

SOME CLINICO-EPIDEMIOLOGICAL AND BIOCHEMICAL OBSERVATIONS OF PARTURIENT HAEMOGLOBINURIA IN NILI-RAVI BUFFALOES (*BUBALUS BUBALIS*)

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ABSTRACT

This study was executed to know the clinico-epidemiology of parturient haemoglobinuria (PHU) in buffaloes. A total of 60 buffaloes suffering from PHU were randomly selected from field cases occurring in Faisalabad, Toba Tek Singh and Jhang districts of Punjab, Pakistan. Sixty clinically healthy buffaloes of similar description from the same localities were also included in the study as control. The most prominent clinical sign was passing of red to coffee coloured urine. Milk production in affected buffaloes was significantly ($P<0.001$) reduced, while respiration and pulse rates were accelerated ($P<0.001$). Ruminal motility was significantly weak and reduced alongwith characteristic straining while defecating. The case fatality was 15%. A significantly higher occurrence of PHU was observed in the winter season (41.8%). Maximum number of affected buffaloes (25.0%) was in 4th lactation. Twenty seven (45.0%) buffaloes developed haemoglobinuria in the post calving period and majority (59.3%) of these were within 1-23 days of calving. Of the 33(55.0%) PHU affected pregnant buffaloes, 18 (54.6%) were in their third trimester. The highest cases of PHU were observed in buffaloes producing 10 or more litres of milk/day (42.5%). Recurrence of PHU was observed in 18.3% buffaloes. Berseem constituted a major component (65%) of fodders of most of the PHU affected buffaloes. Significantly lowered serum phosphorus, copper and selenium, while higher potassium, iron and molybdenum, were found in affected buffaloes as compared to healthy buffaloes. It was concluded that PHU mostly affects high yielding buffaloes in their 3-5th lactation particularly in early lactation or advanced pregnancy. Disease is strongly associated with Berseem feeding in winter season.

Key words: Buffaloes, parturient haemoglobinuria, epidemiological factors, clinical signs.

INTRODUCTION

Pakistan is primarily an agriculture country and livestock play a vital role in its economy by providing principal sources of essential items of human diet like milk and meat and other byproducts which account for 49.1% of agriculture value added and about 11.4% of the GDP. Buffalo population is approximately 25.5 millions out of a total 134.1 million heads of livestock in the country and contributes 67 and 28% of 28624 and 1810 thousand tones milk and meat production, respectively in the country per year (Anonymous, 2005). Thereby, buffaloes play an important role in the agricultural economy of Pakistan.

Parturient haemoglobinuria (PHU) is one of the major and economically important diseases of dairy animals in Pakistan, India and elsewhere in the world (MacWilliams *et al.*, 1982; Chugh *et al.*, 1996; Pirzada and Hussain, 1998). It is an acute disease of high yielding buffaloes and cows characterized by hypophosphatemia, intravascular hemolysis, haemoglobinuria and anemia (Radostits *et al.*, 2000). In a survey conducted during 1996 in Punjab (Pakistan), 9 and 5% mortality due to haemoglobinuria in buffaloes and cattle has been reported which translates into estimated annual losses of Rs. 490.2 and 153.1 millions, respectively (Anonymous, 1996).

Hypophosphatemia is documented consistently in the affected animals (Chugh *et al.*, 1996). Dietary phosphorus deficiency and/or rations containing cruciferous plants are suspected causes of severe hypophosphatemia and have been associated with hemolytic anemia in cows (MacWilliams *et al.*, 1982). Copper deficiency is also an etiological factor of PHU, 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 (Smith *et al.*, 1975).

Information on the epidemiology and blood biochemical aspects of PHU in Nili-Ravi buffaloes in Pakistan is quite scanty, and these are important for planning a control strategy for the disease. The present study was, therefore, planned to investigate clinico-epidemiological and biochemical aspects of PHU in Nili-Ravi buffaloes.

