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PERSPECTIVES OF PARTURIENT HEMOGLOBINURIA (PPH) IN BUFFALOES

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ABSTRACT

PPH is a sporadic phosphorous deficiency metabolic disorder affecting adult buffaloes around the world both during advanced pregnancy and post partum period. Feeding of cruciferous plants, sugar cane tops, sugar beets exacerbate phosphorous deficiency either due to their low phosphorous content or an account of certain inhibitor factors that prevent proper absorption of dietary phosphorous. The serum phosphorous levels in affected buffaloes are as low as 0.9 mg/dl and usually half of the normal (5.0 mg/dl) values. The low phosphorous disrupts the phospholipid layer of RBCs resulting in hemoglobinuria, loss of appetite, reduction in milk production and death if animals are unattended. Buffaloes can be treated by the IV infusion of a 20% solution of sodium acid phosphate (60-80 gm dissolved in 300-400 ml of dw) along with supportive therapy. The more severely affected buffaloes require blood transfusion and supportive therapy. It is safe to terminate pregnancy to save the buffalo. The epidemiology, possible etiological factors, risk factors, pathogenesis, clinical findings, clinical pathology, diagnosis, therapy and prevention are mentioned in this manuscript.

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INTRODUCTION

Parturient hemoglobinuria also known as red water, hypophosphatemia or post parturient hemoglobinuria is an acute life threatening disease of high yielding buffaloes and cattle characterized by hypophosphatemia, intravascular haemolysis, hemoglobinuria, and anemia (Akhtar *et al*., 2006). It has been recognized since last more than 45 years back (Hussain, 1955; Awad and El-Latif, 1963; Nagpal *et al*., 1968) and considered to be the most frequent metabolic disorder of parturient buffaloes (Purohit *et al*., 2014) compared to milk fever and ketosis. A higher incidence has been recorded for buffaloes compared to cattle (Bhikane and Syed, 2014). The disorder is highly fatal owing to intravascular haemolysis and severe anemic anoxia (Gahlawat *et al*., 2007). In cattle the condition is common during the post partum period however, in buffaloes the condition is mostly observed during advanced pregnancy or within a month of parturition (Bhikane *et al*., 2004; Purohit *et al*., 2014).

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Epidemiology

The disorder is of sporadic occurrence in buffaloes in India (Bhikane *et al*., 2004; Gahlawat *et al*., 2007; Iqbal *et al*., 2011), Pakistan (Muhammad *et al*., 2001; Akhtar *et al*., 2007; Khan and Akhtar, 2007; Akhtar *et al*., 2008; Durrani *et al*., 2010), Egypt (Radwan and Rateb, 2007; Ghanem and El-Deeb, 2010), Brazil (Neto *et al*., 2007; Barbosa *et al*., 2013), Sri Lanka (Sivakanesan, 1999) and Iran (Dalir-Naghadeh *et al*., 2006). Adult buffaloes are usually affected with higher frequency during advanced pregnancy (8-9 months) and the immediate post partum period (1-60 days post partum) (Bhikane *et al*., 2004; Dalir-Naghadeh *et al*., 2006; Gahlawat *et al*., 2007; Durrani *et al*., 2010). A rare case of PPH was recorded in a buffalo 16 weeks post partum (Khan and Akhtar, 2007). During gestation a higher incidence was recorded in a

few studies (Akhtar *et al*., 2006; Dhonde *et al*., 2007) while others recorded a higher incidence during the post partum period (Bhikane *et al*., 2004). Akhtar *et al*. (2007) mentioned the deficiency of phosphorous in soils with resultant deficiency in fodders as the main reason why buffaloes develop PPH. The 3rd – 6th lactation are considered to have the highest incidence of the disease (Bhikane and Syed, 2014; Soren *et al*., 2014).

