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Clinical, Epidemiological and Clinic-Pathological aspects of Hypophosphatemia in Buffaloes in Egypt

Ahmed Zaghawa[1]; Hany Yousef [2]; Mohamed Nayel[1]*; Ibrahim Mabrouk[2]; Walid Mousa[1]; Yahia Abo-nab3 [3] Ahmed Elsify[1]; Akram A. Salama[1] and Emad Ghazy[4]

[1]Department of Animal Medicine and Infectious Diseases (Infectious Diseases), Faculty of Veterinary Medicine, University of Sadat City, **Egypt.**

[2]Department of Animal Medicine and Infectious Diseases (Animal Medicine), Faculty of Veterinary Medicine, University of Sadat City, **Egypt.**

[3]Veterinarian at the Directorate of Veterinary Medicine, Menofiua governorate, **Egypt**.

[4]Department of clinical pathology, Faculty of Veterinary Medicine, Kafrelsheikh University, **Egypt.**

***Correspondence: mohamed.aboalez@vet.usc.edu.eg** **Received: 14 Nov. 2019, Revised: 19 Dec. 2019 Accepted: 20 Dec. 2019 e-Published: 26 Dec. 2019, Reviewed by: Prof. Dr. Aly Metwally, Prof. Dr. Abdelkhalk Elshaik**

Hypophosphatemia is recognized as a major and economic metabolic disorder of highly incidence in buffaloes and cattle particular in Egypt. This study spot high lights on some clinical, epidemiological and clinic-pathological changes in hypophosphatemic buffaloes. Clinical examination of 60 affected and another 60 apparently healthy buffaloes was carried out. The results of clinical examination revealed anorexia, anemia, marked decrease in milk yield and secretion of bloody milk in the affected cases. The epidemiological risk factors revealed statically significant higher incidence of hypophosphatemia in winter season, at early lactation, and in the late stage of pregnancy with percentage 38.33%, 59.26%, 54.55% respectively. Also the high incidence was observed in between 3rd and 4rd parity at high producing buffaloes with 21.66 to 25% respectively. The major clinico-pathological findings reported a significant reduction in erythrocyte and haemoglobin values of the affected buffaloes to 3.60±1.02 and 5.80±1.35 respectively. Meanwhile higher level of erythrocyte sedimentation rate was recorded. Regarding to the blood glucose, urea, bilirubin, and creatinine values were significantly higher in the affected buffaloes with 76.05±7.77 and 49.65±7.81 mg/dl, 24±2.49 and 2.10±0.36 mg/dl respectively). The main biochemical changes was the lower serum phosphorus, copper and selenium level to 1.88±0.62 µg/dL, 65.38±5.99 µg/dL and 12.27±3.l6 µg/dL respectively with significantly ($P<0.05$) lower than in healthy buffaloes. In conclusion, severe anemia, coffee-coloured urine and marked decrease in milk yield were the main clinical features in hypophosphatemic buffaloes. The higher incidence of this syndrome was occurred in high yielding buffaloes at last stage of pregnancy. Also, early stage of lactation and 3rd-4rd parity were associated with higher incidence of hypophosphatemia in Egyptian buffaloes.

Keywords: Hypophosphatemia, anaemia, phosphorus, erythrocyte, buffaloes.

INTRODUCTION

Hypophosphataemia is a seasonal sporadic metabolic disorder in buffaloes and cattle which occurred due to prolonged grazing on green barseem resulted in inadequate phosphorous content, haemoglobinurea and reduction in red blood cells count and synthesis of ATP (Rizk et al., 1999). It more recognized mainly in early and highly lactating buffaloes (Chugh et. al., 1996). Moreover, the high incidence was correlated to the late pregnancy and early milking period due to the rapid and continues losses of phosphorus in milk secretion at the first stage of lactation and low content of phosphorus in feed especially in the last gestation period (Larsen et al., 2001; Bhikane et al., 2004; Purohit et al., 2014). From epidemiological point of view, the incidence of hypophosphatemia appeared to be more pronounced in buffaloes than cattle (Bhikane and Syed, 2014), as well as several risk factors have considered putative risk factors with high incidence of hypophosphatemia in buffaloes and cattle including seasonal variation, stage of lactation, lactation season, age of animal, stage of pregnancy and feeding regime pattern (Resum et al., 2017). As results of the intravascular haemolysis severe anemic anoxia and death may occurred in sever chronic cases (Gahlawat et al., 2007). The main recognized symptoms include inappetence, pale mucous membrane, reduction of milk production, locomotors disturbances, lameness and haemoglobinuria with recumbency in severe complicated cases (Hoda, 2006). The pronounced coffee-coloured of urine was a major clinical signs noticed in most affected cases with bloody milk in some cases(Khan and Aktar, 2007).The low value of serum phosphorus in haemoglobinuric animals is main laboratory evident (Sherif, 2012). Moreover, low level of glutathione peroxidase, glucose-6-phospume dehydrogenase and reduced glutathione (GSH) with high levels of hemoglobin and albumin were detected in buffaloes urine (Kumar et al., 2014). Furthermore, intravascular haemolysis causing lowering the mean values of erythrocyte, haemoglobin, haematocrit values and higher erythrocyte sedimentation rate (Mahmood et al., 2013). So, the current study was conducted to describe the clinical, epidemiology and clinic-pathological aspects that associated with hypophosphatemia in buffaloes in Egypt.

