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A RATIONAL APPROACH TO DIAGNOSIS, TREATMENT AND CONTROL OF PARTURIENT HAEMOGLOBINURIA (RED WATER) IN BUFFALOES AND CATTLE

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INTRODUCTION

Parturient haemoglobinuria (PH; colloquially known as 'Rut Mootra' in Punjabi) is an acute disease of high yielding buffaloes and cows associated with hypophosphataemia and characterized by intravascular haemolysis, haemoglobinuria, straining during defecation, anaemia and death (Pirzada and Hussain, 1998). The animals are particularly susceptible during the first 8 weeks of calving although cases do occur during pregnancy. As compared to cow, buffaloes are more prone to this disease which may be due to higher sensitivity of erythrocytes of buffalo to saponins present in different fodders (Raza-Hasan and Singh, 1992). The incidence of this disease in different districts of Pakistani Punjab in buffaloes varied from 0.02–4.44% whereas in cows the corresponding values ranged from 0.007–0.03% with a lower intensity in rice growing areas (Raz *et al.*, 1988). Drought and foggy weather are associated with a higher incidence of the disease. A considerable proportion (18.1%) of animals were found to be repeat affectees during either the same lactation/gestation or during one of the previous lactations/gestations (Muhammad *et al.*, 2001b).

PATHOPHYSIOLOGY

Parturient haemoglobinuria appears to be a disease of multi-factorial aetiology. Phosphorus deficiency is widely believed to be associated with this disease (Wang *et al.*, 1985; Heuer and Bode, 1998; Pirzada and Hussain, 1998). The exact pathogenesis of intravascular haemolysis is not clear and may involve several factors. Deficiency of PO_4 has been documented to impair the glycolysis in bovine RBCs (at glyceraldehyde-3 phosphate dehydrogenase step) resulting in depletion of ATP (Wang *et al.*, 1985; Ogawa *et al.*, 1989). ATP is the direct energy source for maintaining cation gradients across the RBC membrane and is involved in the maintenance of RBC membrane shape and integrity (Agar and Board, 1983). It is noteworthy that all animals suffering from hypophosphataemia do not develop intravascular haemolysis (Mohamed and El-Bagoury, 1990; Yates, 1990). Metabolic acidosis caused by ketosis may exacerbate hypophosphataemia by causing renal excretion of PO_4 as dihydroxyglucophosphate (Tasker, 1980).

The pressure of high level of oxidants or the failure of antioxidants has also been suspected as factor in intravascular haemolysis of PH. The copper containing enzyme, superoxide dismutase, selenium containing enzyme glutathione peroxidase, and vitamin E all protect against oxidation damage to RBCs (Singari *et al.*, 1989; Yates, 1990). Indian workers (Chugh and Mata, 1997), on the basis of a good response to vitamin C, considered parturient haemoglobinuria as an antioxidant-responsive disease. Numerous plants contain oxidants and precursors of oxidants and are known to cause intravascular haemolysis. Cruciferous plants (*Brassica compestris* or 'Sarsoon', cabbage, turnips, kale, rape, etc.) are particularly known for causing PH and Heinz body anaemia because of their high S-methylcysteine sulfoxide content, which is converted by rumen flora to dimethyldisulfide. Once absorbed into circulation, dimethyldisulfide causes precipitation of haemoglobin, leading to haemolysis (Yates, 1990). Sugar beets ('chaqunder') alfalfa (lucerne) and berseem (*Trifolium alexandrinum*) are thought to contain saponins as haemolytic factor.

DIFFERENTIAL DIAGNOSIS

Several other diseases also cause reddish urine. These can be broadly grouped into two categories. First category includes those associated with haematuria (intact RBCs in urine) due to such conditions as kidney infections, acute septicaemia, shock, local trauma in urogenital tract, urinary calculi, tumours, and enzootic haematuria. Urine in haematuria is red but if allowed to stand, RBCs will settle at the bottom of the container. Second category includes those conditions (other than PH) which are associated with reddish discoloration of urine due to lysis of RBCs in the blood vessels. These conditions include toxic chemical poisoning, sweet-clover and other plant poisonings, leptospirosis, babesiosis, photosensitization, bacillary haemoglobinuria, intake of cold water in large quantities, auto-immune haemolytic anaemia, inherited intra-corpuscular defects and *Clostridium perfringens* type A infection. The association of haemoglobinuria with early lactation or advanced pregnancy, absence of fever or very mild transient fever, non-responsiveness to antibiotics and antiprotozoans (e.g., Diminazine, Imidocarb dipropionate 'Imizol') and the knowledge of the occurrence of the disease in the area should be considered in differential diagnosis. Demonstration of

stained with Giemsa's stain can be helpful in differentiating PH from babesiosis.