Incidence

The overall incidence of the condition is low. In most countries the disorder is sporadic and of low incidence affecting high producing buffaloes however, it was mentioned that in certain areas in India (Haryana, Rajasthan, Uttar Pradesh and Punjab) the disorder is much more prevalent (Purohit *et al*., 2013) due to poor quality of roughages fed to animals in Rajasthan (Mathur, 2015) and other places. Purohit *et al*. (2014) analyzed the calving records of 529 Surti buffaloes at a organized University farm and found no single case of parturient hemoglobinuria. Analysis of clinical records of 729 buffaloes to the referral clinic revealed 20.02% cases of parturient hemoglobinuria (Purohit *et al*., 2014). In Punjab (Pakistan) the incidence was recorded to vary from 0.02 %– 4.4% (Muhammad *et al*., 2000). In another survey in Maharashtra (India) the incidence was 0.70% (Bhikane *et al*., 2004). A seasonal incidence of the occurrence of the disorder has been recorded in many studies with many studies recording a higher incidence during winter (Bhikane *et al*., 2004; Khan and Akhtar, 2007; Sharma *et al*., 2014; Soren *et al*., 2014) while a few studies recorded a higher incidence during summer (Dhonde *et al*., 2007; Jain *et al*., 2009). About 60% cases recorded during winter season could be due to cold stress in recently calved animals (Bhikane *et al.,* 2011). The seasonal incidence is probably related with the availability of phosphorous in the soil and the diets fed to buffaloes (Akhtar *et al*., 2006; Akhtar *et al*., 2007). Moreover, stress during the hot summer months exacerbate the condition.

Risk factors

In a recent analysis it was found that age, lactation number, stage of pregnancy, post partum period, previous history of hemoglobuinuria and ingestion of cruciferous plants were considered putative risk factors for development of PPH in bovines (Resum *et al*., 2017). In a previous clinical study it was shown that buffaloes are more prone to PPH compared to hypocalcemia (Purohit *et al*., 2014). Pregnancy beyond 6 months and lactation are considered the putative risk factors for the development of the disorder in buffaloes with the relative risk and odds ratio for pregnancy and gestation being 1.71 and 3.77 (Samad, 1997). It has been mentioned that buffaloes once affected with parturient hemoglobinuria are 21 times more likely to have low serum profiles of phosphorous and are thus likely to suffer from the condition again (Heuer and Bode, 1998). A recent study found that age (≥7 years), pregnancy (≥7 months), lactation (≥3), post-partum period (≤60 days), previous history of parturient hemoglobinuria and ingestion of cruciferous plants were significant risk factors for the disorder in buffaloes, whereas feeding of cottonseed cake, use of mineral mixture or drugs, previous history of disease other than parturient hemoglobinuria were non significant factors in the development of the disease (Mahmood *et al*., 2012). The highest risk of occurrence of PPH is in 3rd to 6th parity (Rana *et al*., 2006; Soren *et al*., 2014). Peak occurrence was recorded in the 5th parity (Mahmood *et al*., 2012). The highest cases of PPH were observed in buffaloes producing 10 or more liters of milk/day (42.5%) (Rana *et al*., 2006; Khan and Akhtar, 2007). However, a few studies found little association of milk production with the development of PPH in buffaloes (Akhtar *et al*., 2008; Mahmood *et al*., 2012).

Etiology

The etiology of parturient hemoglobinuria appears to be multifactorial yet poorly understood. However a consistently low serum phosphorous in affected buffaloes reflects deficiency of phosphorus as the putative cause for the condition. Dietary deficiency of phosphorous has been considered a common cause of the condition (Dalir-Naghadeh *et al*., 2006; Jain *et al*., 2012; Singh *et al*., 2012). Feeding of sugar cane tops, sugar beet, kale, mustard, cabbage and lucerne (Sivakanesan, 1999) or berseem (Muhammad *et al*., 2001; Akhtar *et al*., 2006), gram/lentil straw (Jain *et al*., 2012), sorghum straw (Bhikane *et al*., 2004; Dhonde *et al*., 2007), sugar beet pulp (Iqbal *et al*., 2011) cabbage and turnip leaves (Mc Williams *et al*., 1982) wheat/ rice straw (Singh *et al*., 2012) or paddy straw (Kumar *et al*., 2014) precipitate deficiency of phosphorous either due to their low content of phosphorous or due to presence of inhibitory factors such as metallic ions which interfere with the absorption and assimilation of dietary phosphorous (Sivakanesan, 1999). Addition of maize husk and barley grit caused an outbreak of nutritional hemoglobinuria in recently calved buffaloes at a farm (Chhabra *et al*., 2015). The low phosphorus content in many of the feeds fed to cattle was summarized by Mc Williams *et al*. (1982). Areas with soil deficient in phosphorous would produce crops deficient in phosphorous (Akhtar *et al*., 2007) with resultant phosphorous deficiency. High levels of molybdenum (Patel *et al*., 2002; Akhtar *et al*., 2006) or potassium in the feed or high salt content in the water (Neto *et al*., 2007) can exacerbate phosphorous deficiency. Higher potassium was recorded in roughages and soil in rice growing areas of Pakistan with concurrent low calcium and phosphorous in the serum of buffaloes raised in these areas (Pasha *et al*., 2012). Similar findings were also recorded in an arid area in India with recommendations for supplementing the buffalo diets with phosphorous and calcium (Sharma *et al*., 2010).

