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The Point Prevalence, Etiological and Hemato-Biochemical Investigations of Post-Parturient Haemoglobinuria (PPHU) in Buffalo Population in Tehsil Bhalwal, Pakistan

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Abstract

The current study was designed to explore all possible etiological features including mineral composition of soil and their effects on hemato-biochemical profile in buffaloes suffering from post-parturient hemoglobinuria. The total 384 buffaloes were randomly selected from Tehsil Bhalwal, District Sargodha, Pakistan. The post parturient hemoglobinuria was observed during the period of four months, from November 2016 to February 2017. The parameters including point prevalence of PPHU and hemato-biochemical profile were investigated. The data was analyzed using T-test and regression analysis through SPSS software. Out of 384 animals, 40 (10.4%) were confirmed with PPHU. All hemato-biochemical profile showed significant difference between healthy and affected groups instead mean corpuscular hemoglobin concentration (MCHC), serum calcium and glucose, which were not significant. Vital signs including pulse and respiration showed significant difference but heart rate was found non-significant between both groups. The results regarding mineral analysis of the soil showed significant difference in phosphorus and copper and non-significant difference in calcium and molybdenum. Moreover, strong positive correlation was associated with phosphorus and molybdenum level while moderate positive correlation was attributed to the calcium and copper level in the soil. It is concluded that post parturient hemoglobinuria in buffaloes of Tehsil Bhalwal was associated with variation of soil composition, particularly the deficiency of phosphorus which is main cause of hemoglobinuria. It is necessary to fulfill the minerals requirement of soil and animal's feed to overcome the problems of PPHU in affected areas.

Keywords: Point Prevalence, Hemato-biochemical, Post-parturient Haemoglobinuria, Buffaloes

Introduction

Dairy animals play a vital role in economy of the country in terms of milk and meat production(Mufti et al. 2014). The buffaloes are a significant livestock resource with more than 96% of the population located in Asia comprising 16.4% of population in Pakistan(Warriach et al. 2015). Buffaloes contribute about 68% out of total milk production in Pakistan(Bilal et al. 2006). They are considered as the principal milk production animals of Pakistan(Yousaf et al. 2016).

Among major disorders of buffaloes, post-parturient haemoglobinuria affects a substantial amount of buffaloes each year after initial lactation and it is an active hazard to the milking buffaloes, not only in Pakistan but also in the whole world (Ghanem et al. 2010; Mahmood et al. 2013). This condition is considered as emergent economic significance with growing number of cases in Pakistan(Dalir-Naghadeh et al. 2006).The exact pathogenesis is not yet known but phosphorus reduction or hypophosphatemia is considered to be a major predisposing factor in high milk producing dairy animals. Decrease in phosphorus level in RBCs causes increase in osmotic tenderness of RBCs and intravascular hemolysis. Copper depletion also cause oxidative trauma to RBCs which is associated with consumption of cruciferous plants, dietary phosphorous insufficiency, and saponin from berseem (Brechbühl et al. 2008; Neto et al. 2007). This is a critical disease condition with accompanying hypophosphatemia is described by intravascular hemolysis and anemia with potentially lethal results (Durrani et al. 2010; Reddy et al. 2014). A possible indicator of the bioavailability of feed phosphorus in dairy cows is the plasma inorganic phosphatelevel (Montiel et al. 2007) and hemoglobin level to assess the presence of anemia(Turgut 2000).

The clinical signs including bright red to coffee colored urine, a slight rise in body temperature, malaise, a sharp decline in milk yield with low mortality has been observed (Dhillon et al. 2007).

Hypophosphatemia eventually results because of reduction inerythrocytic glycolysis and ATP synthesis which leads to haemoglobinemia and hemoglobinuria (Constable et al. 2016; Akhtar et al. 2007; Singari et al. 1991).The viable structure and function of mammalian red blood cells depend on glucose metabolism (Constable et al. 2016).Severe stress due to intravascular hemolysis and glucocorticoids release in post-parturient hemoglobinuria affects all activities of various body organs mainly liver, heart and kidneys (Akhtar et al. 2008). Glucose-6-phosphate dehydrogenase protects hemoglobin from oxidative degeneration as it is part of intra-erythrocytic metabolic chain. The precipitation and formation of Heinz bodies takes place by denaturation of globin from the depletion of glucose-6-phosphate dehydrogenase (Mahmood et al. 2013). During third to sixth lactation of adult dairy buffaloes, this disease is observed more frequently with progressive weakness and recumbency (Durrani et al. 2010). Molybdenum and copper deficiency are also associated with hypophosphotemia following haemoglobin (Hb) urea (Ahmad et al. 2013; Kahn et al. 2005; Akhtar et al. 2006). Copper deficiency is induced by hyper-molybdenosis which is regarded as additional key factor triggering post parturient haemoglobinuria (Kahn et al. 2005).

There is a large buffalo population in and around Tehsil Bhalwal, District Sargodha, Pakistan. These cities are currently being reported with the cases of post-parturienthaemoglobinuria. Consequently, present study has been designed to explore all possible etiological features including mineral composition of soil of the area which was being used for fodder cultivation and their possible effect on biochemical examination in buffaloes suffering from post-parturient hemoglobinuria.

Materials and Methods

Study Area

The samples were collected from Bhalwal – a tehsil of district Sargodha during the month of November 2016 to February 2017. Its coordinates include 32° 15’ N latitudes and 72° 54’ Elongitudes. The average annual rainfall is 403 mm.

