

Clinico-haematobiochemical profile in chronic anemic crossbred cattle

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Received: 20 April 2009; Accepted: 23 September 2009

ABSTRACT

Crossbred cows (250) of 3–9 years of age, were screened for gastrointestinal parasites and blood protozoan/rickettsia and acid-fast bacilli (AFB) in faeces. Anemic, apparently healthy crossbred cows (43), negative for gastrointestinal parasites, AFB in faecal smears and protozoa/rickettsia in blood smear were finally sampled to establish the etiology of chronic anemia in crossbred cows. The blood samples were analyzed for haematological parameters, viz. Hb, PCV, TEC, red cell indices, red cell morphology and regenerative response in blood smear. Bone marrow aspirate examination was done to calculate M:E ratio and to assess iron stores in ten anemic cows to see regenerative response. Blood was also analyzed for biochemical parameters, viz. iron biochemistry, total bilirubin, TPP, albumin, globulin, A:G ratio, fibrinogen, PUN and TPP:Fib. ratio. Anemia was mild in 55.8%, moderate in 30.3% and severe in 13.9% of crossbred cows. Normal plasma iron and normal TIBC was observed in 55.9% of anemic cows. Hypoproteinemia and hypoalbuminemia was detected in only 4.65% cows, low A:G ratio in 16.3% and low TPP: Fib. ratio in 11.6% of anemic cows. Plasma urea nitrogen was significantly low in anemic cows. Mean M:E ratio was low (0.6 ± 0.05) in anemic as compared to non-anemic (1.02 ± 0.03) cows suggestive of regenerative anemia. Bone marrow stores were adequate in 7 and low in 3 anemic cows. Considering morphology of red cell, iron biochemistry, A:G and TPP:Fib. ratio, tentative diagnostic possibilities of idiopathic chronic anemia was evolved as anemia of chronic inflammatory diseases in 30.2%, early iron deficiency in 16.3%, iron deficiency in 9.3%, nutritional factors other than iron in 16.3% and etiology was obscure in 9.3% of chronically anemic cows. *Trypanosoma evansi* infection was etiology of chronic anemia in 18.6% cows.

Key words: Bone marrow aspirate, Cattle, Chronic anemia, Fibrinogen, Hemogram

Anemia in cattle is of great clinical importance because of the direct and indirect economic losses. Direct losses are due to mortality and morbidity and indirect losses are in the form of reduced production and physical performance. Baseline surveys in Punjab showed that anemia is widely prevalent in crossbred cattle compared to buffaloes and incidence of chronic anemia varied from 30 and 25% (Singh 1999), 26.4 and 13.2% (Singh 2002) and 30 and 25% (Heigopal 2003) on hemoglobin and packed cell volume basis respectively. Though, the anemic cows were apparently healthy, subclinical effects are expected because of physiological adjustments in cardiovascular system (Jain 1986). Sarkar *et al.* (1996) reported infertility in the form of increased services per conception and delayed postpartum ovarian activity in crossbred anemic cows and high mortality rate of 36.5% in anemic crossbred cattle due to debility and weakness followed by pneumonia in 25% of anemic crossbred cattle (Chavai *et al.* 2001). The present article is a

study on clinico-haematobiochemical profile in chronic anemic crossbred cattle of Punjab.

MATERIALS AND METHODS

Crossbred cows (250) with mean age range of 3–9 years, were screened from rural dairy units of Punjab. Cows having ($Hb < 8 \text{ gdl}^{-1}$ and $PCV < 24\%$) were categorized as anemic. These anemic cows were examined on 3 occasions for gastrointestinal (GIT) parasites and AFB in faeces and blood smears for protozoan/rickettsia, viz. *Babesia* spp or *Theileria* spp or *Anaplasma* spp infection. Cows (43) found negative for GIT parasites and blood protozoa/rickettsia were finally taken up to determine the etiology of chronic anemia. Thirty-five non-anemic cows ($Hb > 8 \text{ gdl}^{-1}$ and $PCV > 24\%$) were selected as healthy control. History about the feeding regime, weight loss, and consistency of faeces, urine discoloration, any previous illness or any underlying disease was recorded. Anemic animals were examined for general body condition, color of conjunctival mucous membrane and heart rate.