Thus the dietary cation/ anion ratio seem important in maintaining the homeostasis of phosphorous and calcium in the serum. The parathyroid hormone secreted during periods of calcium stress increases the renal and salivary secretion of phosphorous which can be detrimental to maintenance of normal phosphorous concentrations. This is one reason that hypocalcemic animals tend to become hypophosphatemic (Goff, 2000). Copper deficiency is also an etiological factor of post-parturient hemoglobinuria as its deficiency reduces the activity of the copper-containing enzyme superoxide dismutase, which is a part of the erythrocyte protective mechanism against oxidative stress (Purohit *et al*., 2013). Low copper was recorded in buffaloes affected with parturient hemoglobinuria (Akhtar *et al*., 2006). Ingestion of cold water was shown to precipitate hypophosphatemia in a pregnant Egyptian buffalo (Awad and El-Latif, 1963).

Pathogenesis

The pathogenesis of erythrocyte destruction leading to anemia and hemoglobinuria in PPH continues to be poorly known. All

animals suffering from hypophosphatemia do not develop intravascular haemolysis (Mohammed and El-Bagoury, 1990; Yates, 1990). There is an association with hypophosphatemia and a low dietary intake of phosphorus, and it is presumed that the drain of lactation causes further depletion of phosphorus reserves. The dependence of mammalian red blood cells on glucose metabolism as the main source of energy for viable function and structure makes them highly vulnerable to factors inhibitory to the glycolytic pathways. The deficiency of phosphorous has been documented to result in depletion of ATP (Samad and Malik, 1996). The proposed mechanism is a marked drop in red blood cell adenosine triphosphate (ATP) and 2,3 diphosphoglycerate (2,3-DPG) synthesis in RBC's in states of phosphorous depletion presumably because of insufficient availability of intracellular phosphate (Wang *et al*., 1985). Fifteen to twenty percent of phosphorous is found in the fluids and soft tissue of cows (Grunberg, 2014). RBC's require ATP among other things to control the cell volume and deformability through active extrusion of sodium (Jubb *et al*., 1990). Without sufficient ATP to power sodium pumps, the intracellular sodium concentration rises and the cells become more rigid and, as a result, rupture as they pass through capillary beds (Goff, 2000).

The reduced ATP levels along with decreased membrane phospholipids (which help in maintaining the shape and integrity of red cells) were considered responsible for the change in the shape of red blood cells with resultant echinocytosis/ sphero-echinocytosis which are prone to hemolysis (Rana and Bhardwaj, 1988). Hypophosphatemia alone is rarely a sufficient cause of RBC fragility. Often such cows are on diets that are also deficient in selenium, copper and energy (Goff, 2000). Cruciferous plants are known to cause hemoglobinuria because of their high s-methyl cysteine sulfoxide content which is converted by ruminal microflora to dimethyl-sulfoxide (Muhammad *et al*., 2001). Once absorbed into circulation dimethyl sulfoxide causes precipitation of haemoglobin leading to haemolysis (Yates, 1990). Sugar beets, alfalfa and berseem are thought to contain saponins as haemolytic factors (Mohammed and El-Bagoury, 1990; Muhammad *et al*., 2001). Low phosphorous intake by cows during early lactation might produce hypophosphatemia as a result of the sudden and increasing loss of phosphorous through milk (Mc Williams *et al*., 1982). Similarly it was noted that heavy drainage of phosphorus through milk, particularly in high milk yielding buffaloes, leads to hypophosphatemia (Bhikane *et al*., 2004). It has been observed that during advanced gestation, more phosphorus and calcium are required for the developing fetus and absence of supplementation leads to hyphosphatemia (Digraskar *et al*. 1991). Moreover, high calcium to phosphorus ratio observed during gestation in buffaloes (Hanif *et al*., 1984) results in decreased phosphorus absorption from the intestinal tract and ultimately leads to hypophosphataemia.