Treatment

1. Blood Transfusion

Transfusion of large quantities of whole blood may be the only treatment that might save the life of a severely affected animal. A delay of 12 hours often leads to an irreversible state. A minimum of 5 litres of whole blood to a 450 kg buffalo is recommended. Since isoagglutinins do not occur in cattle (Gibbons *et al.*, 1970), possibly also not in buffaloes, cross matching of donor and recipient blood is not required at least at the time of first transfusion. Nevertheless, adrenaline, or a corticosteriod-antihistamine combination (e.g., Solon-M Selmore Agencies, Pakistan) should be on hand to deal with any clinical predicament associated with blood transfusion.

The most convenient source of blood for blood transfusion is slaughterhouse. After the throat has been cut, the first gush of blood is allowed to escape, and then the freely flowing blood is collected as aseptically as possible in a clean bucket containing a solution of sodium citrate (anticoagulant) in sufficient amount to give a final concentration of 2.5 g per litre of blood. To ensure a proper mixing of anticoagulant with blood, the bucket should be swirled during collection. One million units of crystalline penicillin and 1 g of dihydrostreptomycin should be added to each gallon (4.5 L) of blood and filtered through sterile gauze before transfusion. (Gibbons *et al.*, 1970)

2. Phosphorus and Dextrose Therapy

When phosphorus deficiency is considered to be a predisposing cause, short of blood transfusion, the standard treatment consists of intravenous administration of 60 g of sodium dihydrogen phosphate (be sure that the chemical formula of drug is $NaH_2PO_4.2H_2O$ and not Na_2HPO_4 because the later is toxic), popularly known as sodium acid phosphate, in 300 mL of distilled water. The same dose is given subcutaneously as well as orally (Caple, 1986; Raz *et al.*, 1988; Radostits *et al.*, 1990). Repeat the treatment, if required, at 12 hours interval. Daily oral dosing with sterilized bone meal (120 g b.i.d.) or dicalcium phosphate (DCP) is also recommended. The response to sodium acid phosphate can be described as variable at best. Although most texts of veterinary medicine e.g., those by Smith (1990) and Radostits *et al.* (1990) have recommended water as a solvent of $NaH_2PO_4.2H_2O$, it would be better to use 10% dextrose instead (Muhammad *et al.*, 2001a), because:

i. glucose reduces the toxicity of sodium dihydrogen phosphate which is common with repeated parenteral administration of sodium acid phosphate and is manifested by anorexia;
ii. dextrose serves as a source of energy, which is needed by an anorectic, ketotic animal; and
iii. like potassium and magnesium, phosphorus is an intracellular ion and fluids containing dextrose promote translocation of phosphorus into cells (Macintire, 1997).

Administration of organic phosphorus (e.g., Inj. Fosfan – Selmore Agencies, Pakistan; Inj. Catosal, Bayer, Germany) @ 25–35 mL IM, daily for three days alongwith inorganic phosphorus (Sodium dihydrogen phosphate, E. Merck, Germany), given IV (in 10% dextrose), SC, and orally, has shown to give better cure rates (Muhammad *et al.*, 2001a).

N.B. For parenteral administration, be sure about the purity of sodium acid phosphate. The pure salt is granular and crystalline. If one is unsure about its purity, administer through mouth only. Alternatively, one can use calcium hypophosphite (30 g in 100 mL of 10% dextrose IV (Caple, 1986).