The blood samples were collected from each cow (15–20 ml) in heparinised and (2–3 ml) in disodium EDTA mineral

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free screw capped glass vials. The blood samples were examined for Hb, PCV and TEC within 5–10 h of collection (Jain 1986). Reticulocyte response was observed by new methylene blue staining. Ziehl's Neelson staining of faecal smears was done for demonstration of acid-fast bacilli (Carter 1975). Bone marrow aspiration, from dorsal end of 9 to 11th ribs was collected aseptically using Klima needle from 10 anemic, and 5 healthy cows. Bone marrow aspirate smears were prepared, air-dried and fixed (Freeman 2000). Bone marrow aspirate smears were stained by giemsa and heamatoxylin and eosin stain, for myeloid to erythroid ratio (M:E) (Bruce and Blue 2000). Bone marrow iron stores were evaluated by staining of bone marrow smears with Prussian blue stain (Luna 1968). Simultaneously blood samples were collected from healthy and anemia cows for TLC and DLC. Plasma was analyzed for total plasma Fe and total iron binding capacity (Caraway 1962) and per cent transferrin saturation was calculated. Total plasma proteins and albumin were estimated by biuret method and BCG method respectively. Globulins were determined by taking the difference in total plasma proteins and albumin and A/G ratio was derived. Plasma fibrinogen was estimated using heat total precipitation refractometer method (Jain 1986). Concentration of plasma total bilirubin (Malloy and Evelyn), urea nitrogen (Ritcher and Laponite) and total plasma cholesterol were estimated by commercial kits. Univariate analysis was done to compare for multiple comparison between groups to find out significant difference between them, using SPSS^{12.0} Software.

RESULTS AND DISCUSSION

Mean values of red cell parameters of non-anemic and anemic crossbred cows are presented in Table 1. Anemia was mild, moderate and severe in 55.8, 13.9 and 30.3% of crossbred cows respectively (Table 2). Considering PCV values, anemia was mild, moderate and severe in 55.8, 27.9% and 16.3% of crossbred cows (Table 3). Mild to moderate

Table 1. Red cell parameters of non-anemic and anemic cows (mean±SE)

Parameter	Non-anemic (n=35)	Anemic (n=43)
Hb (gdl ⁻¹)	10.6±0.21 ^a (9.10–14.6)	6.82±0.16 ^b (4.40–8.70)
PCV (%)	33.6±0.76 ^a (28.0–48.0)	20.6±0.54 ^b (8.00–28.0)
TEC (10 ⁶ ml ⁻¹)	6.63±0.19 ^a (5.00–9.84)	4.86±0.13 ^b (2.05–6.85)
MCV (fl)	50.9±0.92 ^a (38.3–60.6)	42.8±0.82 ^b (24.9–54.4)
MCH (pg)	16.2±0.34 ^a (12.4–20.1)	14.2±0.27 ^b (10.4–18.5)
MCHC (%)	32.3±0.72 ^a (24.8–34.9)	33.5±0.64 ^b (24.2–37.5)

Table 2. Classification of anemia on basis of hemoglobin in anemic cows (mean±SE)