Phosphorus deficient soils are common in dry tropical countries like India and Pakistan (Akhtar *et al*., 2008). Although many soils are naturally deficient in phosphorus, heavy leaching by rain and constant crop removal also contribute to phosphorus deficiency in soil. It was noticed that fodders grown on phosphorus deficient soils are consequently low in phosphorus content, and thereby prolonged feeding on such fodders can lead to hypophosphatemia (Akhtar *et al*., 2007). A significant decrease in erythrocyte count, haemoglobin concentration, and haematocrit in affected buffaloes, indicating severe anaemia was recorded in hemoglobinuria affected buffaloes (Soren *et al*., 2014). Intravascular haemolysis probably occurs due to impaired glycolytic pathway and depletion of ATP in erythrocytes results from phosphorus deficiency (Malik and Samad, 1996). Subnormal concentration of ATP predisposes red blood cells to altered functions and structure, causing a loss of normal formability, and an increase in fragility, ultimately leading to haemolysis. In vitro studies on buffalo erythrocytes revealed that the uptake and fate of glucose was dependent on the concentration of phosphorous in the medium. When erythrocytes were incubated in a synthetic medium deficient in phosphorous all classes of cellular phosphates including ATP were significantly depleted and these changes could be revoked by addition of phosphorous to the medium (Malik and Samad, 1996). Erythrocytes from parturient hemoglobinuria affected buffaloes revealed significant depletion of phosphorous, ATP and acid labile phosphates (Samad and Malik, 1996). In vitro incubation of these erythrocytes in a phosphorous containing medium replenished the cellular phosphates (Samad and Malik, 1996).

A possible mechanism for parturient hemoglobinuria could be the reduction in the glutathione content in red blood cells (Bhardwaj *et al*., 1988; Sarma *et al*., 2014) and the increased malondialdehyde levels in erythrocytes of affected buffaloes (suggestive of oxidative stress) (Sarma *et al*., 2014). A drastic reduction in the glutathione content of red blood cells in parturient hemoglobinuria affected buffaloes was recorded (Singari *et al*., 1989; Mata *et al*., 1994b). A low activity of glucose-6 phosphate dehydrogenase (Singari *et al*., 1991), red cell catalase (Bhardwaj *et al*., 1988) and a significant increase in SGOT, SGPT and alkaline phosphatase was recorded in parturient hemoglobinuria affected buffaloes suggesting the possible alterations in these enzymes in precipitating hemolysis. Oxidative stress has been suggested as a possible putative factor for hemolysis (Gahlawat *et al*., 2007). Significantly ($p \leq 0.05$) increased serum GGT activity in buffaloes with PPH indicated stress (Kataria *et al*., 2013). Development of the disease in pregnant buffaloes during advanced pregnancy is presumed to be due to increased demand of the developing fetus (Raz *et al*., 1988; Chugh *et al*., 1996) coupled with dietary deficiency (Dhonde *et al*., 2007). It was also mentioned that from the seventh month onwards pregnant buffaloes have a significantly wider ratio of blood Ca/P compared to those in the earlier stages of pregnancy (Hanif *et al*., 1984).

Clinical findings

The first notable clinical sign in affected buffaloes is the passage of red to coffee colored urine (Bhikane *et al*., 2004; Akhtar *et al*., 2006; Akhtar *et al*., 2008; Jain *et al*., 2009) within 20±10 days before or after parturition and rectal temperature of 101^0 F to 103^0 F (Kumar *et al*., 2014; Purohit *et al*., 2014). The pulse rate, temperature and respiratory rates are increased in PPH affected buffaloes (Dhonde *et al*., 2007). Enlarged lymph nodes, pale mucous membranes, decreased capillary refill time (1-2 per sec), dyspnoea, weak pulse, tachycardia, panting and mild salivation are some of the observed clinical findings in affected buffaloes (Madheswaran *et al*., 2017). Pale mucous membrane, tachycardia, tachypnea are recorded in affected buffaloes (Shalini *et al*., 2015). The urine from hemoglobinuria affected buffaloes produces moderately stable foam when it falls on the ground