Point Prevalence Recording

Point prevalence of buffaloes affected with post parturient hemoglobinuria was observed during the period of four months, from November 2016 to February 2017. The sample size was determined to three hundred and eighty four animals (n=384) at 95% confidence interval in addition to 5% precision level with expected prevalence of 50% as previously described by Thrusfield (2013).

$$n = 1.96^2 \text{ x } Pexp(1 - Pexp)/d^2$$

n = required sample size, Pexp= expected prevalence, and d= desired absolute precision

Inclusion and Exclusion Criteria

Buffaloes showing signs of haemoglobinuria along with parturition history, pale mucous

membranes, mild tachycardia and dyspnea were assumed as affected with post-parturient haemoglobinuria while animals suffering from other diseases affecting red urine were omitted from the study after validation of diagnosis through standard laboratory methods (Constable et al. 2016). Then the blood samples were processed for haematological analysis for the final confirmation of positive haemoglobinuric buffaloes. The data regarding animal's milk yield and parity were also entered in data capture form.

Collection of Samples

Blood samples were taken from affected and healthy animalswere obtained to study parameters such as blood parasites, haemoglobin (Hgb), erythrocyte count (RBC), white blood count (WBC), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet (PLT) count,erythrocyte sedimentation rate (ESR), alkaline phosphatase, glucose, total bilirubin, blood urea nitrogen, creatinine, phosphorus, calcium, copper, molybdenum, glucose, and urine analysis explained by Abdellah et al. (2014); Ali et al. (2014).

Statistical Analysis

The data was analyzed using T-test and regression analysis with the help of SPSS software.The levelof significance was kept P≤0.05. Data were presented as mean ± SD (Daniel, 2022).

Results

The present study was carried out in Tehsil Bhalwal of district Sargodha, Punjab, Pakistan on post parturient hemoglobinuriain the month fromNovember 2016 to February 2017. From all suspected as well as healthy animals the blood samples were collected, and processed at Department of Pathology, University of Veterinary and Animal Sciences, Lahore. The informations regarding various parameters were collected and then investigated to obtainresults which are stated as follows.

Point Prevalence Results

Out of three hundred eighty four (n=384) buffaloes observed during the period of four months (November 2016 to February 2017), forty seven (n=47) revealed hemoglobinuria. Out of forty seven suspected animals, seven (n=7) were screened out during differential diagnosis through standard laboratory procedures (Constable et al. 2016). Consequently, forty animals (n=40) were confirmed with post parturient hemoglobinuria. Therefore, the point prevalence observed during the period of four months was 10.4% (40/384).

Hematological Results

Results of the study showed significant (P ≤0.05) difference among various hematological parameters including hemoglobin (Hb), total erythrocyte count (TEC), hematocrit (Hct), total leukocyte count (TLC), erythrocyte sedimentation rate (ESR), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). However, mean corpuscular hemoglobin concentration (MCHC) was not significant (P≥0.05).

Serum Biochemical Results

The results of study regarding serum biochemistry reveal significant difference (P≤0.05) of various parameters including alkaline phosphatase (ALP), copper, blood urea nitrogen (BUN), phosphorus, creatinine, total bilirubin and molybdenum. On the other hand, no significant change (P≥0.05) was found among healthy and affected animals regarding blood glucose and serum calcium levels.

Physiological Parameters

Comparison of physiological parameters revealed no significant body temperature differences among the two groups. However, there was significant elevation in pulse and respiration rates in hemoglobinuric buffaloes.

Soil Mineral Analysis

The results regarding mineral analysis of the soil shows significant (P ≤0.05) difference in phosphorus and copper among normal and PPHU affected soils. However, soil levels of calcium and molybdenum didn't differ significantly (P≥0.05).

Correlation of Soil and Serum Minerals Level

The R is actually correlation coefficient. It shows strong positive correlation as if correlation coefficient (R) is more than ≥0.60 and moderate relationship if correlation coefficient (R) is ≥ 0.50 and ≤ 0.60. Results of the linear regression analysis among the mineral levels of soil and serum of animals revealed the strongest positive correlation of phosphorus levels, followed by the molybdenum level. While the calcium and copper levels showed moderate positive relationship. Regression relationship revealed that serum values can be achieved by adding soil value in the equations described above showing significance.

Mean values of various parameters of normal and PPHU affected buffaloes are given in **Table 4.1** and graphical representation is shown in **Figures 4.1-4.4.**