Parameter	Mild anemia (7–8 gdl ⁻¹) (n=24)	Moderate anemia (6–7 g dl ⁻¹) (n=13)	Severe anemia (<6 gdl ⁻¹) (n=6)
Hb (gdl ⁻¹)	7.65±0.06 ^a	6.46±0.08 ^b	5.23±0.15 ^c
PCV (%)	22.6±0.35 ^a	19.9±0.62 ^b	18.3±1.30 ^c
TEC (10 ⁶ ml ⁻¹)	5.36±0.13 ^a	4.56±0.16 ^{ab}	4.11±0.23 ^b
MCV (fl)	42.8±1.06 ^a	44.0±1.52 ^a	43.4±0.37 ^a
MCH (pg)	14.4±0.30 ^a	14.38±0.52 ^a	12.8±0.50 ^a
MCHC (%)	33.9±0.63 ^a	32.8±1.03 ^a	29.0±1.34 ^a

anemia was a usual feature of non-regenerative anemia of inflammatory diseases, chronic infections, gastrointestinal worms, ectoparasites infestation or infection of *Eperythrozoon wenyoni* (Morris 2001). Mean value of TEC was significantly lower compared to non-anemic cattle. Total erythrocyte count was less than 5×10⁶ ml⁻¹ in 53.5% of anemic cows. Low erythrocyte count with hemoglobinemia, hyperbilirubinemia, hemoglobinuria and hyperbilirubinuria was a feature of hemolytic anemia (Jain 1986).

Mean values of MCV, MCH and MCHC were significantly lower in anemic cattle compared to non-anemic cattle. However, mean values of red cell indices were similar in mildly, moderately and severely anemic cows (Table 3). Anemia on the basis of red cell indices was normocytic-normochromic in 70%, normocytic-hypochromic in 13.9% and microcytic-normochromic in 16.1% of anemic cows. Freshly prepared new methylene blue (NMB) stained smears also did not reveal reticulocytosis in any of the anemic cows. Peripheral blood smear examination of anemic cows revealed normocytic-normochromic, macrocytic-hypochromic, microcytic-hypochromic and normocytic-hypochromic anemia in 64%, 23%, 7% and 6%, respectively in anemic cows. Thus anemia was characterized as non-regenerative

Table 3. Classification of anemia on basis of packed cell volume in anemic cows (mean±SE)

Parameter	Mild anemia (23–24%) (n=24)	Moderate anemia (19–22%) (n=12)	Severe anemia (<19%) (n=7)
Hb (gdl ⁻¹)	7.45±0.12 ^a	6.78±0.22 ^b	5.55±0.22 ^c
PCV (%)	22.6±0.17 ^a	20.4±0.19 ^b	16.5±0.57 ^c
TEC (10 ⁶ ml ⁻¹)	5.39±0.13 ^a	4.64±0.14 ^{ab}	4.03±0.18 ^a
MCV (fl)	42.4±0.99 ^a	44.3±1.12 ^a	40.2±1.32 ^a
MCH (pg)	13.9±0.32 ^a	14.7±0.58 ^a	13.8±0.44 ^a
MCHC (%)	33.0±0.64 ^a	33.2±1.08 ^a	33.7±1.74 ^a

Means with different superscripts in row differ significantly ($P \leq 0.05$). Figures in parentheses show range. (n) refers to number of cows.

on the basis of erythrocytic indices. Whitney (1999) emphasized that erythrocytic morphology noted on careful examination of blood films, must always be considered in conjunction of the calculated red cell indices. Calculated erythrocytic indices are also less useful in cattle because of higher degree of anisocytosis and thereby resulting in unreliability of their reference range (Weiss and Perman 1992). Underlying cause of normocytic- normochromic anemia might be early iron deficiency, hypoproliferative anemia due to selective depression of erythropoiesis as in chronic infections, uremia and hypothyroidism due to flourosis. Macrocytic-hypochromic anemia in cattle was a feature in remission anemia. Normocytic-hypochromic and microcytic-hypochromic anemia resulted as a consequence of early iron deficiency. Microcytic-hypochromic anemia was purely a feature of iron or copper deficiency (Jain 1986). Erythrocytic indices might vary in cattle due to anisocytosis, which was also observed in blood smear of non-anemic cows in this study. A proportion of cows with normocytic-normochromic anemia on the basis of calculated red cell indices, revealed macrocytic-hypochromic anemia on examination of blood smears. Thus microscopic erythrocyte morphology examination revealed that macrocytosis was probably indicating regenerative bone marrow response. Normocytic-normochromic anemia in ruminants cannot be always categorized into regenerative or non-regenerative by simply examining peripheral blood smears (Cole *et al.* 1997) and bone marrow examination becomes essential for its differentiation. Also there was discrepancy in the morphological classification of anemia as determined from calculated red cell indices and from peripheral blood smear examination. Therefore bone marrow aspirate examination was done in 10 anemic cows to confirm whether anemia was regenerative or non regenerative and also to access iron status for the differentiation of iron deficiency and inflammatory diseases anemia.

