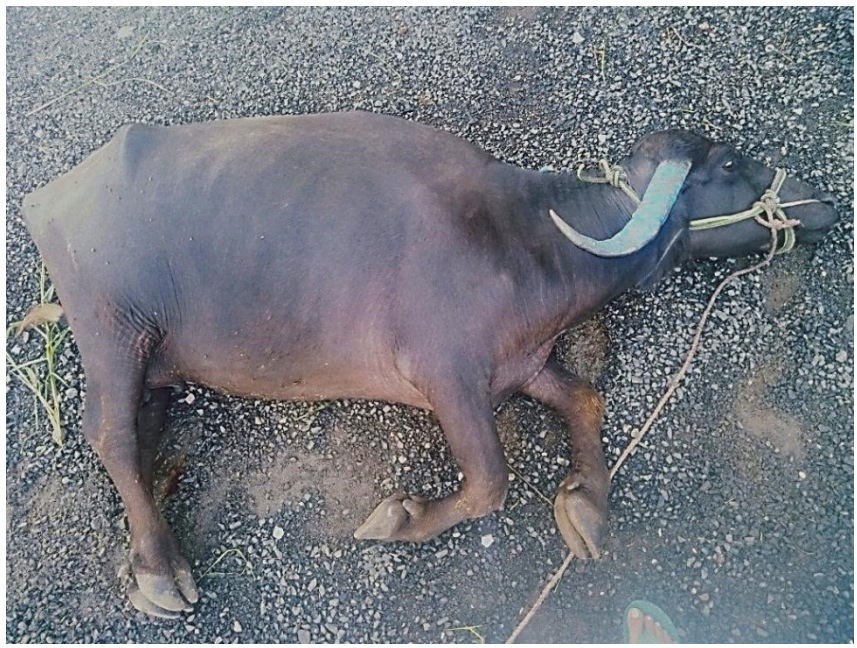

Figure 1. Buffalo suffering from Phosphorus deficiency hemoglobinuria in lateral recumbency due to weakness attributed to severe hemolytic anemia before blood transfusion.

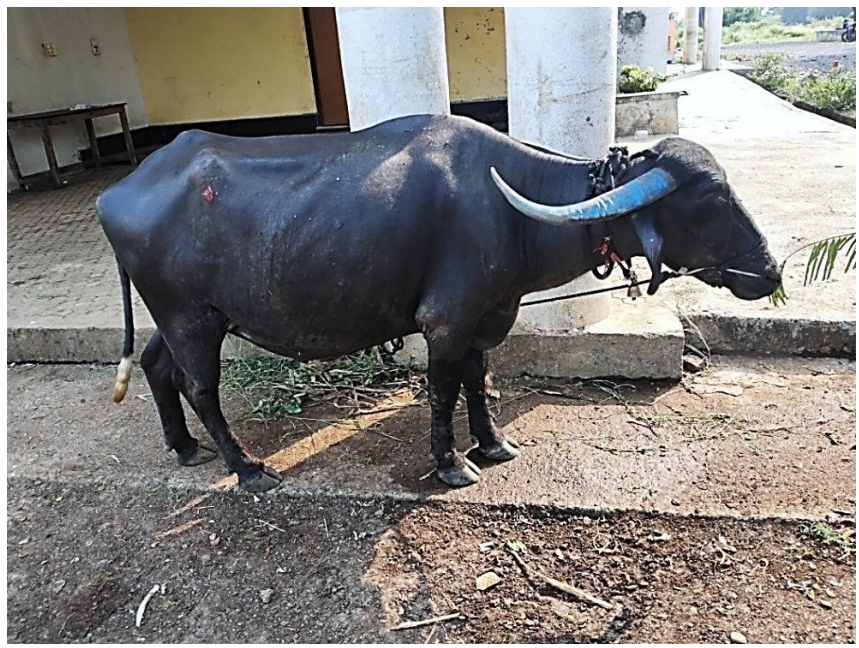

Figure 2. Same buffalo able to stand and walk immediately after whole blood transfusion.

Table 1. Vital and hemato-biochemical parameters in Group 1 and 2 buffaloes suffering from PDH before treatment (day 0) and after recovery (day 21).