3. Antifibrinolytic Agents and Oxygen Releasers

The knowledge of biochemical alterations occurring in parturient haemoglobinuria has been utilized to develop new treatments for this condition. Taking cues from previously reported observation on haemoglobinuric buffaloes (Rao *et al.*, 1977; as cited by Chugh *et al.*, 1987) pointing to a marked increase in fibrinolytic activity, Chugh *et al.*, (1987) conducted therapeutic trials with two antifibrinolytic drugs viz. epsilon-amino caproic acid (EACA) and para-amino methyl benzoic acid (PAMBA) in 43 buffaloes suffering from haemoglobinuria. Of the 30 buffaloes treated with EACA (20 g powder dissolved in 540 mL of 5% dextrose saline and injected IV daily until urine became clear), 27 (90 %) were cured. Out of 13 buffaloes treated with PAMBA (300 mg of drug mixed with 540 mL of 5% dextrose saline and administered IV daily until recovery), 12 (92.3%) recovered completely. Four buffaloes, which died during the trials, had also shown some response to these drugs. More recently, Goel *et al.* (1989) also working on the premise that increase in the fibrinolytic activity is associated with PH found Botropase (a blood coagulant and antifibrinolytic drug prepared from the venom of the snake, *Bothrops jararaca*) effective in 17 (94.4%) of the 18 clinical cases of PH. Although a good percentage of animals respond to either sodium acid phosphate or antifirbrinolytic drugs by clearance of urine, the clinically recovered animals still die due to anaemic anoxia associated with low haemoglobin. In order to address this important pathological residual defect, Goel *et al.* (1988) introduced another variable into the treatment of PH. They conducted trials with inosine (Sigma Chemical Co., St. Louis Missouri, USA) (0.5 g in 5–10 mL of dilute HCL mixed with 540 mL of 5% dextrose saline given IV once daily for 2–3 days) or 10

g of sodium acid phosphate as oxygen releasers in buffaloes which had recovered from PH but were still suffering from respiratory distress due to anaemic anoxia. All animals treated with inosine survived. In another series of trials, 27 of 28 buffaloes with low level of haemoglobin were successfully treated with a combined therapy of PAMBA plus sodium acid phosphate and inosine.

4. *Copper Sulphate and Copper glycinate*

Researchers from Indian Punjab (Dhillon *et al.*, 1972) successfully treated 15 buffaloes (on berseem fodder) with 2 g of copper sulphate ($CuSO_4$) in 500 mL of water PO daily for 3–5 days. They contended that soil in Punjab carry very high molybdenum contents. The fodder, in particular berseem grown on such soils carry very high molybdenum contents. The excess of this element reduces the phosphorus contents of the body by interfering with its absorption from gastrointestinal tract and also by increasing its (phosphorus) elimination through urine. Copper is antagonistic to molybdenum. Parenteral administration of copper glycinate (@ 1.5 mg/kg but not more than 500 mg dissolved in 540 mL normal saline and infused IV) was found effective in the treatment of nutritional haemoglobinuria and babesiosis in crossbred cattle (Randhawa *et al.*, 1992). Similarly, IV infusion of copper glycinate plus oral administration of DCP was found more effective than 10% calcium glycerophosphate (IV), DCP (orally), and copper glycinate (IV) alone in the treatment of puerperal haemoglobinuria in buffaloes (Randhawa *et al.*, 1994).

5. *Use of Antioxidants*

In parturient haemoglobinuria of buffaloes, antioxidant potential of red blood cells becomes very low (Singari *et al.*, 1989; Mata *et al.*, 1994) and haemolysis is possibly due to oxidative damage of RBCs. Vitamin C is a known antioxidant because it scavenges the oxygen-free radicals and maintains the sulphydryl groups of various enzymes in a reduced state. Chugh and Mata (1997) conducted therapeutic trials with ascorbic acid (Vitamin C) in 25 buffaloes suffering from parturient haemoglobinuria. These included 11 buffaloes which had not received any treatment before the commencement of these trials (Group-I) and 14 buffaloes which had earlier been treated with sodium acid phosphate for one to three days but had not still responded (Group-II). Ascorbic acid in the dose of 7.5 g was administered as IV infusion in 500 mL normal saline solution once daily for 1–4 days. The therapeutic efficacy in Group-I and -II was found to be 81.8 and 71.4 percent, respectively. C-Cor (Marvi Labs., Karachi) is a brand of injectable vitamin C available in Pakistan in 10 mL ampoules, each mL containing 100 mg of vitamin C.

6. *Role of Vitamin D*

Synthetic analogue of vitamin D (1 alpha hydroxy vitamin D) has been shown to increase the absorption of both calcium and phosphate from the small intestine (Care, 1994). Inj. Vit. AD_3E (Prix Pharmaceutica, Pakistan) is an injectable formulation containing vitamin D_3 alongwith vitamins A and E.

7. *Role of Glycerine*

Glycerine is a 3-carbon molecule which can be utilized in glycolysis to produce energy.

