Received: 04 September 2023, Accepted: 03 October 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 8, 1650-1656

Investigation on Post-parturient Hemoglobinuria (PPH) in Buffalo: A case Control Study

Mariam Almubarak[1], Mohamed Marzok[1,2*], Adel Almubarak[1], Sayed Elhawari[1], Sabry El-Khodery[3], Magdy Elgioushy[4]

[1]Department of Clinical Sciences, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia.

[2]Department of Surgery, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt.

[3]Department of Internal Medicine, Infectious Diseases and Fish Diseases, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt.

[4]Department of Animal Medicine, Faculty of Veterinary Medicine, Aswan University, Aswan 37916, Egypt.

***Correspondence**
Corresponding author: Mohamed Marzok
E-mail address: mmarzok@kfu.edu.sa

Abstract

Post-parturient hemoglobinuria is a non-infectious hemolytic metabolic disorder of buffalo and cattle characterized by intravascular hemolysis, anemia, and hemoglobinuria. It may be highly fatal, if not diagnosed rapidly and treated promptly. The present study determines the clinical and biochemical alterations as well as, the risk factors associated with PPH in buffalo in Egypt. Sixty-eight buffalo displaying symptoms of hemoglobinuria, along with 24 healthy buffalo for comparison were involved. Each animal participating in the study underwent a comprehensive clinical examination, and blood and urine samples were collected for further analysis. A questionnaire was designed for the hypothesized risk factors. To determine the risk factors at the animal level, the logistic regression model was utilized. Clinically, PPH-affected buffalo had much lower ruminant movements than healthy ones, meanwhile their respiratory and pulse rates dramatically increased. The serum levels of phosphorus (P), calcium (Ca), magnesium (Mg), glucose-6-phosphate dehydrogenase (G6PD), and glutathione peroxidase (GPX) showed a significant decrease in PPH-affected buffalo ($p<0.05$), whereas potassium (K), aspartate amino transferase (AST), alanine amino transferase (ALT), and glucose serum levels all demonstrated a significant increase. ($p<0.05$) In the final logistic regression model, which was used to identify risk factors, there was a significant correlation between PPH and parity ≥4 (P = 0.036, OR: 3.76, 95% CI: 1.09-12.96), hypophosphatemia (P = 0.003, OR: 5.55, 95% CI: 1.78-17.27), and hypocalcaemia (P = 0.044, OR: 3.39, 95% CI: 1.03-11.15), on the animal level. In conclusion, PPH–affected buffalo may demonstrate several biochemical changes, as well as the identification of PPH-associated risk factors may provide a useful approach for the prevention and control of PPH in buffalo.

KEYWORDS
Metabolic disorders, Buffalo, Biochemical alterations, Hypophosphatemia, Risk factors.

INTRODUCTION

Post-parturient hemoglobinuria is a sporadic metabolic disorder that affects high–yielding dairy cattle and buffalo all over the world (Purohit *et al*., 2018). This disorder is characterized by intravascular hemolysis, anemia, hemoglobinuria, and anemia (Sharma *et al*., 2020). Post-parturient hemoglobinuria is not commonly observed in beef cattle compared to dairy cows (Rahmati *et al*., 2021). The primary cause for this disparity is the greater phosphate losses through milk in dairy cattle, especially during periods of low phosphorus intake (Macwilliams *et al*., 1982; Yadav *et al*., 2023). These conditions may include transitory feed intake depression during the periparturient phase (Grünberg, 2014). PPH has been documented to affect buffalo more than cattle (Bhikane and Syed, 2014). Large ruminants are most vulnerable to PPH from the third to sixth gestation period (Constable *et al*., 2016). The risk period for PPH occurrence is the transition from pregnancy to lactation (Whitaker, 1999). However, PPH can occur during the period from six to eight months of pregnancy (Kumar *et al*., 2019). Post-parturient hemoglobinuria can lead to large financial losses because of reduced milk production, high mortality rates, and expensive treatment cost, even though the disease could only affect a few animals in the herd (Deeba and Bashir, 2019).

The exact cause and pathophysiology of post-partum hemoglobinuria remain unknown, despite the fact that hypophosphatemia is frequently observed (Stockdale *et al*., 2005). Moreover, copper deficiency and possible hemolyzing substances contained in certain feeds have been reported as potential causative or predisposing factors for hypophosphatemia (Constable *et al*., 2016). The most common metabolic problem found in parturient buffalo is post-parturient hemoglobinuria (Purohit *et al*., 2014).

Phosphorus (P) is an essential macromineral for animal health, due to its several crucial biological roles (Goselink *et al*., 2015). It is essential for the structural integrity of tissues, cells, and molecules in all living organisms. This includes providing structure and strength to bones and playing a role in cell membrane structure through the phospholipid layer (Grünberg, 2014). Additionally, phosphorous has a regulating function for blood pH via the phosphate buffer system (Zhang *et al*., 2017). Phosphorous is also one of the building units of adenosine triphosphate (ATP), which is an energy compound that living organism uses for storing and releasing energy (Dunn and Grider, 2020). ATP serves as the primary source of energy for almost all cellular processes(Neupane *et al*., 2019). Hypophosphatemia slows ATP generation and red blood cell glycolysis (Sharma *et al*., 2018).

ISSN: 2090-6277/2090-6269/

This might cause hemoglobinemia and hemoglobinuria as a result of making RBCs more fragile, susceptible to structural and functional alterations(Resum *et al*., 2017).

Several factors can alter serum phosphorus levels. The dietary cation-anion ratio is one of these factors. Anionic diets may provide more P availability than cationic diets (Beighle *et al*., 1988). Blood phosphorus levels may be negatively impacted by reduced blood calcium levels. When calcium levels drop, parathyroid hormone is released, which lowers serum phosphorus levels.(Centeno *et al*., 2019). A low level of serum phosphorus might result from a high calcium- to- phosphorus ratio in the diet (Heaney and Nordin, 2002).

