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HAEMATOLOGICAL AND BIOCHEMICAL RISK FACTORS OF PARTURIENT HAEMOGLOBINURIA IN BUFFALOES

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ABSTRACT

A case-control epidemiological study was carried out to estimate the haematological and serum biochemical risk factors associated with parturient haemoglobinuria on 30 haemoglobinuric and 60 apparently healthy buffaloes selected from district Chakwal during December, 2010 and January, 2011. Non coagulated and coagulated blood samples were collected and tested for various haematological and biochemical parameters. Significantly ($P<0.05$) decreased total erythrocyte count ($3.58\pm1.03\times10^6$/µl), haemoglobin concentration (6.90±1.39g/dl), haematocrit (19.26±4.28%), serum inorganic phosphorous (2.67±0.79mg/dl) and selenium (15.77±4.95µg/dl) whereas significantly ($P<0.05$) increased mean corpuscular volume (60.72±8.49fl), mean corpuscular haemoglobin (21.46±2.52pg), erythrocyte sedimentation rate (129.20±21.25mm/1st hour) and serum molybdenum (115.33±30.08µg/dl) were recorded in haemoglobinuric buffaloes with an odds ratio of 26, 17.81, 28.95, 7.50, 21, 12.25, 26 and 11 respectively. Non significant ($P>0.05$) difference was recorded between haemoglobinuric and healthy buffaloes with respect to mean corpuscular haemoglobin concentration and serum copper level with an odds ratio of 0.50 and 0.87 respectively. Hienz bodies were not detected on stained blood smears of haemoglobinuric as well as healthy buffaloes. It was concluded that low red cell count, haemoglobin concentration, haematocrit, serum inorganic phosphorous and selenium whereas; increased mean corpuscular volume, mean corpuscular haemoglobin and serum molybdenum content are potential risk factors associated with parturient haemoglobinuria.

Key words: haemoglobinuria, risk factors, hypophosphataemia, haemolytic anaemia, haemolytic syndrome

INTRODUCTION

Among major disease problems of buffaloes, parturient haemoglobinuria is a potent threat to the milking buffaloes of India and Pakistan affecting a considerable number of buffaloes every year during advanced pregnancy and early lactation (Dalir-Naghadeh *et al.,* 2005; Gahlawat *et al.,* 2007; Akhtar *et al.,* 2008; Ghanem and El-Deeb 2010). It is a non infectious haemolytic syndrome of buffaloes and cattle which is characterized by intravascular haemolysis, anaemia and haemoglobinuria (Akhtar *et al*., 2007a, b). The exact pathogenesis is not yet known however associated risk factors include ingestion of cruciferous plants, saponin from berseem, dietary phosphorous deficiency, decreased serum copper and selenium whereas increased molybdenum (Pirzada *et al.,* 1989; Radostits *et al.,* 2007; Neto *et al.,* 2007; Brechbuhl *et al.,* 2008). Excess of molybdenum in soil and fodder reduces phosphorous contents of the body by interfering with its absorption from gastro-intestinal tract and increasing its elimination through urine (Akhtar, 2006). Hypermolybdenosis also induces copper deficiency leading to reduced activity of copper containing enzyme superoxide dismutase which is part of erythrocyte protection mechanism against oxidative stress. This process helps in rendering them vulnerable to Heinz body formation as a result of feeding on poisonous plants (Smith *et al.,* 1975; Kahn and Line, 2005; Radostits *et al.,* 2007).

MATERIALS AND METHODS

Thirty haemoglobinuric buffaloes were selected from district Chakwal during the months of December, 2010 and January, 2011. Clinical diagnosis of disease was made on the basis of characteristic signs (haemoglobinuria and straining during defecation) and epidemiological features (advanced pregnancy or recent parturition) which was further validated by ruling out other diseases causing red urine (haemoparasites and leptospirosis) through standard laboratory techniques (Cole *et al.,* 1973; Anwar *et al.,* 2005). Sixty apparently healthy buffaloes maintained under similar conditions were also selected from the same areas as controls. Blood samples were collected from jugular vein of each animal with the help of sterillized disposable syringe into 3ml sterile vacuum tubes containing EDTA (ethyl diamine tetra acetic acid) @ 1mg/ml for haematological studies. Blood samples were also collected from each animal into 4ml sterile vacuum tubes without anticoagulant and serum was separated for biochemical analysis.