MATERIALS AND METHODS

Experimental animals

A total of 60 Nili-Ravi buffaloes suffering from PHU were randomly selected from field cases occurring in Faisalabad, Toba Tek Singh and Jhang districts of Punjab province of Pakistan. Sixty clinically healthy

buffaloes of similar description from the same localities were also included in the study as control. The disease was diagnosed clinically based on specific signs such as haemoglobinuria and characteristic straining while defecating in lactating or pregnant buffaloes (Digraskar *et al.*, 1991). Other clinical signs were recorded and the possibility of other diseases causing a reddish discoloration of urine like babesiosis, leptospirosis and bacillary haemoglobinuria was ruled out through laboratory tests.

Epidemiological data

Epidemiological observations in relation to season, parity, stage of lactation, stage of pregnancy, milk yield, recurrence of disease and type of fodders fed were recorded in all the PHU affected buffaloes. Year was divided into four seasons i.e., winter (November to February), spring (March to April), summer (May to August) and autumn (September to October). PHU affected buffaloes were divided into 6 groups i.e. 1-6 on the basis of their parities. Stage of lactation was divided into 1-23, 24-60 and 61-270 days post calving. Stage of pregnancy/gestation was divided into first (1-3 months), second (>3-6 months) and third (>6 months till parturition) trimester. To calculate relationship of milk production (litres/day) with PHU, affected lactating buffaloes were categorized into low (<5), medium (5-9) and high (≥ 10) producing.

Biochemical studies

Blood samples were collected from the jugular vein of each animal without anticoagulant. Serum was separated and stored in aliquots at -20°C until analysis for various minerals. All serum samples were prepared by wet digestion following the procedure of Richards (1968). Briefly, 0.5 ml serum sample was digested with 10 ml concentrated nitric acid in a 100 ml digestion flask first at low temperature for about 15-20 minutes till the contents were clear and then with 5 ml perchloric acid for 15 minutes. The solution in the flask was heated vigorously until 2-3 ml colorless material was left. After cooling, the contents were diluted up to 20 ml with redistilled water in a volumetric flask and preserved for the analysis of minerals.

Calcium, copper, iron, molybdenum and selenium were determined by using an atomic absorption spectrophotometer (Varian Spectr AA-5) and the analysis for phosphorus was done with the help of spectrophotometer (Philip Model 1100). Potassium levels were determined with flame photometer (Jenway FFP-7).

Data analysis

The data thus collected were analyzed by using analysis of variance and Chi-square test using Minitab computer programme (Anonymous, 2003). Pearson correlation was calculated in between various clinical signs of the disease.

RESULTS

Clinical signs

The colour of urine in haemoglobinuric buffaloes ranged from red (28%), dark red (20%) to coffee colored (52%) depending upon the severity and duration of illness, the difference was significant ($\chi^2 = 7.006$, $df = 2$, $P = 0.05$). Other symptoms included dullness, anemia and dehydration. Milk production in affected buffaloes before disease (7.54 ± 3.40 litres/day) was significantly ($P < 0.001$) higher than the milk production after disease (4.50 ± 2.68 litres/day). The body temperature was within normal range. Respiration and pulse rates were significantly ($P < 0.001$) accelerated, whereas ruminal motility was significantly weak and reduced (Table 1).

Table 1: Comparison of clinical parameters (mean \pm SD) in healthy and PHU affected buffaloes ($n = 60$ each)

Parameters	Healthy	PHU affected
Rectal temperature ($^{\circ}\text{F}$)	100.51 $\pm 1.00^a$	101.14 $\pm 1.39^a$
Pulse rate (per minute)	54.75 $\pm 3.78^a$	72.97 $\pm 9.90^b$
Respiration rate (per minute)	14.65 $\pm 1.93^a$	21.58 $\pm 4.27^b$
Ruminal motility (per 2 minutes)	3.60 $\pm 0.50^a$	2.32 $\pm 0.99^b$

Values with different superscripts in a row differ significantly ($P < 0.001$).

In the advanced stage of the disease, anorexia was well marked, accompanied with ruminal stasis and severe straining while defecating. However, some animals continued eating normally for the first day after passing of discoloured urine and showed loss of appetite subsequently. The feces were normal to hard or sometimes even loose in consistency. Dyspnoea, tachycardia, and icterus in 5% cases were observed. The duration of the disease varied from 3-9 days and the case fatality was 15%.