The role of oxidative stress (OS) in the development of PPH was the subject of several studies (Fayed *et al*., 2018b; Gahlawat *et al*., 2007). During the periparturient period, OS can occur when there is an imbalance between the production of oxidants and the capacity of antioxidants to neutralize them. this can cause cellular damage and dysfunction (Sordillo and Mavangira, 2014). Several studies performed in the last decade indicate that adult dairy cows experience OS around the time of calving (Castillo *et al*., 2006; Tsuchiya *et al*., 2020). The ability of erythrocytes to use glucose and make ATP is impacted by phosphorus deprivation, which lowers glutathione levels and limits its synthesis, making the cells more vulnerable to the harmful effects of oxidants (Sarma *et al*., 2014).

To the best of the authors' knowledge, the epidemiology and risk factors of PPH in buffalo in Egypt are scarce. Therefore, the aim of the present study was to conduct an epidemiological investigation and the contributing factors associated with PPH in dairy buffalo in northern Egypt.

MATERIALS AND METHODS

Study animals

A total of 68 buffalo suspected to suffer from clinical PPH were investigated in Qalyubia, Dakahlia, and Dammietta governorates. Simultaneously, 24 clinically healthy (buffalo) from the same localities were also included in the study for analysis and comparison. Clinical signs, epidemiological observations, serum biochemistry and urinalysis were recorded in all the animals. The present study has been approved by Mansoura university Animal Care and use Committee (MU-ACUC:VM.R. 23.09.124). A questionnaire was constructed to explore the hypothesized risk factors. The questionnaire included data concerning each animal and the farming system. Risk factors on animal level were proposed (Table 1).

Blood samples and analyses

Two types of jugular venous blood samples (10 ml each) were collected from the selected animals. The first blood sample was collected with anti-coagulant for hematological studies (total erythrocytic count, hemoglobin concentration and packed cell volume), while the second was collected without anticoagulant then the blood serum was obtained and kept frozen at -20°C till biochemical analysis. Hematological examination for each sample was performed using electronic cell counter (MS 9, France).

Biochemical Analysis of Serum

Macro-element (calcium, phosphorus, magnesium), and electrolytes (Sodium and potassium) were measured spectrophotometrically by using commercial test kits (Tecodiagnostics, 1268 N. Lakeview AVE ANAHEIM, CA 92807, USA) according to the standard method. Estimation of erythrocytic glutathione peroxidase (GSH-PX) activity in three times washed red blood cells was done according to standard technique (Allen *et al*., 1975). Glucose 6 phosphate was measured using commercial test kits (Span diagnostics LTD plot No 336.338 SachiN 394230 (India). Aspartate amino transferase (AST), alanine amino transferase (ALT)were measured using commercial test kits (SEppim S.A.S zone industrial 61500 france).

Urinalysis

Urine samples were collected from each animal using a sterilized catheter into clean, dry, sterilized brown colored glass bottles, brought to laboratory immediately and processed for gross, microscopic, and biochemical analysis. These urine samples were examined for color, pH, proteins, and blood (hemoglobin). For microscopic examination, urine sediment was obtained by centrifuging the sample at 3000 rpm for 5 minutes. A drop of sediment was placed on a clean, grease free glass slide, covered with a cover slip and examined under the microscope to identify erythrocytes, leukocytes (pus cells), epithelial cells, casts, crystals, and bacteria. The maximum, minimum and mean number of RBCs, WBCs, and epithelial cells per high power field per case was calculated and scored accordingly.

Statistical analyses

For all statistical analyses, GMP SAS a commercial statistical software, was used (SAS for Windows, version, SAS Inc, USA). As a first step, descriptive statistics, and the distribution of contributing factors for cases of PPH were accomplished. On animal levels, the logistic regression analysis was conducted to test the association between and the possible risk factors. In an initial step, univariate logistic regression statistics were conducted. In such a process, the category of the cattle (diseased or not) was the dependent dichotomous variable, but the proposed risk factors were the independent variables. Then, independent factors with a significant association ($P<0.1$) were included in the multivariate backward stepwise logistic regression analysis. The regression coefficient (β), odds ratio (OR), confidence interval (CI: 95%), standard error, and P value were the parameters in the results for each variable. For interpretation of the OR, value of OR greater than one indicates that the ill-thrift is more likely to take place than not, while OR less than one indicates that the PPH is less likely to occur than not. For hematological and biochemical parameters, unpaired student t-test was used as the data were found normally distributed. Results were considered significant at $P< 0.05$ in every statistical analysis.

RESULTS

Clinical Signs

The most prominent clinical sign in all PPH-affected buffalo was the passing of red to coffee colored urine depending upon the severity and stage of the disease. Other symptoms included dullness, anemia, and dehydration. The body temperature was within normal range. Respiration and pulse rates were significantly ($P<0.05$) accelerated, whereas ruminal motility was significantly weak and reduced (Table 1).

In the advanced stage of the disease, anorexia was well marked, accompanied with ruminal stasis and severe straining while defecating. However, some cases continued eating nor-

Table 1. Comparison of clinical parameters in healthy and PPH-affected buffalo.

Parameters	Healthy buffalo (No=24)	PPH –affected buffalo (No=68)
Rectal Temperature (°C)	37.5 ± 0.29	38.4 ± 0.28
Pulse rate (per minute)	54.73±3.88[a]	72.6± 9.90[b]
Respiration rate (per minute)	14.55±0.93[a]	24.47±4.87[b]
Ruminal motility (per 2 minutes)	3.60±0.5[a]	2.52±0 89[b]
Color of urine	Amber yellow	Red or coffee color

Variables with different letters in the same row differ significantly (P<0.05).