MATERIALS AND METHODS

2.1.Animals examination and samples

A total number of 60 buffaloes with hypophosphataemia and haemoglobinuria were selected from Moneufia governorate from December 2012 till December 2014. In addition to 60 clinically healthy buffaloes were examined as a control group. The clinical diagnosis depend on the basis of haemoglobinuria and specific symptoms in buffaloes in early lactation or in late pregnancy. Clinical signs, were described and differential diagnosis with other diseases associated with reddish discoloration of urine (such as babesiosis, bacillary haemoglobinuria and leptospirosis) was excluded through confirmatory tests. Also, epidemiological observations in relation to season, parity, stage of lactation, stage of pregnancy and milk yield were recorded in all the PHU affected buffaloes.

2.2. Blood Samples

Samples of blood were collected into test tubes containing EDTA for estimation of TEC count, Hb concentration, PCV and ESR as recommended by (Marinai and Prox., 1973). Blood smears from each animal were stained with Giemsa stain, and examined to exclude blood haemoprotozoan parasites (Beutler, 1971). The different types of leukocytes was estimated as described by (Benjamin, 1978) while, differential leukocytes counts for neutrophils, lymphocytes, monocytes, eosinophils and basophils were done according to previous report (Bush., 1975) .

2.3. Biochemical determinations

Serum samples collected from buffaloes were analyzed for the determination of blood glucose using diagnostic kit (Cat.# GL 2586, Randox Laboratories Ltd., C.K.) according (Benjamin., 1978); Blood urea concentration according to (Roy., 2001) ; Serum creatinine value according to Westergren's method (Khan and Aslam., 2001) . Serum cholesterol using (Cat # 448. Biocon Diagnostik, Germany) as described by (Swecker., 1995); Serum total bilirubin as described by Jendrassik-Gror method according to (Harvey and Boirss.,1997). Serum alkaline phosphatase (ALP) (Cat# 1622, Biocon Diagnosiik, Germany) according to (Kahn and Line,2005); Glucose-6- phosphate dehydrogenase (G6PD) activity in erythrocytes (Cat # PD 410, Randox Laboratories Ltd., U.K.) according to Westergren's method (Swecker., 1995).

2.4. Minerals Analysis

Atomic absorption spectrophotometer (Varian SpectrAA-5) Bauer (1982) was used to estimate the values of calcium, copper, iron, molybdenum and selenium in serum samples. While spectrophotometer (Philip Model 1100) - Kelly (1984) was used for phosphorus value. The flame photometer (Jenway PFP-7) Radostitis (2007) was applied to estimate the Potassium levels.

RESULTS

3.1. Clinical findings of buffaloes affected by hypophosphatemia

The most common clinical sign in hypophosphatemic buffaloes was haemoglobinuria (red, dark-red to coffee colored urine). Anemia, dehydration and dullness were also recorded signs. Significantly higher milk production was recorded in healthy buffaloes with (7.54 to 10.40 liters /day) than the milk production in affected buffaloes (2.70 to 4.68 liter /day). Normal body temperature was recorded within normal range. Significantly increase in the pulse and respiration rates with ($P<0.05$). Meanwhile, significantly reduction in ruminal motility and weakness as shown in (Table1). In the late stage, marked anorexia was clear with complete stasis of rumen and marked straining during defecating. Normal to hard faces were observed or may be loose in consistency. Icterus, lameness, dyspnea and tachycardia may be signs that can sometimes observed in some cases. The course of the disease varied and extend from 3-9 days.

2. Epidemiological risk factors associated with hypophosphatemia

2.1. Incidence of hypophosphatemic haemoglobinuria in relation to climatic season

The highest occurrence of PHU was observed in winter season (38.33%) followed by spring (31.67%), summer (21.67%) and autumn (8.33%). Chi-square analysis revealed significant difference in the occurrence of parturient haemoglobinuria between seasons as shown in (Table 2)

2.2. Incidence of hypophosphatemic haemoglobinuria in relation to parity

The highest incidence in affected buffaloes were observed in fourth and third parity (25%) and (21.66%) respectively, followed by first parity (6.67%). The second, fifth, and sixth parities recorded (13.33%), (18.33%), and (15%) respectively. Chi-Square analysis revealed significant difference in the occurrence of parturient haemoglobinuria between parities as shown in (Table; 3).

Table1: Comparison of clinical parameters (Mean± SD) in healthy hypophosphatemic haemoglobinuric buffaloes.

Parameters	Healthy buffaloes (n=60)	hypophosphatemic haemoglobinuric buffaloes (n= 60)	P-Value
Rectal temperature C°)	38.0±0.5	37.9± 0.9	0.067
Pulse rate (per minute)	54.75±3.78 a	72.97±9.90b	0.001
Respiration rate (per minute)	14.65±1.93a	21.58±4.27b	0.001
Ruminal motility(per 2 minutes)	3.60±0.50a	2.30±0.99b	0.001

Different letters in a row means differ significantly ($p < 0.05$)

Table 2: Seasonal occurrence of hypophosphatemic haemo- globinuria in buffaloes (n=60)

Season	No. of affected animals (n=60)	Percent %
Winter	23	38.33
Spring	19	31.67
Summer	13	21.67
Autumn	5	8.33

Table 3: Frequency distribution of hypophosphatemic haemoglobinuria in buffaloes (n=60) with reference to parity.