Table 4.1: Various Parameters of Normal and PPHU Affected Buffaloes

Parameters	**Healthy Buffaloes n= 40**	**PP**PPHU **Buffaloes n= 40**	**P-value**
Hematology Profile			
Hemoglobin (g/dl)	11.09±0.41	6.13±0.64	0.000
TEC (10^6/µl)	6.36±0.22	3.46±0.28	0.000
TLC (10^3/µl)	9.73±0.34	10.43±0.43	0.001
Hematocrit (%)	33.89±1.47	18.72±1.54	0.000
ESR (mm/hr)	68.22±5.54	107.8±6.94	0.000
MCV (fL)	47.36±1.97	60±3.94	0.000
MCH (pg)	17.43±0.67	21.20±0.83	0.000
MCHC (g/dl)	36.16±0.72	35.83±0.65	0.304
Serum Biochemistry			
ALP (u/L)	82.73±14.94	233.2±21.47	0.000
Blood Urea Nitrogen (mg/dl)	33.26±2.72	48.42±3.09	0.000
Creatinine (mg/dl)	1.32±0.16	2.04±0.20	0.000
Glucose (mg/dl)	84.60±12.97	82.33±6.56	0.628
Total Bilirubin (mg/dl)	0.50 ± 0.08	1.04 ± 0.06	0.000
Calcium (mg/dl)	9.82 ± 0.52	9.94 ± 0.31	0.541
Phosphorus (mg/dl)	5.3 ± 0.34	1.94 ± 0.32	0.000
Copper (µg/dl)	118.5 ± 3.59	66.34±3.60	0.000
Molybdenum (µg/dl)	54.41±6.80	175.3±15.54	0.000
Physiological/Vital Signs			
Temperature	100.46±0.28	100.78±0.45	0.072
Pulse	54.91±1.56	74.49±3.41	0.000
Respiration	14.33±0.56	22.28±1.39	0.000
	Correlation (R) n= 40	**Regression relationship**	
Calcium	0.557	Serum = 5.822 + 0.016 (soil)	0.011
Phosphorus	0.874	Serum = -0.498 + 0.24 (soil)	0.000
Copper	0.584	Serum = 57.867 + 2.58 (soil)	0.007
Molybdenum	0.797	Serum = 117.39 + 51.1 (soil)	0.000

