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Research Article

CLINICO-EPIDEMIOLOGICAL AND HAEMATO-BIOCHEMICAL STUDIES ON BUBALINE PHOSPHORUS DEFICIENCY HAEMOGLOBINURIA

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ABSTRACT

Bubaline phosphorous deficiency haemoglobinuria (PDH) is considered as a key production disease of the buffaloes. The present study was aimed to investigate the epidemiology, clinical and haemato-biochemical aspects of PDH in buffaloes of Marathwada region. The overall prevalence of PDH was found to be 1.72% with highest prevalence in 6–9 year age group, early lactating buffaloes during summer and winter. The prominent clinical signs observed were coffee colored urine, straining while defecation, decreased milk production and anaemia with papery white, pale or icteric pale conjunctival mucous membrane.Vital parameters showed highly significant ($P < 0.01$) increase in heart rate and respiration while decrease in ruminal motility as compared to healthy buffaloes. Haematology showed highly significant ($P < 0.01$) increase in leukocyte and neutrophil count while highly significant ($P < 0.01$) decrease in lymphocyte count, RBC, haemoglobin and PCV values were observed. Biochemical investigations revealed highly significant increase in BUN, creatinine, total and indirect bilirubin while highly significant decrease in serum inorganic phosphorus was observed.

Keywords: Buffalo, Haemato-biochemistry, Haemoglobinuria, Phosphorous Deficiency, Prevalence

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Nutritional haemoglobinuria is an economically important disease of buffaloes observed during advanced pregnancy or early lactation and has been attributed to hypophosphatemia or diets containing cruciferous plants (Chugh *et al.*, 1996). Post-parturient haemoglobinuria (PPH) with clinical syndrome similar to nutritional haemoglobinuria has been attributed to hypophosphatemia (Purohit *et al.*, 2018). PPH is evolving as a major hazard to dairy industry in buffalo rearing countries including India (Gahlawat *et al.*, 2007). The disease is mainly attributed to dietary phosphorus deficiency, and characterized by intravascular haemolysis, haemoglobinuria,severe anaemia and haemoglobinuric nephrosis (Constable *et al.*, 2017). Most of the buffaloes with acute haemolytic crisis succumb to death. Moreover, the economic losses in the form of milk production loss, body condition loss and cost of treatment impart major dent on the pocket of dairy farmers.

Age (>7 years), pregnancy (>7 months), lactation (>3) and post-partum period (≤60 days) are considered to be the major risk factors for the occurrence of PPH in buffaloes (Mahmood *et al.*, 2012). Feeding of dairy buffaloes on forages grown on phosphorus deficient soil for longer duration has been attributed in occurrence of PPH (Akhtar *et al.*, 2007).

The common haemato-biochemical changes observed in buffaloes suffering from PPH were anaemia, abnormal erythrocyte morphology (reticulocytosis, anisocytosis) and decreased serum phosphorus, significantly increased indirect bilirubin, elevated direct and total bilirubin indicative of acute haemolysis (Sarma *et al.*, 2014; Bhikane *et al.*, 2004). Owing to appreciable prevalence of haemoglobinuric syndrome in buffaloes, the present study was planned with objective to study the epidemiological, clinical and haemato-biochemical aspects of bubaline phosphorus deficiency haemoglobinuria (PDH).

MATERIALS AND METHODS

The present study was carried out in Teaching Veterinary Clinical Complex and Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Sciences, Udgir, during November, 2018 to October, 2020. The clinical cases of buffaloes reported from Latur, Parbhani and Nanded districts of Marathwada and Bidar district of Karnataka with history of recent parturition or advanced pregnancy and signs of coffee coloured urine, anaemia, straining while defecation, reduced milk production, anorexia, respiratory distress, pale and/or icteric mucosae, and no response to antibiotic/anti-haemoprotozoan treatment were screened for phosphorous deficiency haemoglobinuria and the disease was confirmed by haemato-biochemical and blood smear examination. All PDH affected buffaloes (n=33) were enrolled in diseased group while 12 healthy, disease free adult buffaloes were enrolled to healthy group (n=12).