Parity number	No. of affected animals	Percent %
1	4	6.67
2	8	13.33
3	13	21.66
4	15	25.00
5	11	18.33
≥ 6	9	15.00

2.3. Incidence of parturient haemoglobinuria related to stage of lactation.

The majority of buffaloes developed haemoglobinuria in the post calving period (59.26%) between 1-23 days of calving followed by (29.63%) in between 61-270 days and(11.11%). In between 24-60 days of calving. Chi-square analysis revealed significant difference in occurrence of parturient haemoglobinuria between various stages of lactation as shown in (Table; 4).

Table 4: Frequency distribution of hypophosphatemic haemo-globinuria in buffaloes (n=27) with reference to stage of lactation.

Stage of lactation (days)	No of Affected animals	Percent %
(1-23)	16	59.26
(24-60)	3	11.11
(61-270)	8	29.63

2.4. Incidence of hypophosphatemic haemoglobinuria related to stage of pregnancy.

Buffaloes in advanced stage of pregnancy were more susceptible for hypophosphatemia as third trimester of pregnancy recorded (54.55%) followed by second trimester (24.24%) and lower incidence in the first trimester (21.2l %). Chi-square analysis revealed significant difference in the occurrence of hypophosphatemic haemoglobinuria between various stages of pregnancy as shown in (Table; 5).

Table 5: Frequency distribution of hypophosphatemic haemoglobinuria in buffaloes (n=33) with reference to stage of pregnancy.

Stage of pregnancy (trimesters)	No. of affected animals	Percent %
First trimester	7	21.21
Second trimester	8	24.24
Third trimester	18	54.55

2.5. Incidence of hypophosphatemic haemoglobinuria related to milk production

The high occurrence of hypophosphatemia was observed in buffaloes producing 10 or more liters of milk/day (42.50%) followed by buffaloes producing 5-9 (37.50%) and less than 5 liters (20%).This clearly indicates that this disease was mostly affects high yielding buffaloes. Chi-square analysis revealed significant difference in the occurrence of parturient haemoglobinuria between lactating buffaloes in as show (Table; 6).

Table 6: Frequency distribution of parturient haemo-globinuria in buffaloes (n=40) with reference to milk yield.

milk yield (liters/day)	No. of Affected animals	Percent %
Low (<5)	8	20.00
Medium (5-9)	15	37.50
High (≥10)	17	42.50

3.2. Haemogram picture in hypophosphatemia in buffaloes :

The results of heamogram pattern showed that low significance with ($P<0.05$) for total erythrocyte counts (TEC), haemoglobin (Hb) and packed cell volume (PCV) in parturient haemoglobinuria affected buffaloes than in healthy buffaloes as shown in (Table 7). While, significantly increase in the mean of ESR value in hypophosphatemic buffaloes (104.13±36.16 mm/ 1 hr) than control buffaloes.

3.3. Leukogram picture in hypophosphatemia in buffaloes:

The total leucocytic counts(TLC) and differential leukocytic counts including monocytes and eosinophils not significantly differ in healthy and hypophosphatemic buffaloes as shown in (Table; 8). The relative (%) and absolute counts of neutrophils in hypophosphatemic buffaloes were significantly ($P<0.05$) higher than in healthy buffaloes. However, the relative (%) and absolute counts of lymphocytes in parturient haemoglobinuria buffaloes were significantly ($P<0.05$) lower than in healthy buffaloes as shown in (Table 8). From the current results it was clear that there was a positive correlation between TLC and neutrophils (r = 0.694, P< 0.01) and with lymphocytes (r = 0.712. P<0.001) and negatively correlated with eosinophils (r = 0.3.38, P<0.0l). Lymphocytes were positively correlated with monocytes (r = 0.278, P<0.05) and negatively correlated with eosinophils (r = 0.293. P<0.05) whereas monocytes were positively correlated with eosinophils (r = 0.459, P<0.00l).