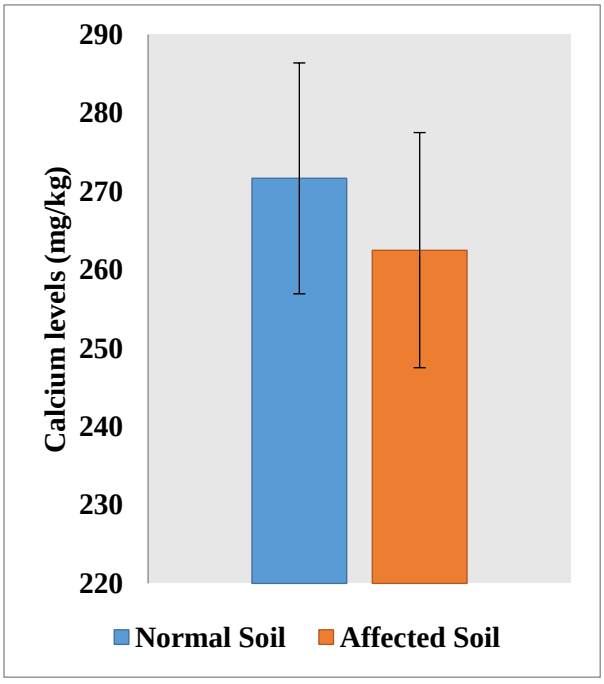

Figure 4.1: Comparison of Calcium Levels in Normal and Affected Soil

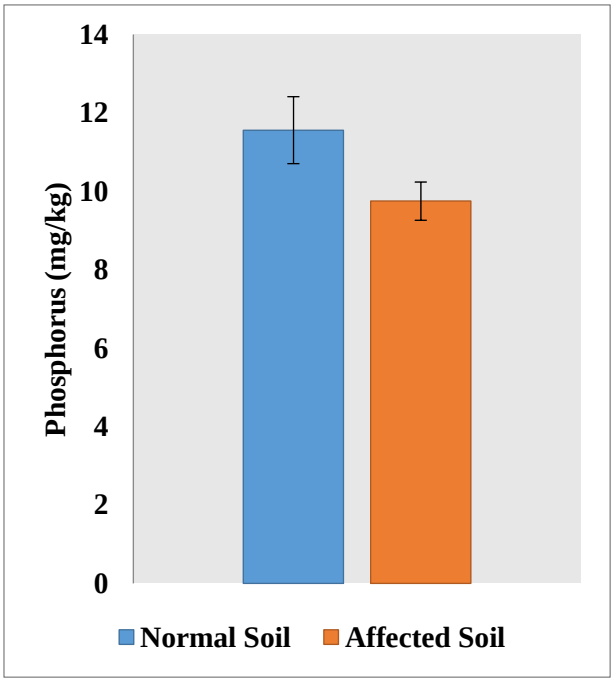

Figure 4.2: Comparison of Phosphorus Levels in Normal and Affected Soil

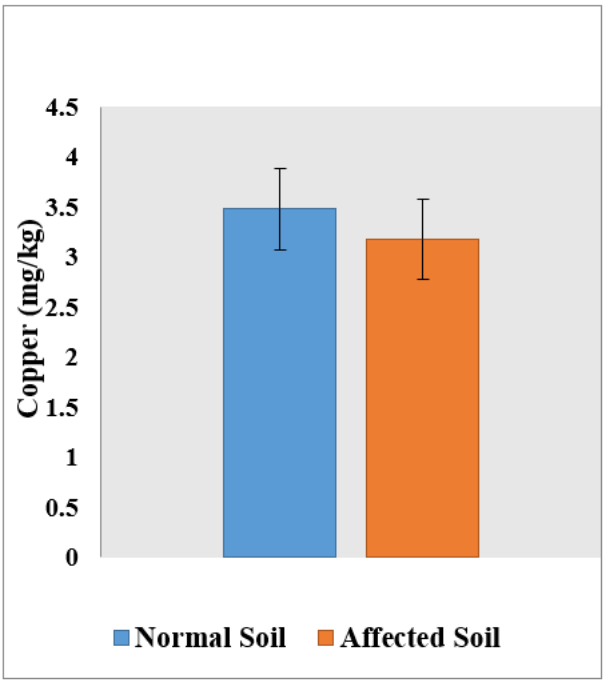

Figure 4.3: Comparison of Copper Levels in Normal and Affected Soil

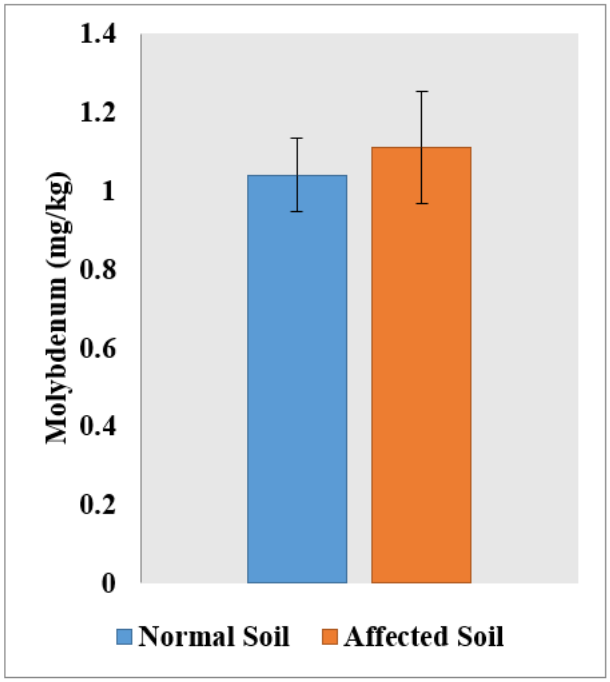

Figure 4.4: Comparison of Molybdenum Levels in Normal and Affected Soil

Epidemiological Observations

The epidemiological observations regarding lactation no. and no. of animals were also described and the percentage wise incidence was evaluated. Observations regarding parity/lactation number reveal the highest incidence rate of 35% among buffaloes at 3rd lactation, followed by buffaloes at 4th lactation (25%), 2nd lactation (17.5%), 5th lactation (15%), 1st lactation (5%) and 6th lactation (2.5%) respectively. Data thus collected regarding milk production showed the highest incidence of PPHU in high yielding buffaloes of more than 9 liters (48%) followed by the buffaloes with milk production of 5-9 liters (37%) and then 1-5 liters (15%). Results are manifested in **Figure 4.5** and **4.6.**

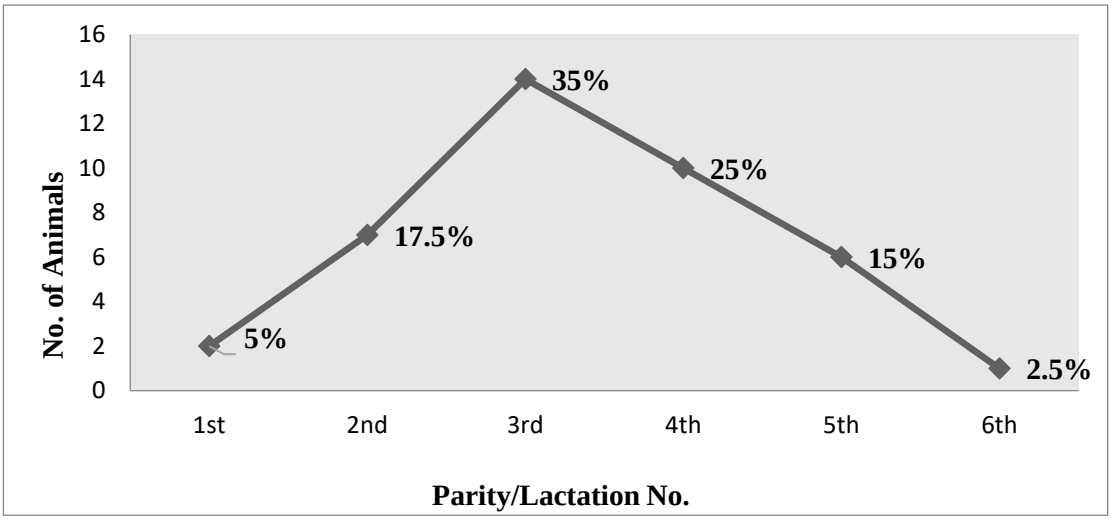

Figure 4.5: Epidemiological Observation of PPHU Affected Buffaloes in Relation to Parity/Lactation Number

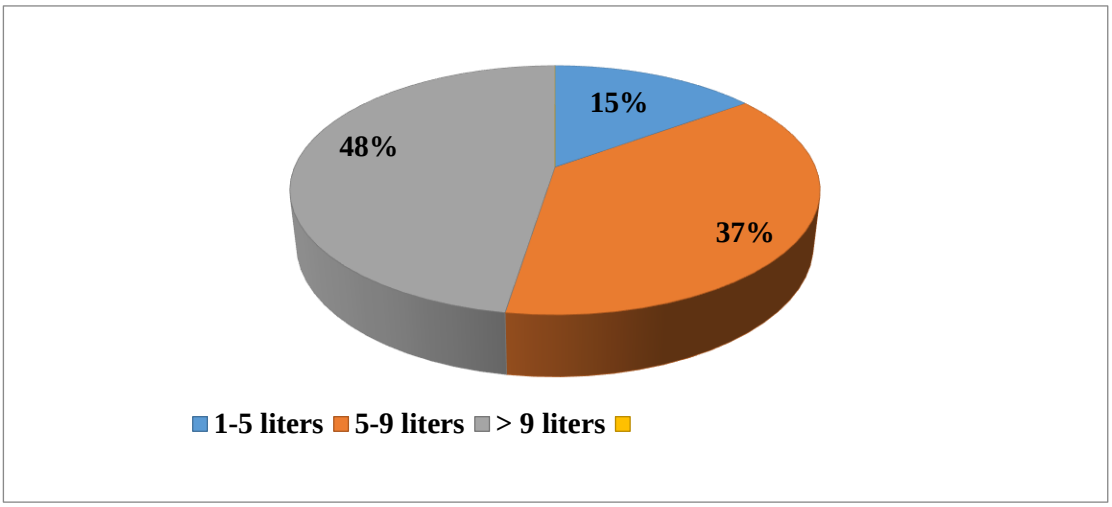

Figure 4.6: Epidemiological Observation of PPHU Affected Buffaloes in Relation to Milk Production