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The prevalence of bubaline phosphorous deficiency haemoglobinuria at hospital was estimated based on data related to age, breed, sex, milk yield, month, season, physiological status, stage of lactation/pregnancy and feeding patterns. Detailed history, clinical signs of study animals and management history was compiled.

Blood samples collected from study animals in EDTA vials were subjected to complete blood count analysis on automated haematology analyser (Diatron Abacus Junior Vet 3.11) and serum samples were subjected to analysis of BUN, creatinine, total bilirubin, direct bilirubin, indirect bilirubin, calcium and inorganic phosphorus using standard diagnostic kits manufactured by Span Diagnostics, Surat, Gujarat on semi-automated biochemical analyser (Chemistry Analyzer-CA 2005 B4B Diagnostic Division, China).

Statistical analysis was carried out as per the methods described by Snedecor and Cochran (1994). The Chi-square (X^2) value was calculated to determine the significance of an association between diseases and hypothesized causal factor. The paired't' test for unequal observations was used for comparison of values in PDH affected and healthy buffaloes.

RESULTS AND DISCUSSION

The epidemiological aspects of bubaline PDH have been depicted in Table 1. The overall hospital prevalence of PDH was 1.72%. The season-wise prevalence of PDH was highest in summer (36.36%) and winter (36.36%) followed by monsoon (21.21%) while lowest during post-monsoon (6.06%). The findings are in agreement with Constable *et al.* (2017) and Dhonde *et al.* (2007) and might be attributed to winter or summer stress and seasonal breeding behaviour in buffaloes. Breed-wise prevalence was found to be highest among Murrah (0.57%) and graded Murrah (0.57%) followed by non-descript (0.46%) and Marathwadi (0.10%) buffaloes. The highest occurrence of PDH in Murrah, graded Murrah and non-descript buffaloes might be attributed to high lactational potential compared to local breeds. Highest prevalence of PDH was observed in 6-9 years age group (0.83%) followed by 0-6 years (0.57%) and least in > 9 years (0.31%) age group. The buffaloes in their 3rd-6th lactations have highest production potential with need of extra nutritional requirements leading to stress on mineral metabolism during these lactations which might culminates in deficiency syndromes (Akhtar *et al.*, 2007). The prevalence of PDH was more among lactating buffaloes (1.2 %) as compared to pregnant buffaloes (0.52%). Buffaloes in advanced pregnancy (>6 month) were at highest risk (70%) followed by mid (3-6 month) pregnancy (30%). Lactation stage-wise highest prevalence of PDH was observed during early (78.26 %) lactation (0-3 month) followed by mid (13.04%) lactation (3-6 month) and late (8.70 %) lactation (>6 month). Highest incidence of PDH was recorded during 3rd lactation (43.48 %) followed by 4th lactation (30.43%). Heavy drain of minerals including phosphorous might be observed during early lactation while the requirement of phosphorous to developing foetus goes to peak at late pregnancy may lead to hypophosphatemia and PDH (Dhonde *et al.*, 2007; Constable *et al.*, 2017). High yielding buffaloes maintained exclusively on dry roughages (84.85%) were at high risk followed by buffaloes fed on dry and green roughages (12.12%) and buffaloes receiving dry roughages along with concentrate (3.03%). Buffaloes fed *ad-libitum* greens were comparatively resistant to PDH.

The major clinical signs of bubaline phosphorous deficiency haemoglobinuria observed in the present study were dark coffee coloured to reddish urine along with inappetence, reduced water intake, reduced body weight, rough skin coat, decreased milk production, frequent urination, straining while defecation, loose to constipated faeces and pale to icteric mucous membranes. Similar clinical findings were also reported by Bhikane *et al.* (2004) and Dhonde *et al.* (2007) in PPH affected buffaloes.