Mean myeloid to erythroid ratio (M:E ratio) was 0.60 ± 0.05 in anemic cows showed predominance of erythroid over myeloid cells and 1.02 ± 0.03 in non-anemic cows showed comparable number of myeloid and erythroid cells. Prussian blue stained smears showed normal hemosiderin (+1 to +2) in 7 of 10 and low hemosiderin (+0.5 to +0.75) in remaining 3 anemic cows. The M:E ratio of anemic cows was thus below 1.0 and was also lower than non-anemic cows (1.02), these observations indicated that anemia was regenerative in anemic cows. It may be used to conclude that normocytic-normochromic anemia in crossbred cows was not always non-regenerative and bone marrow examination was essential for their differentiation. As from the bone marrow examination, it was inferred that anemia was regenerative with adequate iron stores in more number of cows (7/10) and bone marrow regeneration with low iron store in 3 of 10 of anemic cows. It was finally theorized that regenerative idiopathic anemia in 7 of 10 cows was probably either from slow degree of

hemolysis or rapid removal of red cells from the circulation. Other possible cause in 3 of 10 cows with relative low bone marrow iron stores, low plasma Fe and high TIBC was likely to be secondary iron deficiency, might be due to chronic intermittent blood loss.

Mean value of plasma Fe concentration and per cent transferrin saturation in anemic cows were significantly lower than non-anemic cattle (Table 4). Mean values of TIBC of anemic cows were significantly higher as compared to non-anemic cows. Low plasma Fe concentration ($<100 \text{ mgdl}^{-1}$) was observed in 39.5% (17/43) of anemic cows. Normal plasma Fe and normal TIBC; low-normal plasma Fe and normal TIBC; low plasma Fe and high TIBC and normal plasma Fe and high TIBC were detected in 55.9% (24/43), 20.9% (9/43), 18.6% (8/43) and 4.65% (2/43) of anemic cows. Low plasma Fe, high TIBC and low PTS in 18.6% of anemic cows thus suggested iron deficiency as the possible cause of anemia. Primary iron deprivation in adult cows was unlikely as: the soil iron content is many times higher than the plants iron content, physiological losses from animal body are minimal and fodder is contaminated with soil and dust (Underwood and Suttle 1999). Secondary iron deficiency due to chronic blood loss was thus considered as the cause of

Table 4. Comparative biochemical parameters of healthy and AFB shedding cows

Name of parameter	Healthy cows (N=35)	AFB shedding cows (N=45)
Plasma iron (mgdl^{-1})	156.1 ± 7.08^a (92.5–267)	110.8 ± 6.70^b (65.6–233.4)
Total iron binding capacity (mgdl^{-1})	433.5 ± 18.4^a (240–680)	573.6 ± 42.0^b (252.0–1156.2)
Per cent transferrin saturation (%)	39.4 ± 2.71^a (13.9–76.2)	30.3 ± 3.10^b (5.84–92.7)
Protein (gdl^{-1})	8.25 ± 0.09^a (7.36–9.16)	8.08 ± 0.17^a (5.69–11.2)
Albumin (gdl^{-1})	3.19 ± 0.03^a (2.80–3.72)	3.05 ± 0.06^a (1.90–4.00)
Globulin (gdl^{-1})	5.02 ± 0.10^a (3.84–6.03)	5.01 ± 0.16^a (2.90–7.41)
A:G ratio	0.64 ± 0.17^a (0.48–0.91)	0.62 ± 0.02^a (0.32–0.97)
Fibrinogen (mgdl^{-1})	328.5 ± 18.6^a (200–600)	377.7 ± 20.0^a (100–700)
TPP: fibrinogen ratio	28.1 ± 1.71^a (13.3–45.0)	25.8 ± 2.02^a (11.8–74.8)
Total plasma bilirubin (mgdl^{-1})	0.41 ± 0.05^a (0.09–1.20)	0.35 ± 0.20^a (0.03–1.34)
Plasma urea nitrogen (mgdl^{-1})	17.8 ± 0.67^a (11.6–26.7)	11.5 ± 0.90^b (8.03–20.4)
Total plasma cholesterol (mgdl^{-1})	113.8 ± 6.66^a (59.7–210)	101.3 ± 6.68^a (39.50–210)