3.4. Changes in biochemical profile in hypophosphatemic buffaloes :

The values for biochemical parameters were summarized in (Table; 9). Blood glucose and blood urea values in hypophosphatemic affected buffaloes recorded (76.05±7.77 and 49.65±7.81 mg/dl respectively) and were significantly (P<0.05) higher than in healthy buffaloes. Concerning to the value of serum total bilirubin and creatinine values (4.24±2.49 and 2.10±0.36 mg/dl respectively) and significantly (P<0.05) higher than the values in healthy buffaloes. No statistically significant difference between healthy and hypophosphatemic buffaloes (P>0.05) in serum cholesterol level. The serum alkaline phosphates concentration in hypophosphatemic buffaloes was significantly (P<0.05) higher than in healthy buffaloes and recorded (232.5 ± 58.68 U/L). Finally, the concentration of Erythrocytic glucosc-6 -phosphate dehydrogenase (G6PD) in hypophosphatemic buffaloes was significantly (P<0.03) lower than the concentration in healthy buffaloes recording the value (92.28 ± 13.I7mU/10^9 RBCS). Serum glucose level was positively correlated with bilirubin (r= 0.303, P<0.05) and urea as positively correlated with cholesterol (r= 0.299, P<0.05) and creatinine (r= 0.873, P<0.00l). A positive correlation between bilirubin and cholesterol was reported (r (3.342, P< 0,01) and also cholesterol was positively correlated with creatinine (r=0.313, PKO.05) and negatively correlated with alkaline phosphatase (r =0.256, P<0.05). Creatinine was positively correlated with molybdenum (r =0.273. P<0.05) and negative correlation between alkaline phosphatase and G6PD (r = 0265, P<0.05).

Table 7: Haemogram values (Mean ± SD) in healthy and hypophos-phatemic haemoglobinuric buffaloes.

Parameters	Healthy	PHU	P-Value
Total erythrocyte counts (n x 1012/L)	6.21±0.64a	3.60±1.02b	0.001
Haemoglobin (g/dL)	11.03±1.20a	5.80±1.35b	0.001
Packed cell volume (%)	34.34±2.36a	16.85±2.76b	0.001
Erythrocyte sedimentation rate (mm/1 hr)	73.13±29.91a	104.13±36.16b	0.001

*Different letters in a row means differ significantly (p < 0.05).

Table 8: Leukogram picture in hypophosphatemia in buffaloes:

Parameters	Healthy	hypophosphatemic	P-Value
Total Leucocyte Counts(n x 109/L)	9.46 ±2.17	10.03±1.97	0.137
Neutrophlis (%)	35.03±3.84a	43.03±4.54 b	0.001
(Absolute)	3.33±0.92a	4.44±1.11 b	0.001
Lymphocytes%	56.45±4.26a	48.70±2.98b	0.001
(Absolute)	5.32±1.17a	4.93±0.57b	0.023
Monocytes (%)	5.23±0.95	5.12±1.01	0.515
(Absolute)	0.50±0.16	0.51±0.08	0.552
Eosinophils (%)	3.28±0.90	3.15±0.99	0.442
(Absolute)	0.32±0.13	0.31±007	0.698
Basophils (%)	-	-	-
(Absolute)	-	-	-

* Different letters in a row means differ significantly (P<0.05

Table 9: Comparison of biochemical parameters (Mean ± SD) in healthy and hypophosphatemic affected buffaloes

Parameters	Health	hypophosphatemic	P-Value
Glucose (mg/dL)	57.31±6.19a	76.05±7.77b	0.001
Urea (mg/dL)	33.93±5.09a	49.65±7.81b	0.001
Totalbilirubin (mg/dL)	0.57±0.13a	4.24±2.49b	0.001
Cholesterol (mg/dL)	202.73±27.85	207.64±29.08	0.347
Creatinine (mg/dL)	1.34±0.31a	2.10±0.36b	0.001
Alkaline phosphatase (U/L)	80.35±22.89a	232.50±58.68b	0.001
Erytrocytic G6PD (mU/109 RBCs)	108.96±19.61a	92.28±13.17b	0.000

* Different letters in a row means differ significantly (p < 0.05)

Table 10: Serum mineral profile (Mean ± SD) in healthy and hypophosphatemic buffaloes

Parameters	Healthy	Hypophosphatemic	P-Value
Ca (mg/dL)	9.83±1.01	9.86±1.29	0.907
P (mg/dL)	5.41±0.64a	1.88±0.62b	0.001
K (m mol/L)	4.46±0.71a	13.75±1.00b	0.001
Cu (μg/dL)	118.36±5.21a	65.38±5.99b	0.001
Fe (μg/dL)	161.18±14.74a	217.59±11.50b	0.001
Se (μg/dL)	18.42±4.32a	12.27±3.16b	0.001
Mo (μg/dL)	54.82±13.59a	171.53±56.69b	0.001

* Different letters in a row means differ sig significantly (p < 0.05)

3.5. Serum Minerals Profile in hypophosphatemia in buffaloes.

Serum minerals profile in healthy and hypo phosphatemic buffaloes were recorded in (table 10). Serum inorganic phosphorus, copper and selenium values in buffaloes suffering from parturient haemoglobinuria were (1.88±0.62 μg/dL, 65.38±5.99 μg/dL and 12.27±3.l6 μg/dL respectively) with significantly (P<0.05) lower than in healthy buffaloes. Also, the values of serum potassium, iron and molybdenum were (13.75±1.00 μg/dL, 217.59±11.05 μg/dL and 171.53±56.69 μg/dL respectively) with significantly (P<0.05) higher than in healthy buffaloes.