Table 2. Comparison of biochemical parameters in healthy and post-parturient hemoglobinuria (PPH) affected buffalo.

Parameters	Healthy buffalo (No=24)	PPH-affected buffalo (No=68)
Calcium (mg/dl)	9.66+0.32 [a]	6.2 ± 0.24b
Phosphorus (mg/dl)	5.11±0.62[a]	1.80+0. 82[b]
Sodium (mmol/l)	113.5 ± 3.3	120.2 ± 2.9
Potassium (mmol/l)	4.16+0.63[a]	9.12+01.17 [b]
G6PD (U/mg Hb)	5.1 ± 0.11[a]	2.31 ± 0.43 [b]
Glutathione peroxides (U/mg Hb)	33.5 ± 1.2[a]	22.2 ± 1.7 [b]
AST (U/L)	165.6 ± 23.3[a]	216.8 ± 15.6 [b]
ALT (U/L)	63.7 ± 5.1 [a]	79.7 ± 3.35 [b]
Glucose (mg/dl)	56.31± 7.16[a]	71.05±7.57 [b]
Magnesium (mg/dl)	6.18±1.14 [a]	5.16 ± 0.69 [b]

Means with different letters in the same row differ significantly (P<0.05).

mally for the first day after passing discolored urine and showed loss of appetite subsequently. The feces were normal to hard or sometimes even loose in consistency.

Biochemical Analysis

The serum Ca, P, and Mg levels of PPH-affected buffalo significantly decreased when compared to control ones, while the serum K levels significantly increased, but the serum Na levels changed insignificantly. Serum AST, ALT, and glucose levels showed a significant increase in PPH-affected when compared with control ones. Regarding OS parameters, G6PD, and GPX showed a significant decrease (Table 2).

Urinalysis of PPH animals

The color of urine in hemoglobinuric buffalo ranged from red (28%), dark red (20%) to coffee colored (52%) depending upon the severity and duration of illness. The pH of urine in hemoglobinuric animals (8.39±0.26) was significantly higher (p<0.05) than in healthy animals (7.91+0.25). The urine of hemoglobinuric buffalo was positive for hemoglobin (100%) and albumin (95%), but not the urine of healthy animals. Microscopic examination of urine of hemoglobinuric buffaloes revealed no intact erythrocytes but few epithelial cells and crystals (amorphous phosphate and triple phosphate) were present.

Results of epidemiological or Risk factors in PPH -affected Buffalo

The descriptive statistics and findings of the univariate analysis of the variables linked to PPH are presented in Tables 3 and 4. The animal level univariate statistical analysis found an insignificant correlation between PPH and the spring season (P = 0.503, OR: 1.41, 95% CI: 0.56-3.87). Thus, 19.12% of the PPH-affected buffalo were diagnosed during winter, 36.76% during spring, and 44.12% during summer. Buffalo with parity ≥4 was strongly correlated with PPH (P= 0.038, OR: 3.18, 95% CI: 1.07 -9.5). When 68 buffalo with PPH were examined, 16.18% had parity1, 14.7% had parity 2, 23.53% had parity 3, and 45.59% had parity≥ 4. Regarding the stage of location, post-partum hemoglobinuria (PPH) was shown to be significantly correlated with early lactation (P = 0.026, OR: 3.04, 95% CI: 1.14-8.75) 60.3% of the PPH-affected buffalo had their cases detected in the early lactation stage, 19.12% in the middle of lactation, and 20.58% in the late lactation stage. Regarding milk production, it was shown that there was no correlation between PPH and high milk production (P = 0.063, OR: 2.82, 95% CI: 0.94-8.45). The percentage of PPH-affected buffalo with low milk yield was 26.48%, that with moderate milk yield was 30.88%, and that with high milk yield was 42.64%. PPH and barseem feeding were observed to have no connection (P = 0.45, OR: 1.42, 95% CI: 0.56-3.63). Whereas 39.7% of the PPH-affected buffalo were not fed barseem, 60.3% were. Regarding serum electrolytes, the correlation between hypophosphatemia and PPH was significant PPH (P = 0.001, OR: 6.5, 95% CI: 2.3-18.1). While 17.65% of the afflicted buffalo had normal serum phosphorus levels, 82.35% of them had hypophosphatemia. Additionally, a significant association between PPH and hypocalcaemia (P = 0.004, OR: 5.1, 95% CI: 1.7-15.29). Where buffalo with hypocalcaemia represented 57.35% and those with normal calcium levels represented 42.65%. Whereas the association between PPH and hypomagnesmia was insignificant (P = 0.13, OR: 2.11, 95% CI: 0.81-5.4). Only 55.88%, of PPH-affected buffalo had low serum magnesium levels, while, the remaining 44.12% demonstrated normal magnesium levels. PPH and hypernatremia were not significantly associated (P = 0.76, OR: 1.1, 95% CI: 0.44-3.1). 36.76% of the buffalo with PPH had low serum sodium levels, whereas 63.24% had normal levels. Similar to this, there was no association between PPH and hypokalemia (P = 0.92, OR: 0.93, 95% CI: 0.22-3.8). Only 11.76% of buffalo with PPH showed hypokalemia, whereas 88.24% had normal potassium levels.

Only three risk factors were significantly correlated with PPH in the multivariate logistic regression model, including buffalo with parity≥ 4 (P= 0.036, OR: 3.76, 95% CI: 1.09-12.96), hypophosphatemia (P= 0.003, OR: 5.55, 95% CI: 1.78-17.27), and hypocalcaemia (P= 0.044, OR: 3.39, 95% CI: 1.03-11.15) (Table 5).