(Muhammad *et al*., 2001; Reddy *et al*., 2014a). In most cases the appetite is normal but milk yield is significantly reduced (Akhtar *et al*., 2006) and anorexia is marked as the disease progresses. Ruminal movement and milk yield are significantly ($P<0.01$) lower in post-parturient haemoglobinuria affected buffaloes as compared to healthy control buffaloes (Sirari, 2017). Constipation is common and faeces are hard and black tinged (Purohit *et al*., 2014; Soren *et al*., 2014). In a study on 40 buffaloes affected with PPH red/coffee colored urine, constipation and pale mucus membrane were recorded in 100% of the buffaloes (Soren *et al*., 2014; Sirari, 2017). Excessive formation of hemosiderin and its deposition in the gastrointestinal mucosae in hemoglobinuria affected buffaloes could be responsible for gastro intestinal disturbances like ruminal stasis, constipation, straining and dark coloration of the feces (Sharma *et al*., 2014). The body temperature is slightly higher initially but then tends to become subnormal (Bhikane *et al*., 2004; Sharma *et al*., 2014; Soren *et al*., 2014). Heart rate is slightly increased (Bhikane *et al*., 2011). The mucous membranes of the conjunctiva and vulva are discoloured or pale in appearance and affected buffaloes may evidence a foul breath and frothy urine (Durrani *et al*., 2010; Kumar *et al*., 2014). With the progression of the disease jaundice and weakness may be evident with recumbency (Akhtar *et al*., 2006). Laboured breathing and jugular pulsation can be observed during the terminal stages of the disease. Clinical signs mostly appear after 6-8 months of pregnancy (Mahmood *et al*., 2012; Dhonde *et al*., 2007) in pregnant buffaloes and a proportion of buffaloes may abort (Purohit *et al*., 2014). Soren *et al*. (2014) observed that faeces might be firm and dry or fetid and occasionally buffaloes may be diarrheic. According to them, an elevated body temperature (up to 40 °C) in the early stages is a variable sign. At later stages of the condition low temperature is common.

The disease is most commonly observed during summer when animals are fed straw from wheat or paddy, or stoves of maize, sorghum and pearl millet, which are all very poor sources of phosphorous (Bhikane *et al*., 2004). The duration of the disease varies from 3-9 days (Khan and Akhtar, 2007) with fatality up to 15%. Death may occur within a few days. In non-fatal cases, convalescence requires about 3 weeks and recovering animals often show pica. Recovered animals may continue to evidence decreased appetite or respiratory distress (Soren *et al*., 2014). A small proportion of buffaloes may develop a concurrent ketosis. In a recent study, 1/13 hemoglobinuric buffaloes developed ketosis (Gupta *et al*., 2010). Rough hair coat and a stiff gait is noticed in some affected buffaloes (Singh *et al*., 2002) and a proportion of buffaloes may become recumbent. Gangrene at the tip of ears has been observed in pregnant hemoglobinuric buffaloes (Dhonde *et al*., 2007). A proportion of late pregnant buffaloes that develop hemoglobinuria may abort (Purohit *et al*., 2014; Bhikane, 2011) with consequent retention of placenta and development of metritis (Bhikane, 2011). Recurrence of PHU was observed in 18.3% buffaloes (Khan and Akhtar, 2007).

Clinical pathology

In marginal phosphorus-deficient areas, normal non-lactating animals in an affected herd may have serum inorganic phosphorus levels within the normal range. Lactating buffaloes in an affected herd may have moderately low levels of phosphorous below 4 mg/dL without any clinical sign (Ghadge *et al*., 2010) or low values (2- 3 mg/dL) with limb paralysis as a clinical sign (Habib *et al*., 2004). However, in Romanian buffaloes the phosphorous values were recorded as 2.68±1.26 and 2.28±0.92 in pregnant and lactating buffaloes without any clinical change (Serdaru *et al*., 2011). Random samples collected from 95 buffaloes from 67 units of 29 villages in Punjab (India) revealed hypophosphatemia in 26.7% animals of rural dairy units compared to 11.1% of the organized farm (Randhawa *et al*., 2009).