Haematological Studies: Blood samples collected with anticoagulant were analyzed for demonstration of Heinz bodies, erythrocyte sedimentation rate, total erythrocyte count, haemoglobin concentration, haematocrit, and red cell indices (mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration).

Staining solution for Heinz bodies was prepared by dissolving 0.5 gm of methyl violet stain in 100ml of 9g/L (9%) NaCl. The solution was filtered. 1ml of EDTA blood was added to 4ml staining solution (1:4) and suspension was allowed to stand for 10 minutes at 20^{o} C. Then blood films were prepared on glass slides in usual way, dried and examined under microscope for demonstration of Hienz bodies (Anwar *et al.,* 2005).

Erythrocyte sedimentation rate (ESR) was determined by Westergren's method (Khan & Aslam 2001). For other haemogram parameters, blood samples were processed through automated haemotology analyzer Celltac a Mek 6420K (Nihon Kohden Japan) according to manufacturer's instructions.

Serum biochemical analysis: Serum samples of haemoglobinuric and healthy buffaloes were analyzed for determination of phosphorous, copper, molybdenum and selenium. Serum phosphorous was determined through spectrophotometer using diagnostic kit according to manufacturer's instructions whereas; copper, molybdenum and selenium were determined through atomic absorption spectrophotometer after preparation of samples following wet digestion method. 0.5ml of serum sample was digested with 10ml concentrated nitric acid in a digestion flask for almost 20 minutes at low temperature till the clearance of contents and then with 5ml perchloric acid for about 15 minutes. The solution was vigorously heated until about 3ml colourless material was left which was then cooled and diluted with redistilled water in a volumetric flask upto the level of 20 ml. Mineral concentrations in the diluted samples were measured and final quantities were determined by comparing sample reading with standard curves.

Statistical analysis: Data were analyzed to compare the mean values of cases and controls by applying independent sample t-test as well as for calculation of odds ratios and their 95% confidence intervals at 5% significance level using SPSS version 17.

RESULTS AND DISCUSSION

Significantly (P<0.05) decreased total erythrocyte count (3.58±1.03×10^{6}/µl), haemoglobin concentration (6.90±1.39g/dl) and haematocrit (19.26±4.28%) whereas significantly increased erythrocyte sedimentation rate (129.20±21.25mm/1st hour), mean corpuscular volume (60.72±8.49fl) and mean corpuscular haemoglobin (21.46±2.52pg) in affected buffaloes and odds ratios of 26, 17.81, 28.95, 26, 21 and 12.25 respectively indicate severe anaemia which is attributed to intravascular haemolysis in this disease. Non significant (P>0.05) difference was recorded between haemoglobinuric and healthy buffaloes with respect to mean corpuscular haemoglobin concentration with an odds ratio of 0.50. Mean values of haemoglobinuric and healthy buffaloes with respect to each parameter are presented in table 1 whereas odds ratio values of each parameter with 95% confidence intervals are presented in table 2. The findings with respect to haematological parameters of haemoglobinuric animals are in agreement with Dalir- Naghadeh *et al.* (2005); Akhtar (2006); Khan and Akhtar (2007); Radwan and Rateeb (2007) and Durrani *et al.* (2010).