Table 3 Classifications of risk factors in buffalo with PPH and healthy ones.

Variable	PPH Number		Healthy Number	
	(68)	%	(24)	%
Seasons				
Winter:	13	19.12	2	8.3
Spring:	25	36.76	7	29.2
Summer:	30	44.12	15	62.5
Autumn:	0	0	0	0
Parity				
1:	11	16.18	6	25
2:	10	14.7	7	29.2
3:	16	23.53	6	25
≥4:	31	45.59	5	20.8
Stage of lactation or days in milk:				
Early 1-45d:	41	60.3	8	33.33
Mid 46-75d:	13	19.12	10	41.66
Late 76-150d	14	20.58	6	25
Milk production:				
Low (<5 kg/day):	18	26.48	7	29.17
Moderate(5-10kg/day):	21	30.88	12	50
High (>10 kg/day):	29	42.64	5	20.83
Barseem:				
Barseem	41	60.3	12	50
No barseem	27	39.7	12	50
Calcium				
Hypocalcemia	39	57.35	5	20.8
Normal	29	42.65	19	79.2
Phosphorus				
Hypophoshatemia	56	82.35	10	41.67
Normal	12	17.65	14	58.33
Magnesium				
Hypomagnesemia	38	55.88	9	37.5
Normal	30	44.12	15	62.5
Sodium				
Hyponatremia	25	36.76	8	33.33
Normal	43	63.24	16	66.67
Potassium				
Hypokalemia	8	11.76	3	12.5
Normal	60	88.24	21	87.5

DISCUSSION

Post-parturient hemoglobinuria is one of the metabolic diseases that occurs mainly during the transition from pregnancy to lactation (Bhikane and Syed, 2014). Post-partum hemoglobinuria in buffalo can cause a variety of clinical symptoms. The most reported ones include voiding urine that ranges in color from red to coffee, dullness, anemia, decreased milk production, pale mucous membranes, and elevated respiratory and pulse rate (Athar, 2001; Jain *et al*., 2009; Madheswaran *et al*., 2017; Ok *et al*., 2009; Shalini *et al*., 2015). The pale mucous membranes and the significant increase in pulse rate and respiratory rate as well as, the significant decrease in the rate and force of ruminal motility reported in PPH-affected buffalo in this study are in line with those of previous reports (Karapinar *et al*., 2006; Khan and Akhtar, 2007). These symptoms were related to hemolysis of RBCs, a reduction in haemoglobin concentration, and PCV due to the increased fragility of erythrocytes that causes hemolysis, according to Radwan and Rateb (Radwan and Rateb, 2007). The gastrointestinal problems associated with PPH, such as ruminal stasis and constipation may be related to increased hemosiderin production and deposition in the gastro-intestinal mucosa (Digraskar *et al*., 1991). In contrast to our findings, a significant decrease in pulse rate was reported in PPH-affected buffalo compared to healthy ones (Deeba and Bashir, 2019).

In the present investigation, the significant decrease in serum P levels is consistent with previous findings of previous studies (Akhtar *et al*., 2008; Durrani *et al*., 2010; Fayed *et al*., 2018a; Karapinar *et al*., 2006) who reported a similar decrease in serum P levels in buffalo with PPH. As the pregnancy advances, the fetus's need for Ca and P increases; if these needs are not met, hypophosphatemia will ensue. Additionally, hypophosphatemia may be caused by a low phosphorus absorption from the digestive system due to the high calcium -to -phosphorus ratio (Digraskar *et al*., 1991). High P loss during milk production, as well as ingestion of cruciferous vegetables or beetroot products, may be contributing causes to hypophosphatemia (Constable *et al*., 2016).

The significant decrease in serum Ca levels reported in PPH-affected buffalo is previously reported (Deeba and Bashir, 2019; Radwan and Rateb, 2007; Sateesh *et al*., 2017). The increased demand for fetal growth and colostrum outflow are all causes of hypocalcaemia (Pal and Acharya, 2013). However, several studies found that the serum Ca levels of PPH-affected buffalo did not alter significantly (Karapinar *et al*., 2006; Khan and Akhtar, 2007; Ok *et al*., 2009). The present result indicated a considerable rise in serum K levels in PPH-affected buffalo, this is in accordance with recent report (Rashid *et al*., 2021). The elevated levels of K can be linked to the damage of cell membranes (Latimer *et al*., 2003).

The elevated serum ALT and AST levels found in this study are in line with those found in earlier researches in PPH-affected buffalo (Kumar *et al*., 2019; Wakayo *et al*., 2005). The increase in

Table 4 The univariate logistic regression model for animal level risk factors associated with PPH in buffalo.

Variable	[1]β	[2]S.E.	[3]OR	[4]C.I. Lower-Upper	P
Spring season versus other seasons	0.34	0.52	1.41	0.56-3.87	0.50
Parity≥4 versus parity < 4	1.16	0.55	3.18	1.07 -9.5	0.04
Early lactation versus mid and late lactation	1.11	0.5	3.04	1.14-8.75	0.03
High milk yield versus moderate and low milk yield	1.04	0.56	2.82	0.94-8.45	0.06
Barseem feeding	0.36	0.48	1.42	0.56-3.63	0.45
Hypophosphatemia versus normal P levels	1.8	0.52	6.5	2.3-18.1	0.00
Hypocalcaemia versus normal Ca levels	1.63	0.56	5.1	1.7-15.29	0.00
Hypomagnesaemia versus normal Mg levels	0.75	0.49	2.11	0.81-5.4	0.13
Hypernatremia versus normal Na levels	0.15	0.5	1.1	0.44-3.1	0.76
Hypokalemia versus normal K levels	-.07-	0.72	0.93	0.22-3.8	0.92

β: Regression coefficient; [2]SE: Standard error; [3]OR: Odds ratio; [4]CI: Confidence interval at 95%

Table 5 Final multivariate logistic regression model for positive risk factors associated with PPH in buffalo.