Means with different superscripts in a row differ significantly ($P \leq 0.05$); figures in parentheses show range. (n) refers to number of cows.

iron deficiency anemia. Although the blood loss was not established routinely during this study on anemia, however the intermittent urinary blood loss and occult fecal blood loss was not monitored. Hypoferremia was a feature of iron deficiency due to increase iron loss, chronic blood loss, decreased iron intake or intestinal absorption and acute or chronic inflammation (Stockham and Scott 2002). Low plasma Fe concentration, high or normal TIBC and low per cent transferrin saturation was a common finding in iron deficiency anemia (Jain 1986). Early iron deficiency might accompany normal plasma iron and high TIBC (Jain 1986 and Stockham and Scott 2002).

Normal plasma iron and high TIBC in 4.65% cows was considered to be due to early iron deficiency. Anemia of chronic or non-infectious inflammatory conditions caused a fall in TIBC, transferrin saturation and normal to decreased plasma Fe concentration (Mc Gillivray *et al.* 1985), whereas anemia of organ dysfunction or bone marrow dyscrasia did not affect iron metabolism (Cole *et al.* 1997). Normal to decreased plasma Fe and TIBC concentrations was a feature of anemia of chronic infection and inflammatory diseases (Jain 1986, Smith 1997). Normal or low-normal plasma Fe and normal TIBC in 76.7% cows could be attributed to anemia of inflammatory diseases.

Mean concentrations of TPP, albumin, globulins and A:G ratio of anemic cows were not significantly different from non-anemic cows (Table 4). However, the range of plasma proteins was broader in anemic than of non-anemic cows. Frank hypoproteinemia ($TPP < 6.5 \text{ gdl}^{-1}$) and hypoalbuminemia (albumin $< 2.4 \text{ gdl}^{-1}$) was recorded in 4.65% (2/45) of anemic cows. Anemia was normocytic-normochromic in one cow with very low albumin concentration (1.9 gdl^{-1}). Total globulins were higher in 7 of 43 anemic cows. Elevated levels of globulins occurred because of benign plasma cell proliferation in response to persistent antigenic stimulation as in chronic infections, chronic inflammatory diseases or immune mediated diseases (Jain 1986). Albumin/globulin ratio (A:G) was below-normal range in 16.3% (7/43) cows. Therefore chronic inflammatory anemia was suspected in 16.3% of cows.

Mean concentration of plasma fibrinogen in non-anemic and anemic cows were within normal range of 100–700 mg/dl as suggested by Jain (1986) and Radostits *et al.* (2000). The difference in the ratio of total plasma proteins to fibrinogen (TPP: Fib.) between non-anemic and anemic group was also nonsignificant, because of extensive variability of the leukocyte response in chronic inflammation. Estimation of fibrinogen was a useful adjunct to leukogram for the determination of inflammatory response. Though plasma fibrinogen concentration was within the physiological range, low TPP: Fib. ratio (15:1) was present in 5 of 43 anemic cows. Plasma fibrinogen is acute phase proteins, the levels of which are not affected by age, sex, exercise, hemorrhage and repeated bleeding, however elevation suggest