Discussion

Post-Parturient haemoglobinuria is a non-infectious hemolytic syndrome characteristically described by intravascular hemolysis, haemoglobinemia, haemoglobinuria and ultimately anemia. Mostly, it affects the animals producing high milk yield in early lactation. The precise pathogenesis is not well known, but there are many risk factors associated in causing these health problems. Therefore, present study was designed for evaluation of disease problem as well as its distribution and quantification of associated hematological, epidemiological and biochemical risk factors. Findings of the present study were close to the earlier reports with some differences due to dissimilar sample sizes and geo-climatic conditions. The distribution of haemoglobinuria post parturient was determined with respect to severaldescriptive characteristics. A high proportion of parturient haemoglobinuric cases were recorded in the winter season.

Durrani et al. (2010) analyzed a thousand haemoglobinuric buffalo with reference to lactation number, post-partum period and stage of pregnancy. Their results described maximum incidence of disease in 5^{th} lactation than during 7^{th} to 10^{th} month of pregnancy instead within sixty days of postpartum. Ghalawat et al. (2007) demonstrated the existence of haemoglobinuria post parturition within sixty days after parturition and through second to sixth lactation. Akhtar et al. (2006) documented epidemiological data of 60 haemoglobinuric buffaloes and declared highest existence in winters followed by summer, spring and autumn. During post calving period 45% of buffaloes were affected and most of them were in twenty three days of parturation. The total 18.3% animals showed reoccurrence of the condition. Radwan and Rateb (2007) testified peak incidence during advanced pregnancy from the month of May to the month of January. Bhikane et al. (2004) detailed 91 clinical cases and declared that most of cases happened in January and affected buffaloes had greater than four litersof milk yield. Muhammad et al. (2000) investigated epidemiological data of one hundred and eleven effected buffalo and reported highest occurrence in winters followed by spring, summer and fall. Chugh et al. (1996) analyzed the data of a hundred and thirty one haemoglobinuric buffaloes and conveyed that eighty eight percent of buffaloes were affected through 3^{rd} to 6^{th} lactations. Hemoglobinuria was observed in a large number of non-pregnant buffaloes for the period of first four weeks postpartum.

The epidemiological analysis was accompanied with biochemical and haematological risk factors associated with PPHU. There was significant decrease ($P \leq 0.05$) in total erythrocyte count, hematocrit and haemoglobin values while significant increase ($P \geq 0.05$) in ESR of affected buffaloes. Outcomes of the present study regarding TEC, Hb concentration, Hct and ESR are in accordance with the judgments of Mahmood et al. (2013), Durrani et al. (2010), Akhtar et al. (2007) and Dalir-Naghadeh et al. (2005).A significant decrease ($P \leq 0.05$) in TEC, Hb concentration, and Hct in PPHUaffected buffaloes specifies severe anemia. This could be recognized as the result of intravascular haemolysis which results from phosphorus deficiency leading to compromised glycolysis and depletion of intra-erythrocytic ATP.Insufficient concentration of ATP prompts red blood cells to modify structure and function, a loss of normal formability in addition to an increase in delicacy, eventually leading to hemolysis.

The morphological classification of anemia is observed via absolute values or red cell indices comprising MCV, MCH and MCHC. Significantly

decreased (P ≤0.05) MCH while insignificantly changed MCHC indicates macrocytic anemia (Anwar et al. 2005). Elevated level of MCV is also a demonstration of regenerative erythroid response which results in release of immature cells of increased size eventually accompanying hemolysis (Feldman et al. 2000). These cyto-morphological alterations of erythrocytes are credited to decrease in the production of ATP due to compromised glycolytic activity of RBCs. ATP is essential to sustain the shape and integrity of erythrocytes. Due to blood loss, there is decrease in ATP ultimately RBCs lose their plasticity and become rigid and spherocytic (Mahmood et al. 2013; Akhtar et al. 2007).

Khan and Akhtar (2007) stated that blood smear of haemoglobinuric buffalo revealed spherocytes along withmacrocytes. Dali-Naghadeh et al. (2005) documented the shapes as spherocytosis in addition to anisocytosis in buffalo affected with haemoglobinuria. Bhikane et al. (2004) described hypochromic macrocytic anemia in ninety-one affected buffaloes. Finding of present study are consequently in accordance with the previous reports regarding absolute values of erythrocytes.

The level of serum ALP was significantly elevated (P ≤0.05) in PPHU affected buffaloes (233.2 ± 21.47 u/L). Increased serum ALP concentration could be credited to the decreased haemoglobin levels creates general hypoxia because of anemia due to intravascular hemolysis. The cell membrane of liver, heart and kidneys was damaged, with an increase in ALP concentration due to anoxic conditions (Akhtar et al. 2008).