8. *Change in Feed*

Cruciferous fodders (e.g., *Brassica* or 'Sarsoon', turnips, cabbage, kale, rape, etc.), berseem and lucerne should be eliminated from the diet of the animal. The animal should be fed on grains, bran, and concentrates since they are rich in phosphorus. If possible, replace 'Sarsoon', berseem, lucerne, etc. with maize, sorghum, or oat/wheat fodder.

9. *Antigalactagogues and Anabolics*

In high producing non-pregnant animals, IM administration of progesterone @ 10 mL/animal on day 1 and 5 mL on second day along with 15 mL dexamethasone/animal is recommended to suppress the milk production and thus to reduce the loss of phosphorus and other minerals from the body through milk (*Personal communication:* Dr. Fazal-ur-Rehman). Since the animal loses the body condition, single IM administration of an anabolic steroid (e.g., Inj. Deca-Durabolin – Organon Pharma; @ 150–250 mg/adult buffalo) may be helpful (*Personal communication:* Dr. Ghulam Muhammad).

10. *Herbal Treatment*

The herbalist, Levy (1973) recommends treating red water as follows:

In mild cases, after a short laxative, drench with a brew of herb-robert (wild geranium or garden geranium). Follow by a dry but nourishing diet. In more severe cases, give twice daily dosage of the following gruel: White of two eggs, two ounces (1 ounce ≃ 30 g) powdered march-mallow roots, two ounces slippery elm bark, one ounce Orris root, two dessertspoonfuls honey, mixed into one quart of milk-water (equal parts).

A Model Prescription for a Haemoglobinuric Buffalo

A model prescription based on the principles described above and utilizing the drugs readily available in Pakistan is as follows:

Date: January, 7, 2001

Animal: A 7-year old buffalo weighing about 450 kg, in the third month of her 4th lactation and yielding about 16 litres of milk daily.

History: Sick since yesterday. A quack treated the case with a juice of radish ('mooli ka pani') last night, but the condition has remained unresponsive.

Feed: Berseem (30 kg), wheat chaff 6 kg plus concentrate (cottonseed cake) 4 kg/day.

Clinical Signs: Temperature – 101°F; urine – coffee-coloured and produces a moderately stable foam when falls on ground; straining while passing faeces; milk yield – 15 litres/day; appetite almost unaffected.

Rx:

Day 1 (7–1–2001)

(i) Sodium dihydrogen phosphate (E. Merck, Germany) 60 g. Dissolve it in 300 mL distilled water. Add this to 3 litres of Dextrose 10% and give IV.

(ii) Sodium dihydrogen phosphate 60 g. Dissolve it in 300 mL distilled water. Add 10 mL xylocaine 2% with adrenaline (Glaxo-Wellcome, Pakistan) and give SC.

(iii) Sodium dihydrogen phosphate 100 g. Dissolve in ½ litre tap water and drench.

(iv) Inj. Fosfan (Selmore Agencies, Pakistan), 35 mL IM.

(v) Inj. Progesterone (Star Labs., Pakistan) 10 mL IM.

(vi) Inj. Vit. AD$_3$E, (Prix Pharmaceutica, Pakistan) 10 mL IM.

(vii) Vito Mineral-T Powder (Trust Pharmaceuticals,Faisalabad), 100 g PO. Dissolve it in water and drench it in the morning.

(viii) Copper sulphate 2 g, grind it into a fine powder. Dissolve it in 250 mL water, add 250 g vinegar and drench it in the evening.

(ix) Stop berseem feeding and manage the animal on maize or oat ('Javee' in Punjabi) fodder (25 kg) plus grains (maize/wheat) (6 kg) and 2 kg wheat bran. Give a gruel containing candied roses ('gulqund' in Punjabi; ½ kg), glycerine (250 mL), wheat porridge ('dalya' in Punjabi; 1 kg), jaggery ('gurr' in Punjabi; ½ kg), and milk (1½ kg)

Day 2, 3, and 4

If need be repeat sodium dihydrogen phosphate, P.O.; Inj. Fosfan; Vito Mineral-T; and copper sulphate as given under iii, iv, vii and viii on Day 1. Also continue oral administration of gruel as given under ix if needed.