The mean values of physiological parameters (Table 2) revealed highly significant ($P<0.01$) increase in heart rate and respiration rate while decrease in ruminal motility was observed in affected buffaloes as compared to healthy buffaloes. Similar observations were also reported by Bhikane *et al.* (2004); Khan and Akhtar (2007); Soren *et al.* (2014) and Kumar *et al.* (2019). The increased pulse rate and respiration rate might be attributed to on-going destruction of erythrocytes leading to increased severity of anaemia.

Haematological values are depicted in Table 2. The haematological analysis showed highly significant ($P<0.01$) reduction in values of Red Blood Cells, Haemoglobin and Packed Cell Volume in PDH affected buffaloes compared to healthy counterparts. Similar findings of significant reduction in mean erythrocyte count, haemoglobin concentration and packed cell volume in PPH affected buffaloes have been reported by several workers (Bhikane *et al.*, 2004; Dhonde *et al.*, 2007; Khan and Akhtar, 2007; Soren *et al.*, 2014; Sarma *et al.*, 2014; Kumar *et al.*, 2019). Rapid fall in the erythrogram in phosphorus deficiency haemoglobinuria might be attributed to increased haemolysis of erythrocytes due to impaired shape transformation in ATP-deficient erythrocytes due to hypophosphatemia (Samad and Malik,

Table 1

Epidemiological aspects of phosphorous deficiency haemoglobinuria in buffaloes

Parameter	Variable	No. Screened	No. Affected	% Cases	% Prevalence	X^2
Breed	Murrah	278	11	33.33%	0.574	0.01019302**
	Marathwadi	157	2	6.06%	0.104	
	Graded Murrah	567	11	33.33%	0.574	
	ND	915	9	27.28%	0.469	
	Total	1917	33	100%	1.721	
Age	0-6 years	621	11	33.33%	0.574	0.47934875NS
	6-9 years	1070	16	48.49%	0.835	
	> 9 years	226	6	18.18%	0.313	
	Total	1917	33	100%	1.721	
Physiological Status	Lactating	1254	23	69.70%	1.200	0.60502654NS
	Non lactating	663	10	30.30%	0.521	
	Total	1917	33	100%	1.721	
Month	June	122	2	6.06	0.104	0.02920562*
	July	210	4	12.12	0.209	
	August	255		0	0.000	
	September	241	1	3.03	0.052	
	October	139	1	3.03	0.052	
	November	121	1	3.03	0.052	
	December	177	3	9.09	0.156	
	January	160	5	15.15	0.261	
	February	149	4	12.12	0.209	
	March	140	4	12.12	0.209	
	April	84	1	3.03	0.052	
	May	119	7	21.21	0.365	
	Total	1917	33	99.99	1.721	
Season	Monsoon	828	7	21.21	0.365	0.00476254**
	Post-monsoon	260	2	6.06	0.104	
	Winter	486	12	36.36	0.626	
	Summer	343	12	36.36	0.626	
	Total	1917	33	100	1.721	

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

1995). Increased erythrocyte fragility and haemolysis in PPH has been attributed to impaired glycolytic pathway and depletion of ATP in erythrocytes which predisposes red blood cells to alter functions and structure, loss of deformability making red blood cells weak and fragile (Constable *et al.*, 2017).

Leukogram showed highly significant (P<0.01) increase in total leukocyte count, neutrophil count while highly significant (P<0.01) decrease in lymphocyte count in PDH affected buffaloes compared to healthy buffaloes. Similar findings of leukogram were also observed by Bhikane *et al.* (2004); Khan and Akhtar (2007) and Sarma *et al.* (2014) in buffaloes suffering from post-parturient haemoglobinuria.