The levels of serum creatinineand bloodurea nitrogen were significantly higher (P ≤0.05) as 48.42 ± 3.09 mg/dl and 2.04 ± 0.20 mg/dl respectively. It might be due to release of endogenous of corticosteroids, tubular epithelial necrosis and starvation ultimately leading to an increase in blood urea nitrogen values in affected buffaloes. Moreover, decreased renal perfusion due to dehydration, had lead to reduced glomerular filtration rate and elevation in blood urea level (Digraskar et al. 1991; Durrani et al. 2010).

On the other hand, elevated blood urea nitrogen might be the result of failure of the salivary glands to recycle ureaand its non-utilization s in the rumen by the microbe in gastrointestinal problems. A quantity of urea produced by the hepatocytesremains unutilized and goes as such in the vascular system (Singh et al. 1989). In the current study, values of creatinine were also significantly higher (P ≤0.05) in haemoglobinuric buffalo. Benjamin (1978) considered thatreduced glomerular filtration rate will occur when there are concentrations over 2 mg/dl, which affects creatinine parallel to blood urea. There was equally elevation in serum urea and creatinine levels and a positivecorrelation was also established. Urea and creatinine are interrelated as they are waste products of kidneys. There is an elevation in the levels of urea and creatinine as a pathological response when kidneys are not functioning properly (Latimer et al. 2003).

As far as serum glucose level is concerned, there was a non-significant (P ≥0.05) variationamong healthy andaffected buffaloes which is relevant with Mahmood et al. (2013)but it is conflicting to the earlier informations of Akhtar et al. (2006) and Akhtar et al.(2008). They informed significantly (P ≤0.05) higher levels of serum glucose in affected buffalo and owing to anorexia due toglycogenolysis. The main source of energy for ruminants is the production of volatile fatty acids ultimately leading to a continuous loss in appetite. As a result for their energy requirements, the affected individuals have to depend on carbohydrate oxidation metabolism leading to increase inglycogenolysis. Consequently a stress condition is developed leading to release in glucocorticoids in parturient hemoglobinuria might enhance glycogenolysis and gluconeogenesis ultimatelyleading to an increase in hyperglycemia (Latimer et al.2003).

The present study showed dissimilar results to earlier studies regarding glucose levelsaffected animals. It might be because of variation in time of samplings. There was a gradual development of anorexia while former researchers perhaps had performed sampling in the majority of haemoglobinuria affected animals at start of clinical signs before the onset of anorexia and subsequent phenomenon of gluconeogenesis and glycogenolysis.

Present study revealed a significantly (P≤0.05) elevated concentration of total bilirubin in affected buffaloes could be due to intra-vascular hemolysis, hepatocellular damage, anorexia and dehydration which are in agreement with Mahmood et al. (2013). Intravascular hemolysis results in the alteration in functions and structures of liver in addition to decline its capability for bilirubin to be conjugated and secreted due to the progressive development of hypoxic condition (Latimer et al. 2003; Anwar et al.2005).

Significantly (P≤0.05) reduced values of blood inorganic phosphorous concentration of haemoglobinuria affected buffaloes might be result of ingestion of phosphorous deficient feed, cruciferous/toxic plants, decrease in phosphorous as a result of bone formation of fetus and its heavy secretion in high milk yielding animals under phosphorous deficient diets (Akhtar et al. 2006).

Insufficiency of phosphorous got transmitted from soil to fodder and eventually to the animals because of persistent feeding on phosphorous scarce fodder predominantly including cabbage and berseem. Low phosphorous diet with high calcium level induces hypophosphatemia by diminishing concentration of phosphorous from intestinal tract as a result of widespread share of phosphorous and calcium (Akramet al.1990; Akhtar et al.2006; Dua 2009). Moreover, substantial discharge by rain and continuous crop removal are important factors in reducing phosphorus level in soil ultimately lowering the level of phosphorus in soil (Constable et al.2016; Smith, 2000). There was non-significant ($P \geq 0.05$) differencein serum calcium concentration of affected animals is in accordance by the findings of Akhtar et al.(2007b) &Ghanam and El- Deeb (2010)while conflicting to the preceding reports of Radwan and Rateb (2007).

Hypophosphatemia in PPHUaffected animals is constantly acknowledged (Chugh et al. 1996). In the current study, a significant decline in serum levels of phosphorus in buffaloes affected with PPHU has been documented. Hypophosphatemia has also been documented in high milk producing animals due to the heavy secretion of phosphorus in milk (Bhikane et al. 1995). In late gestation, there is increased requirement of phosphorus and calcium for developing fetus if additional phosphorus is not given, thus leading to hypophosphatemia. Furthermore, high calcium to phosphorus ratio eventually results in reduced phosphorus absorption from the intestinal tract in addition to hypophosphatemia (Constable 2016).

Significantly ($P \leq 0.05$) elevated serum molybdenum level ($175.3 \pm 15.54 \mu g/dl$) documented in haemoglobinuric buffaloes coincides with Mahmoodet al. (2013) and Akhtar et al. (2006).The excess of molybdenum lessens phosphorus levels in the body. It proceeds by interacting with phosphorus absorption from the intestinal tract and by enhancing phosphorus elimination through urine (Dhillon et al. 1972).