Significantly (P<0.05) decreased serum inorganic phosphorous (2.67±0.79mg/dl) in haemoglobinuric buffaloes and odds ratio of 7.50 may be associated with prolonged feeding on cruciferous and or/toxic plants particularly berseem, heavy drainage of phosphorous through milk in high producing animals maintained on low phosphorous diets/rations, low phosphorous rations with high calcium content and hypermolybdenosis. Low phosphorous diets with high calcium content cause hypophosphataemia by decreasing phosphorous absorption from the gastro-intestinal tract due to wider ratio of calcium and phosphorous whereas; excess of molybdenum also decreases phosphorous content by interfering with its absorption and increasing its elimination through urine (Bhikane et al., 2004; Akhtar, 2006; Khan and Akhtar, 2007; Dua, 2009). Significantly (P<0.05) increased serum molybdenum level (115.33±30.08µg/dl) recorded in haemoglobinuric buffaloes is in agreement with Digraskar *et al.* (1996) and Akhtar (2006). Significantly (P<0.05) decreased serum selenium level (15.77±4.95µg/dl) of haemoglobinuric buffaloes may be attributed to decreased feed intake due to progressive loss of appetite (Akhtar, 2006).

Non significant (P>0.05) difference recorded between haemoglobinuric and healthy buffaloes with respect to serum copper concentration and odds ratio of 0.87 indicate that copper deficiency is not associated with parturient haemoglobinuria which is contrary to the previously reported results of Digraskar *et al.* (1996); Akhtar (2006); Akhtar *et al.* (2006); Akhtar *et al.* (2007a, b); Khan and Akhtar (2007) and Durrani *et al.* (2010). Decreased serum copper level of haemoglobinuric animals in these previous reports was attributed to increased molybdenum due to interaction of copper with molybdenum and sulfur. Sulfides are produced by the rumen micro organisms due to degradation of sulfur amino acids and reduction of sulphate. These sulfides react with molybdate to form thiomolybdates which bind with copper forming highly insoluble complex and do not release copper rendering it unavailable to the animal leading to copper deficiency (Akhtar, 2006). This

phenomenon is probably not involved in the present study because the serum molybdenum level of haemoglobinuric animals (115.33 ± 5.49µg/dl) although higher than healthy animals (68.43 ± 13.11µg/dl) but it is lower as compared to the previous reports of Akhtar (2006) and Digraskar *et al.* (1996) which were 171.53 ± 56.69µg/dl and 229.34 ± 4.18µg/dl respectively.

Heinz bodies were not detected on stained blood smears of haemoglobinuric and healthy buffaloes. Rana and Bhardwaj (1988) ruled out the possibility of Heinz bodies and involvement of compliment system activation in this disease after investigating cytomorphological changes in erythrocytes of haemoglobinuric buffaloes through scanning electron microscopy whereas; Heinz body formation in erythrocytes of haemoglobinuric animals have been reported by Elison *et al.* (1986); Jain (1993) and Radostits *et al.* (2007).

Two different patterns of parturient haemoglobinuria have been reported from North America and Newzealand (Radostits *et al.*, 2007). Findings of present study are in agreement with the previously reported pattern from North America where low level of serum inorganic phosphorous without hypocuprosis and Heinz bodies was recorded as characteristic finding associated with this disease whereas; contrary to the pattern reported from Newzealand where decreased serum and liver copper with Heinz bodies in erythrocytes were prominent alterations in haemoglobinuric animals but hypophosphataemia was not recorede as consistent finding. Hypocuprosis results in decreased activity of copper containing enzyme superoxide dismutase which is part of erythrocyte protection mechanism against oxidative stress /damage. This process helps in rendering the erythrocytes vulnerable to Heinz body formation as a result of feeding on cruciferous /toxic plants (Smith *et al.*, 1975; Jain, 1993). Previous reports indicate that Heinz body formation in erythrocytes of haemoglobinuric animals is associated with hypocoprosis. In the present study, hypocuprosis was not recorded in haemoglobinuric buffaloes which might be the reason for absence of Heinz bodies in erythrocytes of effected animals.

