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Prevalence of post parturient haemoglobinuria in buffalo and therapeutic trials with toldimfos sodium and tea leaves in Pakistan

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Abstract: The present study was carried out to investigate the prevalence of post parturient haemoglobinuria in buffaloes, and to compare the therapeutic efficacy of toldimfos sodium, sodium acid phosphate, and tea leaves. The prevalence of disease in 1000 animals, grouped according to varying stages of lactation and pregnancy, was recorded. Screening of positive animals based on clinical signs of haemoglobinuria, anaemia, and moderate pyrexia was performed. Urine and blood samples were collected. Blood samples were processed for haematological parameters and biochemical profile. Blood films were made and stained. The animals that were positive for disease were treated with sodium acid phosphate, toldimfos sodium, and tea leaves. Blood samples were collected before treatment and on day 1, 2, and 3 post-treatment, and processed in the laboratory for haematological parameters and biochemical profile. The data were analyzed statistically. Highest prevalence of post-parturient haemoglobinuria was recorded in animals within 4 weeks postpartum (60%). The highest prevalence of disease was seen at lactation 5 (32%). Therapeutic trials showed the highest efficacy with toldimfos sodium (85%) followed by tea leaves (56%) while the lowest efficacy was observed with sodium acid phosphate (18%).

Key words: Buffalo, haemoglobinuria, hypophosphataemia, post-parturient, tea leaves, toldimfos sodium

Introduction

Parturient haemoglobinuria is recorded in buffalo rearing countries particularly in India, Pakistan, and Egypt as described by Pirzada and Hussain (1). Kurundkar et al. (2) described this acute disease in high yielding buffaloes and cows associated with hypophosphataemia and characterized by intravascular haemolysis. Singari et al. (3) reported that hypophosphataemia affected multiparous females and a variety of etiological factors were reported to be associated with the disease in different parts of the world. Heuer and Bode (4) reported that dietary phosphorus deficiency and/or rations containing cruciferous plants were suspected causes of severe hypophosphataemia and have been associated with hemolytic anaemia in cows. According to them,

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copper deficiency is also an etiological factor of postparturient haemoglobinuria, as its deficiency reduces the activity of the copper containing enzyme, superoxide dismutase, which is part of the erythrocyte protection mechanism against oxidative stress. According to Singari et al. (3) hypophosphataemia results in decreased red blood cell glycolysis and ATP synthesis. Subnormal concentration of ATP predisposes red blood cells to altered structure and function, and an increase in fragility and haemolysis, with resultant haemoglobinaemia and haemoglobinuria. Spears (5) reported that haemoglobinuria is often the first clinical sign before anaemia, depression, inappetence, or decreased milk production. According to Spears (5), mucous membranes become pale or may become icteric with the progression of anaemia and with tachycardia breathing becoming rapid and shallow. Affected cows show weakness and recumbency. Heuer and Bode (4) reported that faeces might be firm and dry or fetid and diarrheic. According to them, an elevated body temperature (up to 40 °C) in the early stages is a variable sign. Ogawa et al. (6) reported that the incidence of the disease in the total cattle population is very low with a case fatality rate ranging from 10% to 50%. They further reported that farms with a particularly high incidence are occasionally encountered but usually only 1 or 2 cases are clinically apparent at a time. In the present study, based on the practices of the local farmers, tea leaves, along with toldimfos sodium, were used to observe the recovery of the animals with red coloured urine. We hope that the present study will contribute to a better understanding of this problem in buffaloes in Pakistan.

Materials and methods

Selection and categorization of animals:

One thousand buffaloes were randomly selected during 2007. The prevalence of postparturient haemoglobinuria was recorded at varying stages of lactation and pregnancy. For this purpose different groups were made, including (1) animals within 4 weeks postpartum, (2) non-pregnant and lactating, (3) pregnant up to 3 months and lactating, (4) 3-4 months pregnant and dry, (5) 4-7 months pregnant and dry, and (6) 7-10 months pregnant and dry. The lactation wise prevalence of post parturient haemoglobinuria in the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, and 7^{th} lactation in buffalo was also recorded.

Screening of animals:

Screening of positive animals was based on clinical signs of haemoglobinuria, anaemia, and moderate pyrexia. Samples of urine were collected and centrifuged to differentiate haematuria and haemoglobinuria. Blood samples were collected and processed for haematological parameters and biochemical profiling for calcium and phosphorus. Blood films were made and stained with Giemsa stain and Gram stain to rule out babesiosis, bacillary haemoglobinuria, and leptospirosis.