In healthy buffaloes, no correlation between various clinical signs was observed. However, in PHU affected buffaloes, body temperature and respiration rate showed positive correlation with pulse ($r = 0.340$; $P = 0.008$; $r = 0.469$; $P = 0.000$, respectively) and body temperature also showed negative correlation with ruminal motility ($r = -0.260$; $P = 0.045$; Fig. 1).

Epidemiology

The highest occurrence of PHU was observed in winter season (41.7%), followed by summer (28.3%), spring (23.3%) and autumn (6.7%), the difference in the occurrence of PHU between seasons was significant (Fig. 2). Maximum number of affected buffaloes

(25.0%) were in 4th lactation, followed by 3rd (23.3%), 5th (16.7%), 2nd (15.0%), 6th and above (8; 13.3%) and first lactation (6.7%). Chi-Square analysis revealed non-significant (χ^2 value = 7.112, df = 5, P -value = 0.212) difference in the occurrence of the disease between parities.

Twenty seven (45.0%) buffaloes developed haemoglobinuria in the post calving period and majority (59.3%) of these were within 1-23 days of calving, followed by 61-270 days (29.6%) and 24-60 days of calving (11.1%). Data analysis revealed significant (χ^2 value = 7.104, df = 2, P -value = 0.029) difference in occurrence of haemoglobinuria between various stages of lactation. Of the 33 (55.0%) PHU affected pregnant buffaloes, 18(54.6%) were in their third trimester of pregnancy, followed by second (24.2%) and first trimester (21.2%). Data analysis revealed non-significant difference in the occurrence of the disease between various stages of pregnancy.

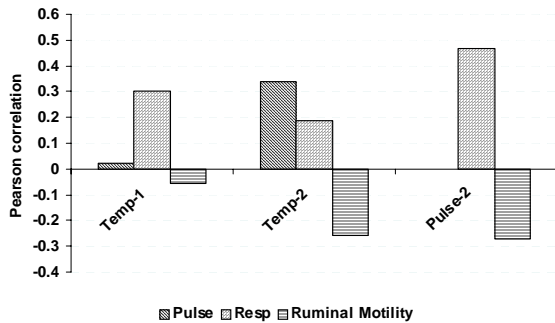


Fig. 1: Correlation of body temperature and pulse rate in healthy (Temp-1) and haemoglobinuria buffaloes (Temp-2: Pulse rate-2) with pulse, respiration and ruminal motility rates.

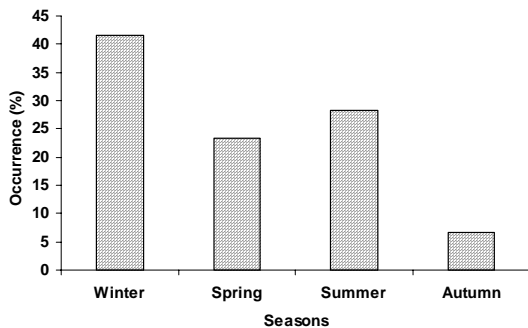


Fig. 2: Seasonal occurrence of parturient haemoglobinuria in buffaloes (n = 60). Data analysis by Chi-square revealed significant difference among seasons (χ^2 value = 12.530, df = 3, P -value = 0.006).

Forty (66.7%) PHU affected buffaloes (lactating and pregnant as well) were analyzed in terms of milk yield. The highest occurrence of PHU was observed in buffaloes producing 10 or more litres of milk/day (42.5%), followed by buffaloes producing 5-9 litres (37.5%) and less than 5 litres (20.0%). Chi-square analysis revealed non-significant difference in the occurrence of haemoglobinuria between various lactation.

Eleven (18.3%) buffaloes were repeat affectees i.e., they had suffered from PHU during one of the previous lactations/gestations or during the same lactation/gestation. Among these, 5(8.3%) and 6(10.0%) buffaloes had suffered once and twice earlier, respectively.