Control and Prevention

Since the aetiology of parturient haemoglobinuria is still unresolved and appears to be multi-factorial, control and prevention of this disorder warrants attention to several factors which are discussed below:

1. Phosphorus Supplementation

When the fodder contains less than 0.14% phosphorus (berseem and lucerne are extremely deficient in phosphorus and extremely rich in calcium; phosphorus and calcium contents of berseem being 0.04 and 0.32%, respectively; El-Latif and Awad, 1964; Pirzada and Hussain, 1998) or when the serum inorganic phosphorus is below 5 mg/100 mL, supplementing a source of phosphorus in ration or in drinking water is recommended. Table 1 gives phosphorus contents and amount (necessary to satisfy total requirements) of various phosphatic supplements for maintenance (Dalling, 1966).

Table 1: Comparative value of various phosphatic supplements for maintenance

Product	Per cent Phos - phorus	Amount necessary to satisfy total requirement (g)		Amount prescribed to supplement phosphorus deficiency (g)	
		Cattle	Sheep	Cattle	Sheep
Bone meal	9.5	100	15	50	7
Bone ash	15.0	64	10	32	5
Degelatin-ised bone meal	13.0	70	12	35	6
Tri-calcium phosphate	14.0	68	10	34	5
Di-calcium phosphate	17.0	56	8	28	4

When a large number of animals are to be dealt with, it is perhaps convenient to administer a water-soluble salt of phosphorus in drinking water. Various water-soluble phosphates along with their phosphorus contents and the theoretical amount to be added to every 1,000 gallons of drinking water are given in Table 2.

Of these, the use of super-phosphate is practical and economical. Before using, it has to be defluorinated. The usual method is to add 110 lb of super-phosphate to 40 gallons of water and stir for 5 minutes. It is then allowed to stand over-night and the clear liquid in which most of the phosphorus has dissolved can be decanted. Add 2 gallons of this concentrated solution to every 100 gallons of drinking water. The aim is to ensure that each gallon of water contains 1–1½g phosphorus. This method may not be completely efficient, due to the variation in seasonal intake of drinking water (Dalling, 1966). When only a few animals are to be treated for phosphorus deficiency, a dose rate of 60 to 100 grams of super phosphate per animal should be used for one to one and a half-month (Hungerford, 1990). The animals on individual basis can also be treated by adding sodium acid phosphate @ 30 g/animal/day, or bone meal @ 100 g/animal/day (Caple, 1986) to the ration. Calgophos™ (Virbac Labs.,

France) is a proprietary preparation available in Pakistan containing phosphates of calcium, magnesium, sodium, iron, manganese, zinc, copper and cobalt. Manufacturer recommends oral administration of 75–100 mL of Calgophos/adult animal/day for 3–5 days. An off-label intravenous administration of this preparation in dextrose has been found safe in buffaloes (Muhammad *et al.*, 1999).

Table 2: **Water soluble phosphates**

Product	Phosphorus (%)	Theoretical amount to be added to very 1,000 gallons
Mono-sodium phosphate	22.5	10lb
Di- sodium phosphate	21.5	10lb
Mono-ammonium phosphate	27.0	10lb
Mono-calcium phosphate	24.0	10lb
Super-phosphate (18.5%, P_2O_5)	8.1	10lb

Where phosphorus deficiency is very wide spread, application of phosphorus fertilizer to the fodder is also recommended. Supply of proteins (oil cakes and concentrates), copper, other minerals and vitamin D should be increased when using super-phosphate as a source of phosphorus in animal diet.

2. Other Minerals and Vitamins Supplementation

i. An optimal intake of other minerals and vitamins (particularly Ca, Cu, Zn, Mn, Iodine, and Mg; Vit. D, E, etc.) should be ensured by supplementing the diet with a Vitamin mineral mixture (e.g., Vito Mineral-T, Trust Pharma, Faisalabad; @ 50–100 g/adult animal/day).

ii. Monthly parenteral administration of Vit. D (e.g., Inj. Vit AD_3E, Farvet-Holland; @ 10 mL for adult cattle and buffalo). Vitamin D increases the absorption of both calcium and phosphorus from the small intestine (Care, 1994).

3. Eliminating or curtailing the intake of cruciferous plants, berseem, lucerne and sugar beets from the ration of pregnant or lactating dairy buffaloes.

DISCLAIMER

Trade names have been used in an effort to make the information contained herein more useful. No endorsement of named products is intended, nor is criticism implied of similar products that are not mentioned.

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