Table 1. Serum phosphorous in healthy buffaloes and buffaloes affected with parturient hemoglobinuria

Serum phosphorous (mg/dl)		Reference
Healthy buffaloes	Buffaloes with parturient hemoglobinuria	
	2.52	Neto et al., 2007
	1.48±0.14	Bhikane et al., 2004
	1.50±0.01	Dhonde et al., 2007
5.50±0.29	1.95±0.06	Jain et al., 2009
3.87±0.21	2.10±0.29	Gahlawat et al., 2007
5.41±0.6	1.8±0.4	Durrani et al., 2010
3.94±0.17	2.16±0.06	Rajbir and Sridhar, 2002
	1.65±0.02	Gupta et al., 2010
	3.68±0.95	Hagawane et al., 2009

Buffaloes with hemoglobinuria evidence extremely low levels of 0.97-2.6 mg/dL of phosphorous (Table 1). The blood glucose, bilirubin, creatinine and serum alkaline phosphatase are elevated in affected buffaloes (Bhikane *et al*., 2004; Dhonde *et al*., 2007; Akhtar *et al*., 2008) suggesting jaundice due to intravascular hemolysis. The blood glucose, serum total bilirubin and serum alkaline phosphatise concentrations in PPH affected buffaloes were significantly higher ($P<0.05$) than in healthy buffaloes (Akhtar *et al*., 2008; Sirari, 2017). The serum enzymes AST, ALT and ALP are significantly elevated in buffaloes with parturient hemoglobinuria (Sirari, 2017). Hemoglobinuria affects the functional activities of different body organs mainly liver, heart and kidneys (Malik and Gautam, 1971). A marked decrease in the PCV, hemoglobin and total erythrocyte count has been recorded in hemoglobinuria affected buffaloes along with Howell Jolly bodies (Bhikane *et al*., 2004). Hemoglobin values ranged from 5.75±0.04 gm/dL to 6.10±0.5 gm/dL in affected buffaloes (Bhikane *et al*., 2004; Dhonde *et al*., 2007; Jain *et al*., 2009; Gupta *et al*., 2010; Durrani *et al*., 2010). The packed cell volume and total erythrocyte count varied from 16.53±0.23 to 20.17±1.38 and 2.83±0.04 $x10^6$/mL to 3.06±0.29 $x10^6$/mL respectively in different studies (Bhikane *et al*., 2004; Dhonde *et al*., 2007; Jain *et al*., 2009; Gupta *et al*., 2010; Durrani *et al*., 2010). Heinz bodies may be present in erythrocytes. The mean values of total erythrocyte counts, hemoglobin concentration and packed cell volume were lower ($P<0.001$), while erythrocyte sediment rate was higher ($P<0.001$) in PPH affected buffaloes as compared to the values in healthy buffaloes (Khan and Akhtar, 2007; Mahmood *et al*., 2013a).

Affected buffaloes evidence macrocytic hypochromic anemia with neutrophilia, higher creatinine and serum urea nitrogen (Bhikane *et al*., 2004; Dhonde *et al*., 2007; Mahmood *et al*., 2013a). Decrease in MCH and MCHC values were recorded in hemoglobinuria affected buffaloes (Sateesh *et al*., 2017). Serum phosphorus, copper and selenium were signifcantly ($P<0.001$) lower, whereas potassium, iron and molybdenum ($P<0.001$) were higher in buffaloes suffering from PHU than healthy buffaloes (Khan and Akhtar, 2007). Experimentally induced hypophosphatemia in cows failed to show effects on

erythrocyte or osmotic resistance indicating that phosphorus depletion alone is unlikely to cause intravascular hemolysis (Grunberg *et al*., 2015). The urine is dark red-brown to black in color and usually moderately turbid. No red cells are present in the urine. Urine analysis reveals a pH of 8.0-9.0 and urine samples are positive for protein and hemoglobin (Bhikane *et al*., 2004) and sometimes ketones and bile pigments (Digraskar *et al*., 1991; Singh *et al*., 1994). Due to probable overload in the liver the serum SGOT, SGPT and ALP levels are increased in affected buffaloes (Singh *et al*., 1992).