DISCUSSION

Hypophosphatemia is considered as non-infectious haemolytic disorder mainly afftected buffaloes and cattle that clinically characterized by anaemia and haemoglobinuria with intravascular haemolysis. As result of decreased serum inorganic phosphorous, the erythrocyte glycolytic activity and Adenosino- tri-phosphate (ATP) production were reduced. ATP is required to maintain the normal form of erythrocytes. Therefore, its decreased level lead to erythrocytes loose their deformability and become rigid (Akhtar et al.,2008). In our study the most prominent clinical findings observed in hypophosphatemic buffaloes were dark-red to coffee colored urine and anemia. Milk yield was significantly reduced in Hypophosphatemic buffaloes. Both pulse and respiration rates were statistically significant elevated (P<0.05) , with significantly weak and reduction in ruminal motility. At the late stage of the disease marked anorexia was present, accompanied with complete stasis of rumen and evidence of severe straining during defecating. Icterus, tachycardia, dyspnea and lameness in some cases may be observed. The similar findings have been reported in different studies of (El Bagory, 1993; Hoda, 2006; Pankaj Kumar et al., 2014).

Epidemiological surveillance declared that some risk factors had significant predisposing effect on the incidence of PHU in bovines such as old age, late pregnancy, post-partum period (Mahmood et al., 2012). In the present study epidemiological and risk factors associated with hypophosphatemia were investigated among the investigated cases, the seasonal incidence was studied and the results reported that (41.67%) of hypophosphatemia cases occurred during the winter months. These come in agreement with (Akhtar et al., 2006) reported a significant higher occurrence of PHU in the winter season (41.8%). In Egypt, Hoda (2006) and Sherif (2012) observed that (68.96%) of parturiem haemoglobinuria cases occurred during the winter months and concluded that the peak incidence of the disease varied from area to area and from year to year in the same area. Meanwhile these results varied from those reported by Karapnar et al., (2004) they reported high prevalence of hypophosphatemia in India in autum (25.19%) followed by summer (14.5%) and lower incidence reported in winter (0.77%) among 131 affected buffaloes. Another study carried out in Pakistan revealed that the disease prevalence was reported around the year although the most of cases occurred from July to September Cheema et al., (1980). Furthermore, Akhtar et al (2008) reported the peak seasonal incidence of haemoglobinuria among buffaloes in Pakistan were found 28 (37.84%) and 17 (22.98%) in summer and winter season respectively among 74 cases of PHU. The high occurrence of Hypophosphatemia in winter months in our study could be related to excessive feeding of berseem (Trifolium alexandrium) as this is the main fodder available exclusively during these months in Egypt.

The higher frequency of Hypophosphatemia was observed in 3rd and 4th within 1-23 days postpartum and in high milk yielding buffaloes. Similar findings were reported high incidence of PHU in 3rd and 4th lactation Jubb et al (1993). Additionally, high incidence in early lactation has also been described previously by (Radwan and Rateb ,2007; Ussama ,2009, Durrani et al., 2010; Pankaj Kumar et al., 2014; Krishna et al., 2014).

The results of the present study indicated that majority of the buffaloes developed haemoglobinuria in the advanced stage of pregnancy (last trimester). These findings were in contact with those of Khan and Aktar (2007), Sherif (2012) and Pankaj Kumar et al (2014). This could be due to the lack of additional requirement of the nutrients for the developing foetus in the last trimester of pregnancy Horst (2010) and Latimer et al (2011).

In the current study the high yielding buffaloes are more prone and susceptible to PHU. This was agree with several studies (Rana and Bhardwaj, 2006; Khan and Akhtar, 2007; Purohit et al., 2018) they showed higher incidence of the disease in high producing buffoles (10 liter or more). However, Akhtar et al., (2008); Mahmood et al., (2012) disagree and claimed that low association between the disease incidence and milk production. This could be explained that buffaloes are usually in peak milk production in their 3rd and 4th lactations which causes heavy stress on mineral imbalance and also drainage of nutrients especially phosphorus from the body Digraskar et al (1996), Latimer et al (2011) , Gahlawat et al (2015) .

Regarding to the changes in haematological and serum biochemical in buffaloes suffering from hypophosphatemia. This study revealed significant decrease in total erythrocyte counts (TEC), haemoglobin Concentaration (Hb) and packed cell volume (PCV) as compared to healthy buffaloes. These were agree with previously reports of (Durrani et al., 2010; Sharma et al.,2014) they recorded lower mean values of erythrocyte count, haemoglobin and haematocrit with hemoglobinuria in diseased buffaloes as well as higher value of erythrocyte sedimentation rate in comparison to control buffaloes. This low values of such parameters (RBC, Hb and PCV) may attributed to the intravascular haemolysis (Stockdale et al., 2005; Khan and Aktar, 2007; Ussama,2009; Sherif, 2012; Pankaj Kumar et al., 2014) .The impairment of the glycolytic process as well as the depletion of ATP in erythrocytes due to inadequate phosphorus content could be a predispose cause to alter the function or structure of RBCs such as a deformities of the normal cell and enhanced the fragility that finally causes haemolysis (Singh and Kumar ,2013) . The erythrocyte sedimentation rate (ESR) was significant higher ($p<0.00$ l) in haemoglobinuric buffaloes. These were in parallel with the results obtained by (Stockdale et al. , 2005 ; Sherif, 2012) . Although, the total leukocyte counts (TLC): monocytes and eosinophils showed non-significant difference, the neutrophil and lymphocyte counts showed significantly increasing and decreasing trend in affected buffaloes respectively. These were in accordance with (Khan and Aktar ,2007) . Moreover, (Montiel et al., 2007) explained that the discrepancy in Increasing and decreasing percentage in neutrophil and lymphocyte counts in the present study may be due to the release of corticosteroids.