Variable	[1]β	[2]SE	[3]OR	[4]CI Lower-Upper	P
Parity≥4 versus parity < 4	1.32	0.63	3.76	1.09 -12.96	0.04
Hypophosphatemia versus normal P levels	1.71	0.58	5.55	1.78 - 17.27	0.00
Hypocalcaemia versus normal Ca levels	1.22	0.6	3. 39	1.03-11.15	0.04
Constant	-0.95-	0.51	0.39		0.06

β: Regression coefficient; [2]SE: Standard error; [3]OR: Odds ratio; [4]CI: Confidence interval at 95%

activities of such enzymes has been attributed to attributed this increase in liver enzymes to the drastic fall in haemoglobin levels as a result of intravascular hemolysis which creates membranes, resulting in the leakage of these enzymes (Akhtar *et al*., 2008).

The significant rise in blood glucose levels observed in PPH-affected buffalo in this study is consistent with the findings of Albayati and Luaibi (2020) in PPH-affected cows, they ascribed the considerable rise in blood glucose levels in PPH-affected cows to a reduction in glucose utilization caused by a P deficit. However, Akhtar *et al*. (Akhtar *et al*., 2008) speculated this increase in blood sugar levels to the anorexia caused by PPH, which impairs the production of volatile fatty acids by the rumen microbiota. As a result, the affected animal must rely on oxidative glucose metabolism, leading to glycogenolysis and subsequent increase in blood glucose levels. On the other hand, this increase in serum glucose levels was attributed to disease-related stress, which raises cortisol levels (Marik and Bellomo, 2013). Oxidative stress can be triggered by the imbalance between antioxidants and oxidants, such as reactive oxygen species and reactive nitrogen species (Leopold, 2023). Excessive levels of free radicals produced during OS may have a number of detrimental effects, such as interference with normal cell division, cell membrane damage, inhibition of vital enzymes, obstruction of vital cellular processes, restriction of energy production, and DNA deterioration. (Kurutas, 2015). According to Möller *et al*. (Möller *et al*., 2022) the pentose phosphate pathway (PPP), one of the main metabolic processes involved in glucose metabolism in red blood cells, is essential in guaranteeing their survival and guarding against oxidative damage.

The significantly decreased values of erythrocytic G6PD activity reported in this study is in line with that previously reported in buffalo with PPH (Fayed *et al*., 2018b). Within the PPP, G6PD mediates the conversion of nicotinamide adenine dinucleotide phosphate (NADP) into reduced nicotinamide adenine dinucleotide phosphate (NADPH) (De Niz *et al*., 2013; Jin *et al*., 2022). This NADPH molecule serves as a reducing agent to provide reduced glutathione (GSH) on a regular basis. GSH scavenges ROS to a significant extent (Leopold *et al*., 2003). The decreased levels of GSH in PPH-affected buffalo may be largely attributable to the decreased erythrocytic G6PD activity, which causes OS to erythrocytes and results in hemolytic disorder (Singari *et al*., 1991).

The significantly decreased activity of GPx reported in PPH-affected buffalo in this study is in accordance with the findings of Zhang *et al*. (Zhang *et al*., 2017), who reported a significant decrease in the activity of GPx in cows with hypophosphatemia. GPx is necessary for free radical elimination (Valko *et al*., 2007). Since, GSH recycling and GSH regeneration regulation are handled by GPx (Chance *et al*., 1979; Salminen and Paul, 2014). Hypophosphatemia could lower GPx activity, which might cause a buildup of lipid peroxidation byproducts. Erythrocyte membrane damage might eventually arise from this accumulation (Zhang *et al*., 2017).

Regarding the identification of the association between PPH in buffalo and different risk factors at the animal level, the high correlation shown between PPH and buffalo of parity ≥ 4 is consistent with the findings of Mahmood *et al*. (Mahmood *et al*., 2012), who found that buffalo with three or more parities were 6.39 times more likely to develop PPH than those with two or fewer parities. A similar conclusion was reported in a recent study in cows (van den Brink *et al*., 2023). The increased stress on the mineral balance during the peak milk production from the third to sixth lactation may be the cause of the rise in the incidence of PPH in buffalo with higher parity (Khan and Akhtar, 2007).

The findings of this study showed that PPH was significantly associated with both hypophosphatemia and hypocalcemia. It was shown that the majority of instances with hypophosphatemia have hypocalcemia as a primary contributing factor. This is probably because blood calcium and phosphorus levels are closely related to one another (Braun *et al*., 2009). Increased parathyroid hormone (PTH) secretion induced by hypocalcaemia produces hypophosphatemia. This occurs on by a rise in phosphorus excretion from the kidneys resulting from high PTH levels, which enables calcium retention. (Chugh *et al*., 1996). Phosphorus, tends to accumulate in the rumen (Oetzel, 2020). In contrast to the findings of previous study (Chugh *et al*., 1996), the results of this investigation indicate that high milk output is not a risk factor for PPH. However, it is analogous to the findings of Mahmood *et al*. (Mahmood *et al*., 2012)), who showed that the odds ratio of PPH in buffalo with a milk output of 8 liters/day was 1.07, demonstrating that PPH is not connected to a high milk yield. This study's findings, which show a negligible correlation between PPH and calving season, are at odds with numerous other researches that found a seasonal pattern in the prevalence of PPH. A higher incidence of PPH cases were recorded during winter (Bhikane *et al*., 2004; Sharma *et al*., 2014; Soren *et al*., 2014). The increased incidence of PPH during winter may be attributed to cold stress in recently calved animals (Bhikane *et al*., 2011). According to the findings of Macwillims *et al*. (Macwilliams *et al*., 1982), post-parturient hemoglobinuria is more common during the winter months, especially when preceded by dry growth. Meanwhile, a few studies (Dhonde *et al*., 2007; Jain *et al*., 2009) found a greater prevalence during the summer. The availability of phosphorus in the soil and the diets supplied to buffalo are likely connected to the seasonal occurrence (Akhtar *et al*., 2007). Additionally, the stress during the hot summer months may exacerbate the condition (Purohit *et al*., 2018). The insignificant association between PPH and berseem feeding reported in this study contradicts the findings of (Khan and Akhtar, 2007), which found a strong association between PPH with berseem feeding. The current study's findings of a negligible correlation between PPH and lactation stage are also contradictory to the earlier research (Mahmood *et al*., 2012) which stated that early lactation (1-60 days) is a substantial risk factor for PPH. However, it is similar to Heuer and Bode (Heuer and Bode, 1998), who concluded that early lactation is not associated with PPH.