Diagnosis: Postparturient hemoglobinuria is characterized by an acute hemolytic anemia in buffaloes calved within the preceding 4 weeks. Other causes of acute hemolytic anemia are not confined to the post calving period. Laboratory examination is usually necessary to confirm the diagnosis and to eliminate hematuria as a cause of the discoloration of the urine. The presence of haemoglobin in the urine can be verified by the Benzidine test or using commercially available dip stick strips (Kumar *et al*., 2014). On centrifugation of the dark coffee colored urine the red blood cells settle at the bottom in hematuria whereas in hemoglobinuria the color remains unchanged. The differential diagnosis of red urine in buffalo was summarized by (Muhammad *et al*., 2001) however, this is not always required. The lecithin cholesterol acyltransferase (LCAT) was found to be low in hemoglobinuria affected buffaloes in a recent study (Ghanem and El-Deeb, 2010) and was thus suggested as a predictor for parturient hemoglobinuria in buffaloes. Significantly lower activity of LCAT was recorded in 37 Egyptian buffaloes 4 weeks prepartum and 23 and 14 of these buffaloes developed ketosis and parturient hemoglobinuria after parturition. The levels of SGOT, SGPT and ALP have been suggested as diagnostic indicators for hemoglobinuria in buffaloes (Singh *et al*., 1992).

Mortality: The mortality rate recorded in different studies vary from 12% (Nagpal *et al*., 1968) to 15% (Akhtar *et al*., 2006; Khan and Akhtar, 2007) however, exceptionally high mortality of 53.5-63.4% was recorded in studies in Pakistan (Cheema *et al*., 1980; Raz *et al*., 1988). The fatality is dependent upon the time of initiation of therapy since the onset of disease. In buffaloes receiving therapy early the mortality is low (Sivakanesan, 1999). Death usually occurs due to anoxic anoxia.

Necropsy findings: The blood is thin and icterus is widespread throughout the body. The liver is swollen, and fatty infiltration and degeneration are evident. Discolored urine is present in the bladder. Grossly, carcasses of buffaloes died of PHU were found anaemic and jaundiced. The kidneys and liver are pale enlarged and congested with deposition of hemosiderin (Akhtar *et al*., 2006). The epicardium and endocardium evidence hemorrhages and the lungs are emphysematous and edematous (Bhikane *et al*., 2004; Akhtar *et al*., 2006). The lungs are emphysemated and oedematous (Akhtar *et al*., 2008). Histopathologically, necrosis of tubular epithelium, deposition of casts along with atrophy of glomeruli, centrilobular necrosis and haemosiderin deposition were the main post mortem findings in buffaloes that died of PHU (Akhtar *et al*., 2008).

Treatment: Sodium acid phosphate 60-80 g dissolved in 300 mL of distilled water as a 20% solution administered IV is the usual therapy administered to affected buffaloes (Bhikane *et al*., 2004; Dhonde *et al*., 2007; Gahlawat *et al*., 2007; Sirari, 2017). This is followed by a similar dose administered SC and oral supplementation of phosphorous (60 gm), copper sulfate (3-5 gm) and cobalt sulfate (100 mg) for a period of 7-10 days. Two to four IV therapies are required for complete recovery (Bhikane *et al*., 2004). The intravenous injection of phosphorus in the form of monobasic sodium phosphate (60g) in 300 ml of sterile distilled water followed by oral administration of the same dose, twice daily for 3 days, has often been effective (Sivakanesan, 1999). Inorganic phosphorous injections are currently suggested to be administered IV (Muhammad *et al*., 2001; Tewari *et al*., 2014) along with fluids (Reddy *et al*., 2014b) with good recovery rates (Bhikane *et al*., 2011) and disappearance of hemoglobinuria within 48-72 h of therapy. In one recent study the administration of inorganic phosphorus @ dose rate of 15 mg/kg body weight facilitated the recovery in hypophosphatemic pregnant Murrah buffaloes (Rashid *et al*., 2018). Ten percent glycerol phosphate calcium (1mL/kg IV) was suggested in one study (Randhawa *et al*., 1994) along with twice daily oral supplementation with 100-120 g of dicalcium phosphate with good recovery in affected buffaloes. Similarly, twice daily oral feeding of 120 gm of dicalcium phosphate or twice daily oral feeding of 200 ml of super juice (Prepared by dissolving one Kg of the single superphosphate fertilizer dissolved in 16 liters of water and kept for 12 h) along with sodium acid phosphate for 7 days resulted in good clinical recovery (Deeba *et al*., 2018).