Table 1: Haematological and serum biochemical values of haemoglobinuric and healthy buffaloes

Parameters	*Mean ± S.D.*		*P-value*
	Cases (n = 30)	*Controls (n = 60)*	
Red blood cells (10^6/µl)	3.58 ± 1.03	6.73 ± 1.28	0.000
Haemoglobin (g /dl)	6.90 ± 1.39	11.20 ± 2.06	0.000
Haematocrit %	19.26 ± 4.28	31.69 ± 5.39	0.000
Mean corpuscular volume (fL)	60.72 ± 8.49	47.97 ± 4.03	0.000
Mean corpuscular haemoglobin (pg)	21.46 ± 2.52	17.39 ± 1.73	0.000
Mean corpuscular haemoglobin concentration (g /dl)	35.82 ± 2.15	36.02 ± 0.97	0.545
Erythrocyte sedimentation rate (mm /1st hour)	129.20 ± 21.25	66.35 ± 37.88	0.000
Phosphorous mg/dl	2.67 ± 0.79	4.01 ± 1.12	0.000
Copper µg/dl	100.80 ± 13.27	97.50 ± 6.38	0.114
Molybdenum µg/dl	115.33 ± 30.08	68.43 ± 13.11	0.000
Selenium µg/dl	15.77 ± 4.95	24.37 ± 6.70	0.000

Table 2: Odds ratios with 95% confidence intervals for haematological and serum biochemical variables of haemoglobinuric buffaloes.

Parameters	*Cut off points*	*Study Groups*		*OR*	*95 % C.I*	*P-value*
		Cases (n=30)	*Control (n=60)*			
Red Blood Cells (10^6/µL)	< 5	26	12	26	7.61 – 88.78	0.000
Haemoglobin g/dL	< 8	24	11	17.81	5.88 – 53.96	0.000
Haematrocrit (%)	< 25	26	11	28.95	8.38 – 99.98	0.000
MCV FL	> 50	28	24	21	4.55 – 96.46	0.000
MCH pg	> 20	22	11	12.25	2.32 – 34.67	0.000
MCHC g/dL	≥ 30	30	60	0.50	0.009 - 26.42	0.511
ESR mm/1st hour	> 80	28	21	26	5.63 – 120.01	0.000
Phosphorous mg/dL	< 2.5	18	10	7.50	2.76 – 20.33	0.000
Copper µg/dL	< 95	12	26	0.872	0.358 – 2.12	0.763
Molybdenum µg/dL	> 70	24	16	11	3.80-31.81	0.000
Selenium µg/dL	< 15	20	14	6.57	2.5-17.27	0.000

REFERENCES

Akhtar, M. Z., A. Khan, M. Sarwar and A. Javaid (2007a). Influence of soil and forage minerals on buffalo parturient haemoglobinuria. Asian-Aust. J. Anim. Sci., 20: 393-398.

Akhtar, M. Z., A. Khan, M. Z. Khan and G. Muhammad (2007b). Haemato-biochemical aspects of parturient haemoglobinuria in buffalo. Turkish J. Vet. Anim. Sci., 31(2): 119-123.

Akhtar, M. Z., A Khan, M. Z. Khan and A. Javaid (2008). Patho-biochemical changes in buffaloes (bubalus bubalis) suffering from parturient haemoglobinuria Pakistan Vet. J. 28 (3): 139-143.

Akhtar, M. Z., A. Khan, T. Zaman and N. Ahmad (2006). Some clinico-epidemiological and biochemical observations of parturient haemoglobinuria in Nili-Ravi buffaloes (Bubalus bubalis). Pakistan Vet. J., 26 (4): 151-156.

Akhtar, M. Z. (2006). Aetiopathology of parturient haemoglobinuria in buffaloes. Ph.D Thesis. Department of Veterinary Pathology, University of Agriculture, Faisalabad.

Anwar, M., M. A.Waqar, F. A. Khan, W. U. Z. Tariq, S. Ahmed, S. Mushtaq, T. A. Ahmed, S. H. Mirza and M. M. Dawood, editors (2005). Manual of Laboratory Medicine, 3rd edition. Armed forces institute of pathology, Rawalpindi, Pakistan, 257 - 288p.

Bhikane, A. U., L. G. Anantwar, A. P. Bhokre and B. W. Narladkar (2004). Incidence, clinico-pathology and treatment of haemoglobinuria in buffaloes. Indian Veterinary J. 81(2): 192 - 197.