CONCLUSION

The current study reveals that PPH is a significant metabolic disorder affecting Egyptian buffalo. PPH can cause many metabolic changes which has a detrimental influence on animal health and production. Identification of PPH-related metabolic changes may aid in determining the pathophysiology of the disease and developing an effective treatment plan. This research also gives the first information on the potential risk factors for PPH in Egyptian buffalo. These findings can be helpful in creating an efficient plan for the prevention and management of such metabolic problems.

ACKNOWLEDGMENTS

The authors acknowledge the Deanship of Scientific Research at King Faisal University for the financial support [GRANT No 4027].

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Akhtar, M.Z., Khan, A., Khan, M.Z., Javaid, A., 2008. Patho-biochemical changes in buffaloes (*Bubalus bubalis*) suffering from parturient haemoglobinuria. Pakistan Veterinary Journal 28, 139-43.

Akhtar, M.Z., Khan, A., Khan, M.Z., Muhammad, G., 2007. Haemato-biochemical aspects of parturient haemoglobinuria in buffalo. Turkish Journal of Veterinary & Animal Sciences 31, 119-123.

Albayati, O., Luaibi, O.K., 2020. Study of hemato-biochemical changes in post-parturient hemoglobinurea in Iraqi local cows. Plant Arch. 2584-588..

Allen, W., Parr, W., Anderson, P., Berett, S., Bradley, R., 1975. Selenium and the activity of glutathione peroxidase in bovien erythrocytes. The Veterinary Record 96, 360-361.

Athar, G.M.M.S.M., 2001. A rational approach to diagnosis, treatment and control of parturient haemoglobinuria (red water) in buffaloes and cattle. Pakistan Veterinary Journal 21, 214-219.

Beighle, D., Tucker, W., Hemken, R., 1988. Interactions of dietary cation-anion balance and phosphorus: effects on growth and serum inorganic phosphorus in dairy calves. Journal of dairy science 71, 3362-3368.

Bhikane, A., Anantwar, L., Bhokre, A., Narladkar, B., 2004. Incidence, clinico-pathology and treatment of haemoglobinuria in buffaloes. Indian veterinary journal 81, 192-197.

Bhikane, A., Ghoke, S., Masare, P., Salunke, V., Syed, A., 2011. A new approach in clinical management of phosphorous deficiency haemoglobinuria (PDH) in buffaloes. Intas polivet 12, 56-58.

Bhikane, A., Syed, A., 2014. Recent trends in management of metabolic disorders of transition cows and buffaloes. Intas Polivet 15, 485-496.

Braun, U., Zulliger, P., Liesegang, A., Bleul, U., Hässig, M., 2009. Effect of intravenous calcium borogluconate and sodium phosphate in cows with parturient paresis. Veterinary Record 164, 296-299.

Castillo, C., Hernandez, J., Valverde, I., Pereira, V., Sotillo, J., Alonso, M.L., Benedito, J., 2006. Plasma malonaldehyde (MDA) and total antioxidant status (TAS) during lactation in dairy cows. Research in veterinary Science 80, 133-139.

Centeno, P.P., Herberger, A., Mun, H.-C., Tu, C., Nemeth, E.F., Chang, W., Conigrave, A.D., Ward, D.T., 2019. Phosphate acts directly on the calcium-sensing receptor to stimulate parathyroid hormone secretion. Nature communications 10, 4693.

Chance, B., Sies, H., Boveris, A., 1979. Hydroperoxide metabolism in mammalian organs. Physiological reviews 59, 527-605.

Chugh, S., Mata, M., Malik, K., 1996. Epidemiological observations on post-parturient haemoglobinuria in buffaloes. Indian journal of animal sciences 66, 1123-1125.

Constable, P.D., Hinchcliff, K.W., Done, S.H., Grünberg, W., 2016. Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats. Elsevier Health Sciences.

De Niz, M., Eziefula, A.C., Othieno, L., Mbabazi, E., Nabukeera, D., Ssemmondo, E., Gonahasa, S., Tumwebaze, P., DiLiberto, D., Maiteki-Sebuguzi, C., 2013. Tools for mass screening of G6PD deficiency: validation of the WST8/1-methoxy-PMS enzymatic assay in Uganda. Malaria journal 12, 1-11.

Deeba, F., Bashir, A., 2019. Investigations on copper and phosphorus deficiency associated post-parturient hemoglobinuria in dairy animals and clinical management with antioxidants. Journal of Entomology and Zoology Studies 7, 426-431.

Dhonde, S., Digraskar, S., Chavan, V., 2007. Phosphorus Deficiency Haemoglobinuria in Buffaloes (*Bubalus bubalis*). Intas Polivet 8, 382-386.