The biochemical analysis (Table 2) showed highly significant (P<0.01) increase in BUN and creatinine values in PDH affected buffaloes as compared to healthy buffaloes. The elevated BUN and creatinine are in agreement with those reported by Bhikane *et al.* (2004) and Akhtar *et al.* (2007). The highly significant (P<0.01) increase in the mean values of total, direct and indirect bilirubin was observed in diseased buffaloes. The elevated

Table 2

Mean values of Physiological and haemato-biochemical parameters in PDH affected and healthy buffaloes

Sr. No.	Parameters	Affected (n=33)	Healthy (n=12)	't' value
1.	Rectal temperature (ºF)	101.8 ± 0.25	101.06 ± 0.13	-2.097^{NS}
2.	Heart rate (bpm)	71.76 ± 2.35	53 ± 0.64	-7.703**
3.	Respiration rate (per min)	27.97 ± 1.44	20.83 ± 0.53	-4.653**
4.	Ruminal motility (per 5 min)	1.18 ± 0.18	4.17 ± 0.24	-9.992**
5.	WBC (×103 /µl)	12.56 ± 0.49	9.18 ± 0.66	-4.097**
6.	Lymphocyte %	39.16 ± 1.55	62.99 ± 2.52	8.047**
7.	Monocyte %	5.08 ± 0.54	5.31 ± 0.57	0.288^{NS}
8.	Neutrophils %	55.75 ± 1.65	31.69 ± 2.84	-7.333**
9.	RBC (×106 /µl)	2.28 ± 0.25	7.09 ± 0.37	10.858**
10.	Hb (gm/dl)	4.73 ± 0.44	8.38 ± 0.47	5.637**
11.	PCV (%)	15.86 ± 1.86	24.35 ± 1.68	3.387**
12.	PLT (×103 /µl)	191.8 ± 18.53	271.17 ± 34.32	2.044^{NS}
13.	BUN (mg/dl)	23.63 ± 2.30	9.93 ± 0.38	-5.881**
14.	Creatinine (mg/dl)	2.07 ± 0.23	0.75 ± 0.05	-5.604**
15.	Total Bilirubin (mg/dl)	2.17 ± 0.17	0.19 ± 0.02	-20.004**
16.	Direct Bilirubin (mg/dl)	0.57 ± 0.08	0.11 ± 0.01	-5.728**
17.	Indirect Bilirubin (mg/dl)	1.58 ± 0.10	0.08 ± 0.01	-14.982**
18.	erum calcium (mg/dl)	8.85 ± 0.17	9.46 ± 0.29	1.810^{NS}
19.	Serum inorganic phosphorous (mg/dl)	1.29 ± 0.14	5.35 ± 0.15	20.265**

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

values of total, direct and indirect-bilirubin in PDH affected buffaloes have been reported by Bhikane *et al.* (2004) and Dhonde *et al.* (2007).

Non-significant decrease in mean values of serum calcium was observed in affected buffaloes. Kumar *et al.* (2019) reported significantly decreased serum calcium concentration in PPH affected buffaloes. Soren *et al.* (2014) also reported decrease in serum calcium values in haemoglobinuric buffaloes. Highly significant (P<0.01) decrease in mean values of serum inorganic phosphorus were observed in PDH affected buffaloes compared to healthy counterparts. Similar findings of hypophosphatemia in buffaloes suffering from post-parturient haemoglobinuria were also reported by Bhikane *et al.* (2004); Gahalawat *et al.* (2007) and Dhonde *et al.* (2007). High Ca and low P in diet which results in wide Ca and P ratio and high molybdenum in diet also affects the absorption of phosphorous from the gut and results in to hypophosphatemia (Kumar *et al.*, 2019).

In conclusion, phosphorus deficiency haemoglobinuria was observed in both advanced pregnant and early lactating buffaloes of Marathwada region of Maharashtra during winter and summer with overall prevalence of 1.72% and was characterized by coffee colored urination, straining while defecation, pale to icteric conjunctival mucous membranes, severe anaemia, neutrophilic leukocytosis, elevated BUN, creatinine, bilirubin levels while significantly reduced values of serum inorganic phosphorus.

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