Significantly ($P \leq 0.05$) reduced serum copper level ($66.34 \pm 3.60 \mu g/dl$) documented in haemoglobinuric buffaloes were found which is in accordance with the findings of Akhtar et al. (2006); Akhtar et al. (2007a,b) and Durrani et al. (2010) and is contrary with Mahmood et al. (2013). Significantly reduced levels of copper might be recognized as the result of a 3-way interaction between copper, sulphur and molybdenum. Due to reduction of sulphate, sulfides are continually produced by the rumen microorganisms. These sulfides give reaction with molybdateto form thiomolybdates that bind to copper, forming an extremely insoluble complex which do not liberate copper even under acidic condition, rendering it inaccessible to animals leading to copper deficiency (Akhtar et al. 2006).

Results of the study showed pronounced influence (87.4%) of phosphorus levels of soil on the serum phosphorus of animals. Soil molybdenum content also revealed marked impact (79.7%) on the animal serum levels. Calcium and copper contents also tend to affect the serum levels in animals. However the impact was moderated showing 55.7% and 58.4% for calcium and copper, respectively.

Outcomes of the present study could be concluded that PPHU observed in buffaloes of tehsil Bhalwal may be attributed to the variation of mineral content of the soil. Most important one is Phosphorus, deficiency of which may lead to hemoglobinuria through various pathways. As conclusion, efficient replenishment of minerals content in fodder producing soil is necessary to avoid the buffaloes from developing post-parturient hemoglobinuria in the affected areas.

Conclusion

From the present study, it is concluded that hemoglobinuria observed in buffaloes of tehsil Bhalwalwas associated with variation of soil composition, particularly the deficiency of phosphorus which is main cause of hemoglobinuria. Therefore, efficient replenishment of minerals content in fodder producing soil is necessary to overcome the disease in buffaloes affecting from post-parturient hemoglobinuria in the affected areas.

Novelty Statement

To the best of our knowledge, present study is the first one describes the influence of minerals content of soil on the serum minerals in buffalo of tehsil Bhalwal of Sargodha district in Pakistan.

References

1. Ahmad K, Khan ZI, Jabeen H, Ashraf M, Shaheen M and Raza SH. 2013. Assessment of heavy metals and metalloids toxicity in buffaloes fed on forages irrigated with domestic wastewater in Bhalwal, Sargodha, Pakistan. Pak. J. Zool. 45(6): 1629-1637.
2. Abdellah M.R., Hamed M.I., Ibrahim D.R., Rateb H.Z. 2014. Serum biochemical and hematological reference intervals for water buffaloes heifers. Journal of south aferican veterinary association. 85(1): 1-7.
3. Akhtar M, Khan A, Sarwar M and Javaid A. 2007a. Influence of soil and forage minerals on buffalo (Bubalus bubalis) parturient haemoglobinuria. Asian Australas. J. Anim. Sci. 20(3): 393.