Digraskar, S., Singh, B., Deshpande, B., 1991. Epidemiology and clinico-pathology of haemoglobinuria in buffalo (*Bubalus bubalis*). Livestock Advisor 16, 32-38.

Dunn, J., Grider, M.H., 2020. Physiology, adenosine triphosphate. Treasure Island (FL): StatPearls Publishing.

Durrani, A.Z., Kamal, N., Shakoori, A.R., Younus, R.M., 2010. Prevalence of post parturient haemoglobinuria in buffalo and therapeutic trials with toldimfos sodium and tea leaves in Pakistan. Turkish Journal of Veterinary & Animal Sciences 34, 45-51.

Fayed, H., Ghanem, M., Abdel-Raof, Y., El-Attar, H., 2018a. Hematobiochemical and urological alterations in buffaloes with post parturient haemoglobinuria. Benha Veterinary Medical Journal 34, 287-294.

Fayed, H., Ghanem, M., Abdel-Raof, Y., El-Attar, H., 2018b. Oxidative stress and antioxidant activity in buffaloes with postparturient hemoglobinuria. Benha Veterinary Medical Journal 34, 57-63.

Gahlawat, I., Singh, K., Kumar, R., 2007. Investigations on oxidative stress in post-parturient haemoglobinuria in buffaloes receiving sodium acid phosphate therapy. Italian Journal of Animal Science 6, 974-977.

Goselink, R., Klop, G., Dijkstra, J., Bannink, A., 2015. Phosphorus metabolism in dairy cattle: literature study on recent developments and gaps in knowledge. Published by Wageningen UR Livestock Research https://research.wur.nl/en/publications/phosphorus-metabolism-in-dairy-cattle-literature-study-on-recent-

Grünberg, W., 2014. Treatment of phosphorus balance disorders. Veterinary Clinics: Food Animal Practice 30, 383-408.

Heaney, R.P., Nordin, B., 2002. Calcium effects on phosphorus absorption: implications for the prevention and co-therapy of osteoporosis. Journal of the American College of Nutrition 21, 239-244.

Heuer, C., Bode, E., 1998. Variation of serum inorganic phosphorus and association with haemoglobinuria and osteomalacia in female water buffaloes in Pakistan. Preventive Veterinary Medicine 33, 69-81.

Jain, R., Nimkar, D., Saksule, C., Dhakad, R., 2009. Haemato-biochemical profile of haemoglobinuric buffaloes. Buffalo Bulletin 28, 184-211.

Jin, X., Li, X., Li, L., Zhong, B., Hong, Y., Niu, J., Li, B., 2022. Glucose-6-phosphate dehydrogenase exerts antistress effects independently of its enzymatic activity. Journal of Biological Chemistry 298, 102587.

KARAPINAR, T., DABAK, M., KIRBAŞ, A., 2006. İki inekte tespit edilen puerperal hemoglobinüri ve tedavisi. Fırat Üniversitesi Doğu Araştırmaları Dergisi 5, 7-10.

Khan, A., Akhtar, M., 2007. Hemato-biochemical andclinico-epidemiological aspects of parturient hemoglobinuria in Nili-Ravi buffaloes. Italian Journal of Animal Science 6, 953-956.

Kumar, A., Thakur, V., Sandeep, P., Harpreet, S., Swati, R., Anita, G., Biswa, R., Bisla, R., 2019. Study on incidence, haemato biochemical changes and therapeutic management of post parturient haemoglobinuria in Murrah buffaloes. The Pharma Innovation Journal 8, 147-150.

Kurutas, E.B., 2015. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutrition journal 15, 1-22.

Latimer, K.S., Mahaffey, E.A., Prasse, K.W., 2003. Veterinary laboratory medicine: clinical pathology. Iowa State Press.

Leopold, J.A., Zhang, Y.-Y., Scribner, A.W., Stanton, R.C., Loscalzo, J., 2003. Glucose-6-phosphate dehydrogenase overexpression decreases endothelial cell oxidant stress and increases bioavailable nitric oxide. Arteriosclerosis, thrombosis, and vascular biology 23, 411-417.

Macwilliams, P., Searcy, G., Bellamy, J.E., 1982. Bovine postparturient hemoglobinuria: a review of the literature. The Canadian veterinary journal 23, 309.

Madheswaran, R., Saranya, N., Lavanya, C., Arulmozhi, A., Balasubramaniam, G., 2017. Clinico-Pathological Features of Post Parturient Haemoglobinuria in She Buffaloes. Indian Vet. J. 94, 49-51.

Mahmood, A., Khan, M.A., Younus, M., Khan, M.A., Iqbal, H.J., Ahad, A., 2012. Case-control study of parturient hemoglobinuria in buffaloes. Pak. Vet. J 32, 375-377.

Marik, P.E., Bellomo, R., 2013. Stress hyperglycemia: an essential survival response! Critical care 17, 1-7.

Möller, M.N., Orrico, F., Villar, S.F., López, A.C., Silva, N., Donzé, M., Thomson, L., Denicola, A., 2022. Oxidants and antioxidants in the redox biochemistry of human red blood cells. ACS omega 8, 147-168.

Neupane, P., Bhuju, S., Thapa, N., Bhattarai, H.K., 2019. ATP synthase: structure, function and inhibition. Biomolecular concepts 10, 1-10.

Oetzel, G.R., 2020. Rational treatments for mineral disorders in fresh cows, In: American Association of Bovine Practitioners Conference Proceedings, pp. 92-101.

Ok, M., Guzelbektes, H., Sen, I., Coskun, A., Ozturk, A.S., 2009. Post-parturient haemoglobinuria in three dairy cows. A case report. Bull Vet Inst Pulawy 53, 421-423.