The results of biochemical changes reported that serum glucose, urea, creatinine, total bilirubin, alkaline phosphatase, potassium, iron and molybdenum were significantly increased. The increase in total bilirubin could be mainly attributed to increase in the unconjuagated bilirubin induced by intravascular haemolytic anemia Meanwhile, erythrocytic gulcose-6-phospbate dehydrogenase, phosphorus, copper and selenium were significantly decreased in hypophosphatemic buffaloes. On the other hand, serum cholesterol and calcium level not changed between hypophosphatemic buffaloes and healthy. This in contact with (Walter, 2011) who achieved that the low level of plasma phosphorus to 1.8mg/dl was due to high consumption of phosphorus at early lactation and late gestation which act as a predisposing factor for hypophosphatemia. The serum alkaline phosphatase (ALP) showed significantly increased value in hypophosphatemia buffaloes (232.50±58.68 UL). This was the same reported by (Singh et al., 1992). The dramatic decrease in haemoglobin levels as a result of generalized intravascular haemolysis that produce condition of hypoxia. When anoxic conditions was occur in vital internal organs such as (liver, heart, and kidneys) causing ALP leakage and cell membranes damage (Singari et al .,1991) . While, the hypophosphatemic buffaloes showed significantly lower activity of erythrocytic glucose-6-phosphate dehydrogenase (G6PD) than in healthy buffaloes. These was previously obtained by (Grunberg et al., 2006) who noticed a reduction in glutathione activity. Moreover, the high level of serum potassium was found which was in contact with (Dabak and Kirbas, 2007).The hyperkalemia can be explained that the release a high K content as result of damage of cell membrane damage and muscles tissues necrosis (Singh and Kumar, 2013). Furthermore, the level of serum molybdenum was significantly higher in hyophsphatemic buffaloes than in healthy buffaloes. This finding was in close agreement with (Dabak and Kirbas, 2007). On the other hand, a significantly lower level of copper in hyophsphatemic buffaloes. This finding was parallel to the results of (Khan and Aktar, 2007 ; Dabak and Kirbas, 2007). The significantly decreased in copper level may be attributed to the interaction with other micro-elements such as molybdenum and sulfur (Pirzada et al ., 1998). The results also detected significantly higher level of serum iron in compared to in healthy buffaloes. These observations correlated with the findings of (Dabak and Kirbas, 2007). This could be due to acute intravascular haemolysis (Singh and Kumar, 2013). The concentration of selenium concentration was significantly lower than in the healthy buffaloes, although, this value remain within the normal dietary requirement (Singh et al .,1992). This may attributed to the progressive loss in appetite (Singh and Kumar ,2013) . In a different manner, non-significant difference between the values of serum calcium in hypophosphatemic buffaloes (9.86±1.29 mg/dL) and healthy were come in accordance with (Hoda, 2006 ; Singh and Kumar, 2013).

CONCLUSION

There is significant higher incidence of hypophosphatemia in winter season, at early lactation, and in the late stage of pregnancy. The major clinico-pathological findings reported a significant reduction in erythrocyte and haemoglobin values. The main biochemical changes was the lower serum phosphorus, copper and selenium.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

All listed authors have made substantial contributions to the research design, the acquisition, analysis, or interpretation of data; and to drafting the manuscript or revising it critically; and that all authors have approved the submitted version.

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REFERENCES

Akhtar, M. Z.; Khan, A,; Zaman, T. and Ahmad. N. (2006) Some Clinico-epidemiological and Biochemical observations of Parturient Haemoglobinuria in Nili-Ravi Buffaloes (Bubalus Bubalis). *Pakistan Vet. J.,* 2006, 26(4): 151-156.

Akhtar, M. Z.; Khan, A.; M. Z.; Khan, M.Z. and Muhammad, G. (2008)

Haematobiochemical aspects of parturient haemoglobinuria in buffalo. Turkish *J. Vet. Anim. Sci*, 31(2): 119 -123.

Bauer, J .D. (1982) In clinical laboratory methods 9 Ed. The C .V Mosby co .II 830, thelium (Kurundkar et al .,1981).

Benjamin, M.M. (1978) Puerperal haemoglobinuria in buffaloes. . 3rd Ed. Iowa State Univ. Press, Ames. Iowa, CSA.

Beutler, E. (1971) Red Cell Metabolism: A manual and of Biochemical Methods. NewYorh, Grune and Straton .146p.

Bhikane, A.U., Ali, M.S., Narladkar, B.W. and Kawitkar, S.B. (1995) Post parturient hemoglobinuria in a crossbred cow and its treatment. *Indian Vet. J,* 72:734-736.

Bhikane, A.U. and Syed, A.M. (2014) Recent trends in buffaloes*. Intas Polivet,* 15(2):485-496.

Bhikane, A.U.; Anantwar, L.G.; Bhokre, A.P. and Narladkar, B.W. (2004) hemoglobinuria in buffaloes. Incidence clinic-pathology and treatment of *Indian Vet J*, 81:192-197.