Processing of samples:

Two types of blood samples were collected from each buffalo; with and without anticoagulant (Na_2EDTA: 1 mg/mL). A haematology analyzer for the determination of blood cell counts, haemoglobin concentration, and haematocrit and erythrocyte sedimentation rate was used for blood samples with anticoagulant. Serum separated from blood samples was collected without anticoagulant and preserved at -20 $^{\circ}$C for further biochemical analysis by a chemistry analyzer. Serum urea, creatinine, calcium, copper, molybdenum, and phosphorus concentrations were determined by spectrophotometer analysis.

Therapeutic trials:

For therapeutic trials, 120 animals were selected out of the animals that had recently parturiated or were near parturition. These cases were confirmed for postparturient haemoglobinuria by clinical signs and laboratory diagnosis. The animals positive for postparturient haemoglobinuria were divided into 4 groups, namely A, B, C, and D. Group A was treated with sodium acid phosphate, orally @ 60 g in 300 mL of water I/V and a similar dose S/c was repeated after 12 h. Group B was treated with toldimfos sodium (Fosfan, 100 mg in 50 mL vial, Samu Chemical Industries, CO.Ltd.) I/M @ 25 cc/animal. Group C was treated with tea leaves, orally @ 250 g boiled in 2 L water. Group D was the control group. Blood samples were collected before treatment and on day 1, 2, and 3 post-treatment. The samples were processed in the laboratory for haematological parameters and biochemical profile. The data were analyzed statistically.

Results

Prevalence of diseases in relation to stage of lactation and pregnancy:

The highest prevalence of post-parturient haemoglobinuria was recorded in animals within 4 weeks of parturition while the lowest one was seen in non-pregnant, lactating, and 4-7 months postpartum, dry animals (Figure 1).

Lactation-wise prevalence of post-parturient haemoglobinuria is shown in Figure 2.

Clinical signs recorded in the control group:

Inappetence, decreased milk yield, discoloration, and frothy urine, dehydration, foul smell from mouth along with firm, dry, and bile stained faeces, pale conjunctiva, moderate rise in temperature, and staggering were observed.

The haematological and biochemical values:

In postparturient animals, haemoglobinuria mean erythrocyte count, haemoglobin concentration, and haematocrit were lower while their erythrocyte sedimentation rate was higher compared to the healthy buffaloes (Table 1).

Urea and creatinine concentrations were higher in the postparturient haemoglobinuria affected buffaloes. Serum phosphorus and copper rates were lower while molybdenum was higher in the postparturient haemoglobinuria affected buffaloes compared to the healthy buffaloes (Table 2).

Efficacy of treatment:

Based on the recovery of urine discoloration, the efficacy of toldimfos sodium was 85% followed by tea leaves 56%, and sodium acid phosphate 18% (Table 3).

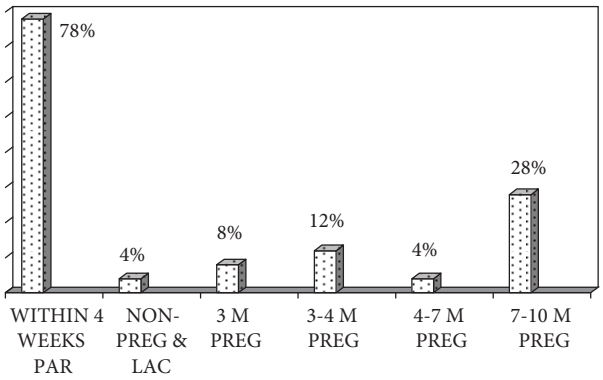

Figure 1. Prevalence of diseases in relation to the stage of lactation and pregnancy.

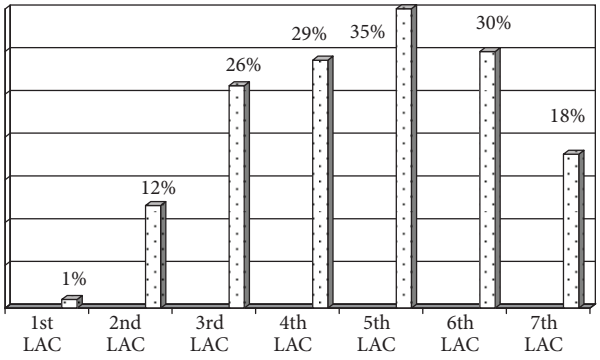

Figure 2. Lactation-wise prevalence of post-parturient haemoglobinuria in buffalo.