Brechbuhl, M., M. Meylan, C. Kunz-Kirchhofer, M. Bodmer, A. Michel and T. Kaufmann (2008). Post-parturient haemoglobinuria in cows kept in the Swiss Alpine region. Tierarztliche Praxis Ausgabe G: Grosstiere – Nutztiere 36 (4): 236 - 240.

Cole, J. R., C. R. Sulzer and A. R. Pulrsell (1973). Improved microtechniques for the leptospiral microagglutination test. Appl Microbiol, 25: 976 – 980.

Dalir-Naghadeh, B., H. A. Seifi, S. Asri-Rezaei and N. Pilevary (2005). Post-parturient haemoglobinuria in Iranian river buffaloes: a preliminary study. Comp. Clin. Pathol. 14: 221 – 225.

Digraskar, S., B. Singh and D. N. Rajguru (1996). Blood chemistry and therapeutic management of metabolic haemoglobinuria in buffaloes. J. Maharashtra Agriculture University, 21(1): 100 – 102.

Dua, K. (2009). Importance of micronutrients and relevance of their supplementation in buffaloes. Pakistan J. of Zoology, supplementary series, number 9: 541 – 549.

Durrani, A. Z., N. Kamal, A. R. Shakoori and R. M. Younus (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.

Ellison, R. S., B. J. Young, and D. H. Read (1986). Bovine post-parturient haemoglobinuria: two distinct entities in New Zealand. New Zealand veterinary J. 34 (4): 7 -10.

Gahlawat, I., K. Singh and R. Kumar (2007). Investigations on oxidative stress in post-parturient haemoglobinuria in buffaloes receiving sodium acid phosphate therapy, Ital. J. Anim. Sci. 6 (Suppl. 2): 974 – 977.

Ghanem, M. M. and W. M. El-Deeb (2010). Lecithin cholesterol acyltransferase (LCAT) activity as predictor for ketosis and parturient haemoglobinuria in Egyptian water buffaloes. Research in Veterinary Science 88: 20 – 25.

Jain, N. C. (1993). Essentials of Veterinary Hematology. Lea and Febiger 200 Chester Field Parkway Box 3024 Malvern, Penunsylvania 19355 – 9725, U.S.A, 206 – 207 p.

Kahn, C. M. and S. Line, editors, (2005). The Merck Veterinary Manual, 9th edition. Merck and Co., Inc. Whitehouse station, N.J., U.S.A., 816 p.

Khan, S. A. and A. Aslam (2001). Manual of Veterinary Clinical Pathology, 1st edition. Maktaba-e-Danishwaran, 8-Alfazal Market, Urdu bazaar, Lahore, 33 – 77 p.

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

Neto, J. D. B., C. M. C. Oliveira, M. D. Duarte, T. T. Albernaz, C. A. de O. Júnior, G. R. Correa and F.R. Correa (2007). Phosphorus deficiency in buffaloes in the state of Paras, Northern Brazil. Ital.J.Anim.Sci. 6 (Suppl. 2): 971 – 973.

Pirzada, W. H., M. Hussain and K. N. M. Khan (1989). A note on parturient haemoglobinuria in buffaloes. J. of Animal Health and Production 9: 20 – 22.

Radostits, O. M., C. C. Gay, K. W. Hinchcliff and P. D. Constable (2007). Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, 10th Ed. Elsevier's Health Sciences 1600 John F. Kennedy Boulevard, Suite 1800, Philadelphia, PA 19103 – 2899, U.S.A. 1682 – 83 p.

Radwan, M. E. and H. Z. Rateb (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 R. M. Bhardwaj (1988). Relationship of intraerythrocytic ATP and membrane phospholipids to red cell shape in Haemoglobinuric buffaloes. Proceedings of II world buffalo congress, India 4: 178 – 183.

Smith, B., D. A. Woodhouse and A. J. Fraser (1975). The effects of copper supplementation on stock health and production. 2: The effect of parenteral copper on incidence of disease, haematological changes and blood copper levels in a dairy herd with hypocuprosis. N.Z. Vet. J. 23: 109 - 112.