Parameter	Group 1			Group 2		
	BT (n=6)	AT (n=5)	't' value	BT (n=6)	AT (n=6)	't' value
Body temperature (^{0}F)	100.98±0.58	100.1±0.19	1.325^{NS}	101.52±0.57	100.12±0.17	2.340*
Respiration rate (/minute)	77.33±3.08	55.2±1.96	5.762**	71.67±6.16	54.67±2.23	2.590*
Heart rate (beats /minute)	35.67±3.52	22.4±0.75	3.361**	26.83±1.94	25.5±1.87	0.500*
Ruminal motility (/minute)	1.17±0.54	4.0±0.32	-4.265**	1.17±0.31	4.33±0.33	-6.985**
Hemoglobin (gm/dl)	3.58±0.39	7.76±0.56	-6.308**	3.07±0.26	7.52±0.36	-10.102**
PCV (%)	10.77±1.38	24.38±0.56	-5.099**	9.16±0.74	23.18±1.07	-10.76**
TEC (x10^9/L)	1.59±0.22	4.28±0.55	-4.880**	1.35±0.13	3.94±0.22	-10.398**
TLC (x10^6/L)	11.39±1.23	7.81±1.20	2.055*	13.74±1.92	10.64±1.66	1.22*
Total bilirubin (mg/dl)	2.15±0.19	0.25±0.07	8.899**	2.18±0.08	0.15±0.01	24.219**
Direct bilirubin (mg/dl)	0.52±0.12	0.14±0.05	2.611**	0.62±0.11	0.08±0.01	4.960**
Indirect bilirubin (mg/dl)	1.63±0.17	0.11±0.04	8.212**	1.56±0.13	0.07±0.01	11.654**
BUN (mg/dl)	19.75±1.80	9.22±0.67	5.472**	24. 18±2.26	11.1±1.10	5.208**
Creatinine (mg/dl)	1.78±0.21	0.48±0.10	5.187**	2.37±0.39	0.96±0.25	3.027**
Calcium (mg/dl)	8.91±0.25	10.49±0.16	-5.120**	8.78±0.26	10.05±0.33	-3.045**
Phosphorus (mg/dl)	1.18±0.16	4.86±0.15	-16.988**	1.41±0.23	4.83±0.14	-12.718**

**-Highly significant (P<0.01), *-Significant (P<0.05).

Table 2. Variations in the values of Hb and PCV in PDH affected buffaloes treated with (Group 2) and without (Group 1) whole blood transfusion.

Sr. No.	Group		Hb (gm/dl)		PCV (%)	
			Mean	Average variation	Mean	Average variation
1	1 (n=6)	0 h	3.58±0.39	-	10.77±1.38	-
		12 h	3.07±0.35	-0.51	9.48±1.35	-1.29
		7th day	5.22±0.52	+1.64	16.07±1.49	+5.3
		21st day	7.76±0.56	+4.18	24.38±2.43	+13.61
2	2 (n=6)	0 h	3.07±0.26	-	9.16±0.74	-
		12 h	3.48±0.25	+0.41	10.47±0.73	+1.31
		7th day	4.18±0.17	+1.11	13.16±0.45	+4
		21st day	7.52±0.36	+4.45	23.18±1.07	+14.02

volume and restoration of tissue oxygenation thereby increasing chances of survival of patients during critical periods. In the present study blood was transfused 3 ml/kg body weight, which although seems less helps in rejuvenating the animal in critical condition. Divers (2005) replaced half of whole blood lost and suggested that the smaller amount of blood transfusion is also beneficial for survival. Similarly, Bell (2006) transfused about 6 liters of blood in 600 kg HF cow and observed an increase in PCV by nearly 5% signifying 2 ml/kg body weight of transfused blood is elevating PCV by 1%. In the present study, whole blood transfusion 3 ml/kg body weight helped to raise PCV by 1.32% about 12 h post-transfusion which is in agreement with the findings of Bell (2006). Similarly, Shah *et al.* (2021) observed 100% recovery rate in group of cattle suffering from severe hemolytic anemia due to hemoprotozoan infections treated with standard treatment for hemoprotozoan infection along with blood transfusion compared to 75% recovery rate in cattle treated only for hemoprotozoan infections without blood transfusion. The hematological analysis 12 h post-treatment showed 14.24% decrease in hemoglobin while 11.97% decrease in PCV values in Group 1 buffaloes compared to pre-treatment values, which indicates ongoing blood loss and severe hemolytic anemia. On the contrary, there was 13.35% rise in hemoglobin and 14.30% rise in PCV values of Group 2 buffaloes 12 h post-transfusion compared to pretreatment values, indicating positive effect of blood transfusion on raising the erythrogram. The elevation of values of erythrogram post-transfusion might be helpful in sustaining oxygenation in transfused buffaloes more effectively than non-transfused buffaloes during critical periods. It could be the reason behind 83.33% recovery rate in Group 1 buffaloes while, 100% recovery in transfused Group 2 buffaloes.

CONCLUSION

It is concluded that based on the availability of donor and blood bags, 3 to 6 ml of whole blood transfusion will be beneficial in therapeutic management of phosphorus deficiency hemoglobinuria along with standard treatment for improving the survival rate.

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