Table 1. Haematological variables (mean ± SD) in healthy and post-parturient haemoglobinuria-affected buffaloes.

Parameter	Healthy*	Post PHU affected
Erythrocyte count (1012/L)	6.2 ± 0.6	2.9 ± 1.5
Haemoglobin concentration (g/dL)	11.0 ± 1.2	6.5 ± 1.5
Haematocrit (%)	34.3 ± 2.4	19.4 ± 3.2
Erythrocyte sedimentation rate (mm/L h)	73.1 ± 29.9	110 ± 35.9
Total leukocyte counts (×109/L)	9.5 ± 2.2	11.0 ± 2.1

*Reported by Akhtar et al. (11)
PHU: Post parturient haemoglobinuria

Table 2. Biochemical variables (mean ± SD) in healthy and post-parturient haemoglobinuria affected buffaloes.

Parameter	Healthy*	Post PHU affected
Urea (mg/dL)	33.9 ± 5.1	50.5 ± 8.2
Creatinine (mg/dL)	1.3 ± 0.3	2.8 ± 0.8
Calcium (mg/dL)	9.8 ± 1.0	10.3 ± 1.5
Phosphorus (mg/dL)	5.41 ± 0.6	1.8 ± 0.4
Copper (µg/dL)	118.4 ± 5.2	63.4 ± 5.8
Molybdenum (µg/dL)	54.8 ± 13.6	185.2 ± 53.9

*Reported by Akhtar et al. (11)
PHU: Post parturient haemoglobinuria

Table 3. Change in urine color.

Drug	# of animals	Day 1	Day 2	Day3
Toldimfos Sodium	30	clear	clear	clear
Tea leaves	30	clear	clear	clear
Sodium Acid Phosphate	30	red	red	clear

The biochemical and haematological values supported the results of clinical recovery. Post-treatment results showed that mean erythrocyte count and haemoglobin concentration were higher for toldimfos sodium followed by tea leaves and sodium acid phosphate, while the total leukocyte count value was lowest for toldimfos sodium followed by higher value for tea leaves and sodium acid phosphate (Table 4).

Discussion

Kurundkar et al. (2), Samad et al. (7), and Stockdale et al. (8) also documented decreased serum phosphorus in affected buffaloes as reported in the present study. Heavy drainage of phosphorus through milk, particularly in high milk yielding animals, leads to hypophosphataemia as reported by Bhikane et al.(9). Digraskar et al. (10) reported that in advanced gestation, more phosphorus and calcium are required for the developing foetus if supplementary phosphorus is not provided, thereby leading to hypophosphataemia. Moreover, high calcium to phosphorus ratio results in decreased phosphorus absorption from the intestinal tract and ultimately leads to hypophosphataemia. Phosphorus deficient soils are common in dry tropical countries like Pakistan. Although many soils are naturally deficient in phosphorus, heavy leaching by rain and constant crop removal also contribute to phosphorus deficiency in soil. Akhtar et al. (11) reported that fodders grown on phosphorus deficient soils are consequently low in phosphorus content, and thereby prolonged feeding on such fodders can lead to hypophosphataemia. Pandey and Misra (12) reported a significant decrease in erythrocyte count, haemoglobin concentration, and haematocrit in affected buffaloes, which indicates severe anaemia. According to them, intravascular haemolysis due to impaired glycolytic pathway and depletion of ATP in erythrocytes results from phosphorus deficiency. Subnormal concentration of ATP predisposes red blood cells to alter functions and structure, causing a loss of normal formability, and an increase in fragility, ultimately leading to haemolysis. In the present study, total erythrocyte count, haemoglobin concentration, and haematocrit in post-parturient haemoglobinuria affected buffaloes decreased and resulted in anaemia.

Table 4. Haematological variables (mean ± SD) post treatment in post-parturient haemoglobinuria affected buffaloes.

Drug	Post-PHU affected	Tolfimfos Sodium	Tea leaves	Sodium Acid Phosphate
Erythrocyte count (1012/L)	2.9 ± 1.5	5.8 ± 0.5	3.5 ± 0.8	3.2 ± 0.9
Haemoglobin concentration (g/dL)	6.5 ± 1.5	8.9 ± 1.0	4.3 ± 0.8	4.3 ± 0.8
Haematocrit (%)	19.4 ± 3.2	3.8 ± 2.0	19 ± 1.5	19 ± 1.5
Erythrocyte sedimentation rate (mm/L h)	110 ± 35.9	86 ± 27.4	70 ± 23.4	69 ± 21.5
Total leukocyte counts (×109/L)	11.0 ± 2.1	9.9 ± 1.9	10.3 ± 2.1	10.9 ± 1.9