inflammation. A ratio of less than 15:1 indicated a relative increase in fibrinogen over plasma proteins and ratio below 10:1 indicated a marked increase in fibrinogen (Jain 1986). Thus low TPP:Fib. ratio also supported anemia of chronic inflammatory diseases in 5 cows. Usually the elevation of plasma fibrinogen leveled off within several days after the active inflammation had stopped and thereafter elevation of plasma gamma globulins occurred in chronic inflammatory disease (Benjamin 1997). Commonly plasma fibrinogen was more in inflammatory, suppurative and neoplastic diseases. Thus low TPP:Fib. probably indicative of anemia of chronic inflammatory diseases in anemic cows. Mean plasma total bilirubin of anemic and non-anemic cows was comparable (Table 4). However plasma bilirubin of cows infected with *T. evansi* varied from $0.75 - 1.25 \text{ mgdl}^{-1}$ and was high in 1 cow only (1.25 mgdl^{-1}). Plasma bilirubin concentration decreased to $0.60 \pm 0.09 \text{ mgdl}^{-1}$ following treatment. Icterus in absence of hemoglobinuria might occur if significant and persistent erythrocyte destruction occurred (Jain 1986). Thus, the mean and range of plasma bilirubin concentration suggested that persistent and remarkable extravascular or intravascular hemolysis was probably not undergoing in the chronically anemic crossbred cows in the present study.

Mean plasma urea nitrogen (PUN) concentration of anemic cattle was lower ($P < 0.05$) than non-anemic cows (Table 4). The concentration of PUN had been demonstrated to be directly proportional to daily protein intake (Payne *et al.* 1974, Whitlock *et al.* 1976, Pelletier 1985). A study by Manston *et al.* (1975) recorded a concentration of 7.5 mg/dl in cows maintained on low protein diet, associated with low Hb and PCV values. Concentration of PUN was less than 7.5 mgdl⁻¹ in 18 of 43 anemic cows. It indicated that anemic cows were probably on low protein diet at time of sampling. Low Hb had also been recorded by Manston *et al.* (1975) in animals on low protein diet.

Cows that had low TPP:Fib. or low A:G ratio and normal plasma Fe and TIBC were attributed to anemia of inflammatory diseases or chronic infection. Cows with normocytic-normochromic anemia, low iron and high TIBC and normal TPP: Fib. or A:G ratio was incriminated to early iron deficiency. Cows with normocytic-normochromic anemia with normal plasma Fe and TIBC and high TPP: Fib. or A:G ratio was considered due to nutritional factors other than iron. Anemic cows showing normocytic-normochromic anemia accompanied by low normal or normal plasma Fe, normal or high TIBC, high TPP:Fib. and A:G ratio were unusual findings where tentative etiology could not be established. Anemia in other 35 crossbred cows when the biochemical and hematological changes were considered for all the cows was idiopathic as clinical, hematological and biochemical changes failed to confirm the exact cause. Considering morphology of red cell, iron biochemistry, A:G and TPP:Fib. ratio, tentative diagnostic possibilities of idiopathic anemia was evolved as anemia of chronic

inflammatory diseases in 30.23%, early iron deficiency in 13.95%, iron deficiency in 11.76%, nutritional factors other than iron in 16.27% and etiology was obscure in 6.97% of chronically anemic cows. *Trypanosoma evansi* infection was etiology of chronic anemia in 18.6% cows.

The present study concluded that anemia was mild to moderate in nature and of normocytic- normochromic and regenerative type in chronic anemic cows. Etiology of anemia was evolved as anemia of chronic inflammatory diseases in 30.2%, early iron deficiency in 16.3%, iron deficiency in 9.3%, nutritional factors other than iron in 16.3% and etiology was obscure in 9.3% of chronically anemic cows. The study also concluded that *Trypanosoma evansi* infection should be ruled out as an etiology of chronic anemia in cows.

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