Pal, P., Acharya, H.R., 2013. Subclinical metabolic disorders in post-partum cross bred HF cattle in central part of Nepal. Int. J. Pharm. Med. and Bio. Sci 2, 57-62.

Purohit, G., Ruhil, S., Daga, M., Gaur, M., Bihani, D., Ahuja, A., 2014. Par-

turition related metabolic disorders in buffaloes: A 10 year case analysis. Ruminant Science 3, 241-244.

Purohit, G., Trilok, G., Amit, K., Atul, S., Mitesh, G., Chandra, S., 2018. Perspectives of parturient hemoglobinuria (pph) in buffaloes. International Journal of Development Research 2, 23513-23520.

Radwan, M., Rateb, H., 2007. Clinical, haematological and some biochemical variations hypophosphataemia in buffaloes before and after treatment at Assiut Government. Journal of Veterinary Medical Research 17, 35-41.

Rahmati, S., Aziz, A., Tawfeeq, M.M., Zabuli, J., Nazhat, S.A., 2021. Clinical features of post-parturient hemoglobinuria in dairy cattle and Buffaloes: A Review. Open Journal of Veterinary Medicine 11, 143.

Rashid, S.M., Amin, I., Ahmad, R., Razak, R., Rashid, S.A., u Rahman, M., 2021. Biochemical and haematological aspect of hypophosphatemia in pregnant Murrah buffaloes. Buffalo Bulletin 40, 389-397.

Resum, N.S., Kour, P., Singh, H., Sharma, N., 2017. Post-partum hemoglobinuria (PPH) in bovine. Theriogenology Insight-An International Journal of Reproduction in all Animals 7, 51-59.

Salminen, L.E., Paul, R.H., 2014. Oxidative stress and genetic markers of suboptimal antioxidant defense in the aging brain: a theoretical review. Reviews in the Neurosciences 25, 805-819.

Sarma, K., Saravanan, M., Kumar, P., Kumar, M., Jadav, R., Mondal, D., 2014. Influence on haemato-biochemical and oxidative indices of post parturient haemoglobinuric (PHU) buffalo. Buffalo Bulletin (December 2014) 3, 343-348 .

Sateesh, A., Roopali, B., Ravindra, B., Abhilash, B., Dhabale, R., 2017. A study on comparision of haemato-biochemical changes in haemoglobinuria buffaloes. The Pharma Innovation 6, 125.

Shalini, A., Sivaraman, S., Vijayakumar, G., Venkatesakumar, E., Ramyadevi, R., 2015. Successful treatment of postparturient haemoglobinuria with acid inorganic phosphorus in a Murrah buffalo. Shanlax Int. J. Vet. Sci 3, 30-32.

Sharma, S., Hashmi, M.F., Castro, D., 2018. Hypophosphatemia. StatPearls. Treasure Island (FL): StatPearls Publishing. Copyright © 2020, StatPearls Publishing LLC. (2020).

Sharma, S., Joshi, M., Singh, D., Khosa, J., 2014. A haemato-biochemical and therapeutic study of postparturient haemoglobinuria in buffaloes. Intas Polivet 15, 523-525.

Sharma, V., Kumar, H., Kumar, S., Yadav, M., Bisht, P., Mishra, A.,2020. Therapeutic management of post-parturient haemoglobunuria in buffalo: A case report. The Pharma Innovation Journal 9, 299-301.

Singari, N., Bhardwaj, R., Chugh, S., Bhandwaj, S., 1991. Status of erythrocytic glucose-6-phosphate dehydrogenase (G6PD) in phosphorus deficiency haemoglobinuria of buffaloes. Indian Vet. J 68, 226-230.

Sordillo, L., Mavangira, V., 2014. The nexus between nutrient metabolism, oxidative stress and inflammation in transition cows. Animal Production Science 54, 1204-1214.

Soren, S., Srivastava, M., Kachhawa, J., Soren, P., Kumari, A., Sharma, A., 2014. Clinical studies on postparturient haemoglobinuria in buffaloes. Intas Polivet 15, 518-522.

Stockdale, C., Moyes, T., Dyson, R., 2005. Acute post-parturient haemoglobinuria in dairy cows and phosphorus status. Australian Veterinary Journal 83, 362-366.

Tsuchiya, Y., Kawahara, N., Kim, Y.-H., Ichijo, T., Sato, S., 2020. Changes in oxidative stress parameters in healthy and diseased Holstein cows during the transition period in Yamagata Prefecture, Japan. Journal of Veterinary Medical Science 82, 955-961.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M., Telser, J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. The international journal of biochemistry & cell biology 39, 44-84.

van den Brink, L.M., Cohrs, I., Golbeck, L., Wächter, S., Dobbelaar, P., Teske, E., Grünberg, W., 2023. Effect of Dietary Phosphate Deprivation on Red Blood Cell Parameters of Periparturient Dairy Cows. Animals 13, 404.

Wakayo, B., Vaungahun, E., Brar, P., 2005. Diagnosis and Treatment of Postparturient Haemoglobinurea in Buffalo; A Case Report. Doi 10, 13140.

Whitaker, D., 1999. Goodger WJ Garcia M., Perera BMAO, Wittver F.: Use of metabolic profiles in dairy cattle in tropical and subtropical countries on small holder dairy farms. Prev. Vet. Med. 38, 119-131.

Yadav, A., Jhambh, R., Singh, Y., Kumar, S., 2023. Clinico-haematobiochemical Profiling and Therapeutic Studies on Hypophosphatemic Dairy Buffaloes. Journal of Animal Research 13, 273-277.

Zhang, Z., Bi, M., Yang, J., Yao, H., Liu, Z., Xu, S., 2017. Effect of phosphorus deficiency on erythrocytic morphology and function in cows. Journal of veterinary science 18, 333.