Berseem (*Trifolium alexandrinum*) constituted the component of fodders of most of the PHU cases (n = 39; 65%). Other fodders offered to the diseased buffaloes included maize (*Zea mays*), sorghum (*Sorghum vulgare*), sugarcane (*Saccharum officinarum*), wheat straw (*Triticum aestivum*), lucerne (*Medicago sativa*) and sarson (*Brassica campestris*). In addition, 2-5 kg of concentrate (cottonseed cake) per day was also fed to 15(25%) affected buffaloes.

Biochemical parameters

Serum phosphorus, copper and selenium were significantly ($P < 0.001$) lower, whereas potassium, iron and molybdenum ($P < 0.001$) were higher in buffaloes suffering from PHU than healthy buffaloes. Calcium concentration did not vary between two groups (Fig. 3).

DISCUSSION

Clinical signs

The most prominent clinical sign in all the PHU affected buffaloes in the present study was passing of red to coffee colored urine depending upon the severity and stage of the disease. Other symptoms observed were dullness, anemia, dehydration and significantly reduced milk production. Similar findings have also been reported earlier in PHU affected buffaloes (Nagpal *et al.*, 1968) and cows (Stockdale *et al.*, 2005). Red or coffee coloured urine and anemia have been attributed to acute intravascular hemolysis (Dhillon *et al.*, 1972). Respiration and pulse rates were significantly accelerated, while ruminal motility was significantly weak and reduced in PHU affected buffaloes, as has also been reported previously (Dhillon *et al.*, 1972; Raz *et al.*, 1988). In the advanced stage of the disease, the PHU affected buffaloes showed marked anorexia that was accompanied with ruminal stasis and severe straining while defecation. However, some PHU affected buffaloes continued eating normally for the first day after passing discolored urine and showed loss

of appetite subsequently. The feces were normal to hard or even loose in consistency. Unthriftiness, tachycardia and dyspnoea observed in the present study could be due to cardiovascular compensatory mechanisms. According to Digiraskar *et al.* (1991), excessive formation of haemosiderin and its deposition in the gastro-intestinal mucosae in PHU affected buffaloes could be responsible for gastro-intestinal disturbances like ruminal stasis, constipation and straining. Deaths in fatal cases could be due to anemic anoxia caused by excessive hemolysis (Radostits *et al.*, 2000).

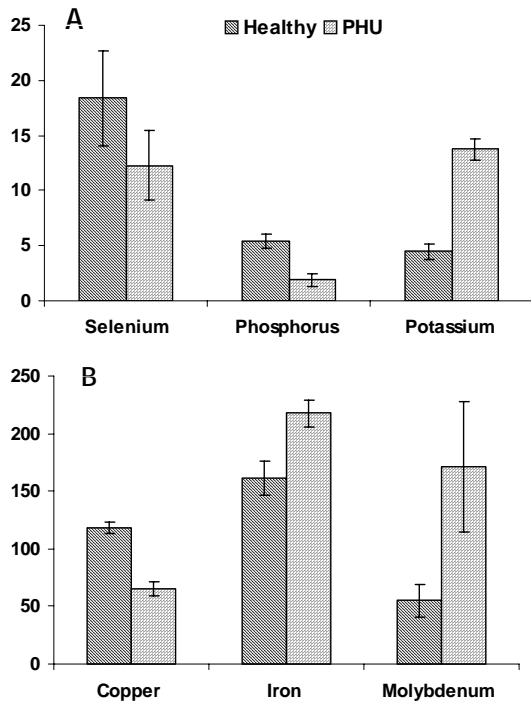


Fig. 3: In part A: Selenium ($\mu\text{g/dL}$), phosphorus (mg/dL) and potassium (m mol/L) and in part B copper, iron and molybdenum ($\mu\text{g/dL}$) showed significant variations in serum of healthy and PHU affected buffaloes.