Bush, B.M. (1975) Veterinary Laboratory Manual. William Heinemenn Med. Books Ltd., London, U.K.

Cheema, R.A.; Ijaz,G.R.; Chaudhry, N.l. and Ashfoque, M. (1980) Studies on epizootiology of parturient haemoglobinuria in a part of Punjab province. J. *Anim. Sci. Pak.,* 2. I l-16.

Chugh, S.K., Maa, M.M. and Malik, K.S. (1996) Lowered antioxidant status of red blood cells in post parturient haemoglobinuria of buffaloes. *Vet. Rec. Commun*, 22(6): 385-388.

Dabak, T.S. and Kirbas, A. (2007) The Post parturient haemoglobinuria in cattle . *Vet. Rec,* 61 : 86.

Digraskar, S.; Singh, B. and Rajguru, D.N. (1996) Blood chemistry and therapeutic management of metabolic haemoglobinuria in buffaloes . *Maharashtra Agri. Univ*, 21(1): 100-102.

Durrani, A.Z.; Kamal, N.; Shakoori, A.R. and Younus, R.M. (2010) Prevalence of post parturient hemoglobinuria in buffalo and therapeutic trials with toldimfos sodium and tea leaves in Pakistan. *Turk J Vet Anim Sci,* 34: 45-51.

Dyson, F.G . (2006)Red Blood Cells of Domestic Mammuls. Elsevier Science Publishing Co. Inc ... Amsterdam, PP: 337-111.

El Bagory, A.M. (1993) Ceding related to digestive disturbances in dairy animals .Ph.D. Thesis, Fac.Vet.Med., Cairo. Univ..

Gahlawat, I.; Singh, K. and Kumar, R. (2015) Investigations on oxidative stress in post parturient hemoglobinuria in buffaloes receiving sodium acid phosphate. *Italian J. Anim. Sci,* 6(Suppl 2):974-977.

Grunberg, K.D.; Heuer, C. and Bode, E. (2006) Variation of serum inorganic phosphorus and association with HaemoGlobinuria and osteomalacia in female water buffaloes in Pakistan. *Prev. Vet. Med*, 27(l-4):15-. Path.,56:442-381

Harvy, H . W and Boirss, M .L. (1997) Clinical Biochemistery of Domestic Animals. 5h Ed London .

Hoda, I.M. (2006) Clinico-Biochemical studies on hypophosphataemia in baffaloes with some therapeutic traits. Ph.D. Vet. Med. Assiut. Univ. Egypt.

Horst, S. K. (2010) Erythrocytic reduced gluiathione instability in post parturient haernoglobinuria of buffaloes. *Indian Vet. J..* 66 : 405- 409.

Jubb, T. F.; Jerrett, I.V.; Browning J.W. and Thomas, K. W..(1993) Haemoglobinuria and hypophosphataemia in post-parturient dairy buffaloes without dietary deficiency of phosphorus. *Aust. Vet . J .* 67(3) : 86 – 89 .

Kahn, C.M. and Line, S. (Eds.). (2005) The Merck veterinary manual,9thedition. Merck and Co., Inc. White house station. N.J., U.S.A. pp. 816.

Karapnar, T. M.; Amjad, M.H. and Ahmad, D.F. (2004) Bovine post-parturient haemoglobinuria in India. *Haryana Agri. Univ. J. Res*., 4(4) : 288-273.

Kumar, C.P., Praveena, G. and Sundar, N.S. (2014) Clinical management of acute post parturient haemoglobinuria in a graded Murrah buffalo. *Intas Polivet,* 15(2): 531-534.

Kelly, B. R. (1984) Veterinary Clinical Diagnosis” 1Ed., Printed in Great Britian by William Clews, Beccles and London.

Khan, A. and Akhtar, M.Z. (2007) Hemato-biochemical and clinico-epidemiological aspects of parturient hemoglobinuria in Nili Ravi buffaloes. Italian. *J. Anim. Sci*, 6(Suppl 2):953-956.

Khan, J. R and Aslam, W. S. (2001) Minerals in Animal Nutrition. 612 ed. Pearson Education (Singapore) Pre. Ltd., Indian Branch. Delhi. Indian.

Latimer, K.S. and Mahaffey, E.A. (2011) Parturient haemoglobinuria in buffaloes. *Pak. Vet. J.* 6: 95 – 87 .

Larsen, T., Moller, G. and Bellio, R. (2001) Evaluation of clinical and clinical chemical parameters in periparturient cows. *J. Dairy Sci,* 84: 1749-1758.

Mahmood, A.; Khan, M.A.; Younus, M.; Khan, M.A.; Iqbal, H. J. and Abdul, A. (2012) Case-control study of parturient hemoglobinuria in buffaloes. *Pak. Vet. J.,* 32:375-377.

Mahmood, A.; Khan, M.A., Younis, M.A.; Khan, M.A.; Ahad, A.; Ahmad, M.; Iqbal, H.J.; Fatima, Z. and Anees, M. (2013) Haematological and Biochemical risk factors of Parturient haemoglobinuria in buffaloes. *J. of Ani. and Plant Sci,* 23(2): 364-368.