PHU: Post parturient haemoglobinuria

Similar observations were reported by Suttle (13), Bhikane et al. (9), and Muhammad et al. (14), indicating a possible positive correlation between the total erythrocyte count and both haemoglobin concentration and haematocrit. Serum molybdenum and copper were significantly high and low, respectively, in postparturient haemoglobinuria affected buffaloes in the present study. As reported by Suttle (13), significantly decreased copper levels were attributed to a 3-way interaction between copper, molybdenum, and sulphur. According to him, this interaction can occur with concentrations of molybdenum and sulphur, which are naturally present in feedstuffs, and is involved in the formation of thiomolybdates in the rumen. Similar observations were reported by Spears, (5). Allen and Gawthorne (15) also reported that sulphides are produced by micro-organisms in the rumen via the reduction of sulphate and degradation of sulphur amino acids. These sulphides react with molybdate to form thiomolybdates, which bind with copper and form an insoluble complex that does not release copper, even under acidic conditions, and renders it unavailable to the animal for utilization, resulting in copper deficiency. Dehydration reported in the present study as a source of decreased renal perfusion resulted in a reduced glomerular filtration rate and increased blood urea level as reported by Finco and Duncan, (16). Alternatively, increased blood urea could be due to the failure of the urea recycling process through salivary glands and its non-utilization by microbes in the rumen during digestive disorders. In the present study, creatinine significantly increased in post parturient haemoglobinuria in buffaloes. Benjamin (17) considered that concentrations over 2 mg/dL lead to a reduced glomerular filtration rate, which affects creatinine in a manner similar to that of blood urea. Both urea and creatinine levels were elevated and positively correlated to each other in parturient haemoglobinuria affected buffaloes. According to Digraskar et al. (10), urea and creatinine are waste products that the kidneys normally filter from the blood and these are interrelated. Latimer et al. (18) also reported that if the kidneys are not working properly, these substances build up in the body, and elevated blood levels of urea and creatinine are indications of pathological kidney function. It was concluded from the present study that phosphorous deficiency plays a key role in causing haemoglobinuria in buffaloes.

During the present study inappetence, decreased milk yield, discoloration, frothy urine, dehydration, foul smell from mouth, pale conjunctiva, constipation, moderate rise in temperature, and staggering were observed. Muhammad et al. (14) reported similar findings. In the present study, haemoglobinuria noticed before anaemia but diarrhea was not noticed as reported by Madsen and Nielsen (19).

In the present study, the highest prevalence was seen at the 5th lactation (32%) while the lowest one was recorded at the 1st lactation (1%). According to Pandey and Misra (12), the disease is usually seen in adult dairy cattle during their 3rd to 6th lactation. Similar observations were made by Farquharson and

Smith (20). Macwilliams et al. (21) reported very low levels of serum phosphorus (0.4-1.5 mg/dL) during the hemolytic crisis. In affected herds, lactating but clinically normal cows have moderate hypophosphataemia levels (2-3mg/ dL); non-lactating cows usually have normal serum phosphorus concentrations. Serum calcium concentration is usually normal and total bilirubin levels are parallel to the intensity of clinical icterus. Smith (22) reported that the recommended treatment for post-parturient haemoglobinuria in North America includes intravenous infusion of sodium acid phosphate (60 g in 300 mL of water), 100 g of bone meal administered as a drench twice a day, transfusion of fresh blood as indicated, and intravenous fluids to maintain hydration. According to him, correction of any phosphorus deficiency or imbalance in the ration along with removal of incriminated feeds may prevent additional cases. Because of inconsistent results with phosphate therapy and the copper-deficient status of affected cows, some workers also suggested parenteral copper (120 mg available copper per cow) as the preferred treatment. Use of tea leaves in haemoglobinuria has not been reported so far. The astringent effect of tea leaves may be due to the presence of tannic acid, which causes the protein precipitation and stops drainages due to the shrinkage of tissues. The protein released due to the haemolysis of erythrocytes is deposited in the vascular system, which may binds with the electrolyte ions present in the plasma due to the action of tannic acid and this protein-bound-electrolyte complexes are not immediately available biologically nor are they vulnerable to sudden loss. Hence, tea leaves, due to their astringent effect, clear the discoloration of urine and can be given as supportive treatment along with toldimfos sodium (4-dimethyle 1-2 methyl phenyl phosphorus acid sodium salt) as an alternative treatment to sodium acid phosphate therapy.

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