4. Akhtar M, Khan A, Zaman T and Ahmad N. 2006. Some clinico-epidemiological and biochemical observations of parturient haemoglobinuria in Nili-Ravi buffaloes (Bubalus bubalis). Pak. Vet. J., 2006, 26(4): 151-156.
5. Akhtar MZ, Khan A, Khan MZ and Javaid A. 2008. Patho-biochemical changes in buffaloes (Bubalus bubalis) suffering from parturient haemoglobinuria. Pak. Vet. J. 28(3): 1-5.
6. Akhtar MZ, Khan A, Khan MZ and Muhammad G. 2007b. Haemato-biochemical aspects of parturient haemoglobinuria in buffalo. Turk. J. Vet. Anim. Sci. 31(2): 119-123.
7. Akram M, Hever C, Tayyab MA, Amjad MH,and Bajwa MA. 1990. Factors associated with phosphorous deficiency syndrome and helminth infestation in dairy buffaloes of rural small holders. Proc. 3rd Int. Cong., Pakistan Vet. Med. Assoc. Islamabad, Pakistan, pp. 91-96.
8. Ali F, Lodhi LA, Hussain R and Sufyan M. 2014. Oxidative status and some serum macro minerals during estrus, anestrous and repeat breeding in Cholistani cattle. Pak. Vet. J.34**:** 532-534.
9. Bhikane A, Anantwar L, Bhokre A and Narladkar B. 2004. Incidence, clinico-pathology and treatment of haemoglobinuria in buffaloes. Indian veterinary journal. 81(2): 192-197.
10. Bilal M, Suleman M and Raziq A. 2006. Buffalo: black gold of Pakistan. Livestock research for rural development. 18(9): 140-151.
11. Brechbühl M, Meylan M, Kunz-Kirchhofer C, Bodmer M, Michel A and Kaufmann T. 2008. Post-parturient haemoglobinuria in cows kept in the Swiss Alpine region. Tierärztliche Praxis Großtiere. 36(4): 236-240.
12. Benjamin MM. 1978. Outline of veterinary clinical pathology. Iowa State University Press. (3):278-280.
13. Chapman H and Pratt P. 1961. Methods of Analysis of Soil, Plants and Water. University of California, Division of Agricultural. Science. USA.1: 1-309.
14. Constable PD, Hinchcliff KW, Gruenberg W and Done SH**.** 2016. Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats. Elsevier Health Sciences. p. 11: 384-385.
15. Daniel WW. 2022. Biostatistics: basic concepts and methodology for the health sciences. John Wiley & Sons, New York. 9th Ed. 346-353.
16. Dalir-Naghadeh B, Seifi H, Asri-Rezaei S and Pilevary N. 2006. Post-parturient haemoglobinuria in Iranian river buffaloes: a preliminary study. Comp. Clin. Path. 14(4): 221-225.
17. Dhillon KS, Singh J, Ashuma KH, Bal MS and Sandhu KS. 2007. Haemoglobinuria in dairy herds. Vet. Rec. 160(8): 276.
18. Digraskar S, Singh B, and Deshpande BB. 1991. Epidomiology and clinico - pathology of hemoglobinuria in buffalo (Bubalus bubalis). Livestock Advisor, 16, pp. 32-38.
19. Dua K. 2009. Importance of micronutrients and relevance of their supplementation in buffaloes. Pak. J. Zool. Suppl. Ser. 9: 541-549.
20. Durrani AZ, Kamal N, Shakoori AR and Younus RM. 2010. Prevalence of post parturient haemoglobinuria in buffalo and therapeutic trials with toldimfos sodium and tea leaves in Pakistan. Turk. J. Vet. Anim. Sci. 34(1): 45-51.
21. Gahlawat I, Singh K and Kumar R. 2007. Investigations on oxidative stress in post-parturient haemoglobinuria in buffaloes receiving sodium acid phosphate therapy. Ital. J. Anim. Sci. 6(2): 974-977.
22. Ghanem MM and El-Deeb WM. 2010. Lecithin cholesterol acyltransferase (LCAT) activity as a predictor for ketosis and parturient haemoglobinuria in Egyptian water buffaloes. Res. Vet. Sci. 88(1): 20-25.
23. Kahn CM, Line S and Aiello S. 2005. The Merck Veterinary Manual. Merck & Co. Inc., Whitehouse Station, NJ.9: 1020-2020.
24. Latimer KS. 2011. Duncan and Prasse's veterinary laboratory medicine: clinical pathology. John Wiley & Sons. (5):370-374.
25. Mahmood A, Khan M, Younus M, Khan M, Ahad A, Ahmad M, Iqbal H, Fatima Z and Anees M. 2013. Haemotological and biochemical risk factors of parturient Haemoglobinuria in buffaloes. J. Anim. Plant. Sci. 23(2): 364-368.
26. Montiel L, Tremblay A, Girard V and Chorfi Y. 2007. Preanalytical factors affecting blood inorganic phosphate concentration in dairy cows. Vet. Clin. Pathol. 36(3): 278-280.
27. Mufti S, Afshan K, Khan IA, Zafar Y, Rizvi SSR, Nazir F and Qayyum M. 2014. Genetic characterization of Fasciola samples from bovine hosts in Pakistan by sequences of ribosomal internal transcribed spacer regions. Pak. Vet. J. 34: 361-366.
28. Muhammad G, Nazir A, Khan M, Sarwar M and Zubair M. 2000. Some epidemiological features of bovine parturient haemoglobinuria in Punjab province of Pakistan. Indian. j. dairy sci. 53(3): 216-221.
29. Neto B, Oliveira C, Duarte D, Albernaz T, Júnior dO, Riet-Correa G and Riet-Correa F.

2007. Phosphorus deficiency in buffaloes in the state of Pará, Northern Brazil. Ital. J. Anim. Sci. 6(2): 971-973.
30. Pösö A, Saukko T, Tesfa A and Lindberg L-A. 2000. Fat infiltration in liver and activity of lecithin: cholesterol acyltransferase in serum of dry and lactating dairy cows. Res. Vet. Sci. 68(2): 169-173.
31. Radwan M and Rateb H. 2007. Clinical, haematological and some biochemical variations hypophosphataemia in buffaloes before and after treatment at Assiut Government. BS. Vet. Med. J. 17(2): 35-41.
32. Reddy BSS, Reddy BS, Venkatasivakumar R and Sivajothi S. 2014. Haemoglobinuria due to hypophosphataemia in last trimester pregnant buffalo. Int. J. Biol. Sci. 2(2): 153-155.
33. Singari N, Bhardwaj R, Chugh S and Bhardwaj S. 1991. Status of erythrocytic glucose-6-phosphate-dehydrogenase (G6PD) in phosphorus deficiency hemoglobinuria of buffalos. Indian. Vet. J. 68(3): 226-230.
34. Singh N, Kumari R, and Akbar MA. 1989. Biochemicalchanges in blood metabolites in buffaloes with indigestion. Indian Vet. J. 66(10): 923-926.
35. Stockdale C, Moyes T and Dyson R. 2005. Acute post-parturient haemoglobinuria in dairy cows and phosphorus status. Aust. Vet. J. 83(6): 362-366.
36. Thompson J and Badger S. 1999. Outbreak of post-parturient haemoglobinuria in an autumn calving dairy herd. New. Zeal. Vet. J. 47(5): 180-183.
37. Thrall MA, Weiser G, Allison R and Campbell TW. 2012. Veterinary Hematology and Clinical Chemistry. Wiley. 2: 250-410.
38. Thrusfield M 2013. Veterinary Epidemiology, Wiley. (3):232.
39. Turgut K. 2000. Veteriner Klinik Laboratuvar Teşhis. Bahçıvanlar Basım Sanayi AŞ. Konya. 885.
40. Warriach H, McGill D, Bush R, Wynn P and Chohan K. 2015. A review of recent developments in buffalo reproduction—a review. Asian. Australas. J. Anim. Sci. 28(3): 451-455.
41. Yousaf A, Laghari R, Shoaib M, Ahmad A, Malhi K, Mughal G, Lakho S and Khetran I. 2016. The prevalence of brucellosis in Kundhi buffaloes in District Hyderabad. Pakistan. J. Anim. Health Prod. 4(1): 6-8.