Marinai, D. and Prox, G.V. (1973) Post-parturient haernoglobinuria in cattle: A Heinz body haemolytic anaemia. *N.Z. Vet. J,* 17(13): 244-363.

Montiel, L.;Tremblay, A.S.; Girard, A.V. and Chorfi, Y.M. (2007) Preanalytic factors affecting blood inorganic phosphate concentration in dairy cows. *Vet Clin Pathol*, 36: 278-280.

Pankaj Kumar, M. Kumar, Jadav, R.K. and Mondal, D.B. (2014) Influence on haemto - biochemical and oxidative indices of post Parturient haemoglobinuric (PHU) Buffalo *. Buffalo Bulle*, 33 (4).

Pirzada, W.H. and Hussain, S.Z. (1998) Parturient haemoglobinuria in buffaloes: a review. *Trop Anim. Health Prod*, 30 (4) 209 - 215.

Purohit, G.N.; Gaur, M.; Saraswat, C.S. and Bihani, D.K. (2013) Metabolic disorders in the parturient buffalo. In: Bubaline Theriogenology, Purohit GN (Ed). International Veterinary Information Service, Ithaca NY (www.ivis.org), Last updated: A5721.1013.

Purohit, G. N.; Trilok, G.; Amit, K.C.; Atul, S.A.; Mitesh, G. and Chandra, S.S. (2018) Perspectives of Parturient Hemoglobinuria (PPH) in Buffaloes. International Journal of Development Research. 2 (10): 23513-23520.

Radostits. O.M.; Gay, L.C.; Blood, D.C. and Hinchcliff, K.W. (2007) Veterinary Medicine : a Text book of the Diseases of Cattle, Sheep, Pigs. Goats and Horses. 9th ed. Saunders. London.

Radwan, M. E. and Rateb, H. Z. (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.

Rana, J.P. and Bhardwaj, R.M. (1988) Relationship of intraerythrocytic ATP and membrane phospholipids to red cell shape in hemoglobinuric buffaloes. Proc II World Buffalo Congress New Delhi, IV:178-184

Resum, N.S.; Kaur, P.; Singh, H. and Sharma, N. (2017) Post parturient hemoglobinuria (PPH) in bovine. Theriogenology Insight, 7(1):51-59.

Rizk, H.I.; Kamel, A.A. and Razek, M.S.A. (1999) Biochemical changes in subclinical cases associated with phosphorus deficiency in buffaloes at sharkia Governorate. *J. Vet. Med. Res.* 1: 165-174.

Roy. B.K. (2001) Textbook of Veterinary pharmacology and Toxicology .1 ed Kalyani publisher, New Delhi .pp.355-357.

Sai Krishna , K.S.; Suresh, K. ; Sreenu, M. and Syaama Sundar, N. (2014) Prevalence of Subclinical Hypocalcaemia and Subclinical Ketosis in Buffaloes. *Buffalo Bulletin*. 33 (1): 107-110.

Sharma, S.K.; Joshi, M.; Singh, D. and Khosa, J.S. (2014) A hematobiochemical and therapeutic study of postparturient hemoglobinuria in buffaloes. *Intas Polivet*, 15(2): 523-525.

Sherif, M.A. (2012) Advanced studies on ketosis and post parturient haemoglobinuria in cattle and buffaloes .Ph.D.Thesis. Fac of Vet. Med.Al mansoura. Univ. Egypt.

Singari, N.A.; Bhardwaj, R.M., Mata, M.M. and Bhardwaj, S. (1991) Status of erythrocyte G-6BD in phosphorous deficiency hemoglobinuria of buffaloes. *Indian Vet. J,* 68:226-230.

Singh, I. D. and . Kumar, R. (2013) Phosphorus deficiency haemoglobinuria in buffaloes in India. *Haryana Agri. Univ. J .Res*, 6(2) : 244-251.

Singh, R.; Bhardwaj, R.M. and Rana, J.P. (1992) Serum enzymes profile in post parturient hemoglobinuria in buffaloes. *Indian Vet. J,* 69:591-593.

Stockdale, C.R.; Moyes, T.E. and Dyson, R. 2005. Acute post-parturient hemoglobinuria in dairy cows and phosphorus status. *Aust. Vet. J,* 83: 362-366.

Soren, S.; Srivastava, M.; Kachhawa, J.P.; Soren, P.; Kumari, A. and Sharma, A. (2014) Clinical studies on post parturient hemoglobinuria in buffaloes. *Intas Polivet*, 15(2):518-522.

Swecker, V.S. (1995) Effect of selenium supplementation on cholesterol lgG concentration in cows grazing selenium-deficient pastures and post suckle serum lgG concentration in their calves. *Am. J. Vet. Res*, 56: 450-453.

Ussama, I. E. (2009) Clinical and biochemical studies on red urine as field problem in cattle. Ph .D .Thesis . Fac .Vet. Med Zag . Univ. Egypt.

Walter, G. (2011) Treatment of phosphorus . balanced disorders. Clinic for Cattle, Univ. Of Vet Med Hannover foundation. *Bischofsholer Damm .* 15, 30173 hand over Germany.