Epidemiology

In the present study, a preponderance (41.67%) of PHU cases occurred during the winter months. With respect to the seasonal occurrence of the disease, the results of the present study varied from those reported in India (Chugh *et al.*, 1996), where maximum occurrence was found in September (25.19%), followed by July (14.5%), December (9.93%), November and May (8.40%), October (7.70%), June (7.60%), January and August (6.10%), April (4.58%) and February and March (0.77%). Another study in the same country revealed the highest occurrence of haemoglobinuria in

buffaloes during February to May (Anonymous, 1985). As reported from Punjab, Pakistan (Cheema *et al.*, 1980), the disease was prevalent round the year but more cases occurred from July to September, although from the same locality, two seasonal peaks (22.98 and 37.84% in January-February and July-August, respectively) have also been reported (Lateef, 1981). Muhammad *et al.* (2000) reported that a preponderance (68.96%) of PHU cases occurred during the winter months in Punjab (Pakistan) and concluded that the peak incidence of the disease varied from area to area and also from year to year in the same area.

In the present study, more frequency of PHU was observed in 3rd and 4th lactations, within 1-23 days postpartum and in high milk yielding buffaloes. Possible explanation for more occurrence of PHU in these stages could be 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 from the body especially phosphorus through milk (Dhillon *et al.*, 1972). As observed previously (Raz *et al.*, 1988; Chugh *et al.*, 1996), majority of the buffaloes developed haemoglobinuria in the advanced stage of pregnancy (last trimester). This could be due to the lack of provision of additional requirement of the nutrients for the developing fetus in the last trimester of pregnancy (Chugh *et al.*, 1996).

In the present study, 18.3% buffaloes were repeat affectees. Among these, 8.3 and 10.0% buffaloes had suffered once and twice earlier, respectively. Chugh *et al.* (1996) reported that out of 51 affected buffaloes, 14 (27.45%) had suffered from haemoglobinuria earlier also. Among these, 12 buffaloes had suffered once earlier, while one had suffered twice and another one thrice earlier. Recurrence could be due to some genetic predisposition in respect of low glucose-6-phosphate dehydrogenase activity in erythrocytes of haemoglobinuric buffaloes (Singari *et al.*, 1991). Once this relationship is categorically confirmed, subsequently it might be worthwhile to eliminate such animals from further breeding programmes for the eradication of the disease.

Berseem (*Trifolium alexandrinum*) constituted a component of the ration of 65.0% PHU cases in the present study. By virtue of its haemolytic saponin contents and low phosphorus contents, this fodder might predispose the animals to haemoglobinuria (Abdel-Latif and Awad, 1964). Dhillon *et al.* (1972) have contended that soils in Punjab (India) contained a high molybdenum contents. The fodders, in particular berseem, grown on such soils carried very high molybdenum contents. The excess of this element reduces the phosphorus contents of the body by interfering with its absorption from the gastro-intestinal tract and also by increasing its elimination through

urine. The excessive feeding of berseem between November and February in Punjab, Pakistan might account for the disproportionately higher occurrence of PHU cases observed during these months in the present study.

Biochemical parameters

Significantly decreased serum phosphorus in PHU affected buffaloes observed in the present study has also been reported (Samad *et al.*, 1979; Kurundkar *et al.*, 1981). Heavy drainage of phosphorus through milk particularly in high milk producing animals leads to hypophosphatemia (Bhikane *et al.*, 1995). Moreover, high calcium:phosphorus ratio results in decreased phosphorus absorption from intestinal tract and ultimately leads to hypophosphatemia (Benjamin, 1978). 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 the phosphorus deficiency in soil. The fodders grown on such soils are consequently low in phosphorus contents and thereby prolonged feeding on such fodders can lead to hypophosphatemia (Radostits *et al.*, 2000; Smith, 2000).

Serum molybdenum and copper were significantly high and low, respectively in PHU affected buffaloes. Significantly decreased copper could be attributed to a three-way interaction between copper, molybdenum and sulfur. This interaction can occur with concentrations of molybdenum and sulfur (Suttle, 1991) that are naturally present in feedstuffs and are involved in the formation of thiomolybdates in the rumen (Spears, 2003). Sulfides are produced by the rumen microorganisms via reduction of sulphate and also degradation of sulfur amino acids. These sulfides react with molybdate to form thiomolybdates which bind with copper and form a highly insoluble complex that does not release copper even under acidic conditions and renders it unavailable to the animal for utilization and results in copper deficiency (Allen and Gawthorne, 1987).

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