In severe cases blood transfusions may be a useful supportive therapy (Muhammad *et al*. 2001). Blood collected from slaughtered buffaloes supplemented with 2.5 gm of sodium citrate (per litre of blood), penicillin and streptomycin is suggested for transfusion in buffaloes (Muhammad *et al*., 2001). Oral supplementation of copper sulphate (2 gm in 500 mL of water) has been advocated for hemoglobinuric buffaloes in areas with high content of molybdenum in fodder (Dhillon *et al*., 1972). Alternatively copper glycinate (1.5 mg/Kg dissolved in 540 mL normal saline and infused IV) has also been suggested (Muhammad *et al*., 2001). Subcutaneous injections of commercially available copper glycinate have also been suggested (Purohit *et al*., 2014). Supplementation of iron and other medicaments would depend upon the general condition and requirement (Singh *et al*., 2002; Dhonde *et al*., 2007; Gupta *et al*., 2010; Kumar *et al*., 2014). Reoccurrence of the condition in recovered buffaloes has been recorded in 18.3% to 27.4% buffaloes (Aktar *et al*., 2006). Antifibrinolytic drugs have been suggested for the therapy of hemoglobinuria on the basis of increased fibrinolytic activity in affected buffaloes (Rao *et al*., 1977).

Epsilon amino caproic (EACA) (20 g dissolved in 540 mL of 5% dextrose and administered IV till recovery) and para-amino methyl benzoic acid (300 mg dissolved in 540 mL of 5% dextrose and administered IV till recovery) have been shown to be 90-92% effective (Chugh *et al*., 1987; Chugh *et al*., 1988). Similarly Botropase (a blood coagulant and antifibrinolytic drug prepared from the venom of snake; *Bothrops jararaca*) has been suggested as a supplement to sodium acid phosphate therapy in buffaloes with good recovery rates (Muhammad *et al*., 2001). Administration of antifibrinolytic drug tranexamic acid (Transamine injection 250mg × 10) along with sodium acid phosphate resulted in 70% recovery rate in affected buffaloes whereas 100% recovery rates were observed when sodium acid phosphate was

administered along with blood transfusion in more severely affected buffaloes (Mahmood *et al*., 2013b). Oxygen releasers like inosine (0.5 g in 5-10 mL of dilute HCl mixed with 540 mL of 5% dextrose administered IV once daily for 2-3 days) have also shown promise in buffaloes that continue to evidence respiratory distress despite a clear urine. The erythrocyte malondialdehyde (MDA), reduced glutathione (GSH) and erythrocyte fragility were high in PPH affected buffaloes indicating oxidative stress and these decreased significantly following therapy with buffered phosphorous with inosine and sodium pyruvate (Yadav *et al*., 2014). Antioxidants have been suggested for therapy of hemoglobinuria in buffaloes because of the possible oxidative stress that may be present in affected buffaloes (Gahlawat *et al*., 2007) with vitamin C being the common antioxidant used. Ascorbic acid either at 5g (Dhonde *et al*., 2007) or 7.5g infused daily IV (Rana *et al*., 2006) along with 500 mL of normal saline (Chugh and Mata, 1997) were found to be 68.5 and 82% effective, respectively. Other antioxidants like vitamin A and E have also been suggested to be helpful (Gupta *et al*., 2010).

Prevention

An adequate intake of phosphorus according to the requirements for maintenance and milk production should be ensured, particularly during late gestation and early lactation. A decrease in the incidence of the disease was reported after copper supplementation of cattle in a copper-deficient area (Smith *et al*., 1975). However, in one study on buffaloes copper was found to be normal in hemoglobinuria affected buffaloes (Rajbir and Sridhar, 2002). By virtue of hemolytic saponin and the low phosphorous contents cruciferous plants like Berseem (Trifolium alexandrium) (Abdel Latif and Awad, 1964) are suggested to be fed mixed with other fodders to reduce the incidence. Supplementation of the diet or water with a source of phosphorous is suggested in deficient areas (Muhammad *et al*., 2001). This includes addition of sodium acid phosphate 30 g/animal/day or bone meal 100 g/animal/day or the use of commercially available preparations (Muhammad *et al*., 2001).

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