CLINICAL REVIEW ARTICLE

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 ‘Pit Mootra’ in Punjabi) is an acute disease of high yielding buffaloes and cows associated with hypophosphataemia and characterized by intravascular haemolysis, haemoglobinuria, strainings 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 foders (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 (Raza 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-fac torsial 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 PO₄ has been documented to impair the glycolysis in bovine RBCs (at glycolaldehyde-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 Beard, 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 PO₄ 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 campestris 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 dimethyl disulfide. Once absorbed into circulation, dimethyl disulfide causes precipitation of haemoglobin, leading to haemolysis (Yates, 1990). Sugar beets (‘chaqander’) 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 sepsisemia, 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 intracorporeal infectants and Clostridium perfingens 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. Diminazene, Imidacarb dipropionate ‘imidol’) and the knowledge of the occurrence of the disease in the area should be considered in differential diagnosis. Demonstration of
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 corticosteroid-antihistamine combination (e.g., Solon-M Selmore Agencies, Pakistan) should be on hand to deal with any clinical predication 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 NaH₂PO₄·2H₂O and not Na₂HPO₄ 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 NaH₂PO₄·2H₂O, 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 1M, daily for three days along with 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 hypophosphate (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 Botropsa (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 antifibrinolytic 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
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 (Core, 1994). Inj. Vit. AD, E (Prix Pharmaceutica, Pakistan) is an injectable formulation containing vitamin D, along with 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 dessert spoonfuls 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 panj’i’) 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 xylol.idine 2% with adrenaline (Glaxo-Welcomme, Pakistan) and give SC.

(iii) Sodium dihydrogen phosphate 100 g. Dissolve in 1 litre tap water and drench.

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

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

(vi) Inj. Vit. AD3-E. (Prix Pharmacutica, 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), glycérine (250 mL), wheat porridge (‘daliya’ 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. Fosfam; 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 etiology 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

<table>
<thead>
<tr>
<th>Product</th>
<th>Percent Phosphorus</th>
<th>Amount necessary to satisfy total requirement (g)</th>
<th>Amount prescribed to supplement phosphorus deficiency (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone meal</td>
<td>15.0</td>
<td>100</td>
<td>15</td>
</tr>
<tr>
<td>Bone ash</td>
<td>13.0</td>
<td>70</td>
<td>12</td>
</tr>
<tr>
<td>Degelatinised bone meal</td>
<td>11.0</td>
<td>52</td>
<td>12</td>
</tr>
<tr>
<td>Tri-calcium phosphate</td>
<td>14.0</td>
<td>66</td>
<td>10</td>
</tr>
<tr>
<td>Di-calcium phosphate</td>
<td>17.0</td>
<td>58</td>
<td>8</td>
</tr>
</tbody>
</table>

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 1–1½ g 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 superphosphate 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. Calgrophos™ (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 Calgphos/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>
<thead>
<tr>
<th>Product</th>
<th>Phosphorus (%)</th>
<th>Theoretical amount to be added to very 1,000 gallons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mono-sodium phosphate</td>
<td>22.5</td>
<td>10lb</td>
</tr>
<tr>
<td>Di-sodium phosphate</td>
<td>21.5</td>
<td>10lb</td>
</tr>
<tr>
<td>Mono-ammonium phosphate</td>
<td>27.0</td>
<td>10lb</td>
</tr>
<tr>
<td>Mono-calcium phosphate</td>
<td>24.0</td>
<td>10lb</td>
</tr>
<tr>
<td>Super-phosphate</td>
<td>8.1</td>
<td>10lb</td>
</tr>
</tbody>
</table>

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 AD₃E, 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).

iii. 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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