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# Comparative bone marrow aspirate studies of anemic and acid-fast bacilli shedding cows to healthy cows

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Bone marrow examination reflects hematopoietic activity of an individual and is indicated in many conditions like non-responsive, megaloblastic or microcytic anemias, persistent neutropenia, thrombocytopenia, drug toxicities, irradiation, lymphoproliferative and marrow infiltrative diseases and to confirm whether normocytic-normochromic anemia is regenerative or nonregenerative. In the study on the etiology of chronic anemia, there was discrepancy in the morphological classification of anemia as determined from calculated red cell indices and from peripheral blood smear examination. Anemia on the basis of red cell indices was normocytic-normochromic in majority of anemic cows. In contrast anemia on basis of red cell morphology was normocytic-normochromic and macrocytic-hypochromic in anemic cows. 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. The present study deals with comparison of bone marrow aspirate examination of anemic and acid fast bacilli shedding anemic cows to healthy cows in order to access the type of anemia on basis of regeneration and bone marrow iron stores.

Bone marrow aspiration, from dorsal end of ninth to eleventh ribs was collected aseptically (Freeman 2000) using needle from 10 crossbred anemic and 10 AFB shedding cows 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) as described by Bruce and Blue (2000). Bone marrow iron stores were evaluated by staining of bone marrow smears with Prussian blue stain (Luna 1968). Prussian blue stained bone marrow aspirate was the only test available to differentiate regenerative and non-regenerative anemia, if the serum iron (Fe) and total iron binding capacity (TIBC) values were equivocal Cole *et al*. (1997). Simultaneously blood samples were collected in dipottasium EDTA from healthy and AFB shedder cows for total leukocyte count (TLC) and differential leukocyte count (DLC). Blood examination provides clues as to the necessity of a bone marrow examination and it is imperative that a blood sample should always be collected along with the bone marrow specimen for proper evaluation (Jain 1986).

Mean values of TLC and DLC of healthy and AFB shedding cows were in the normal reference range as suggested by Radostits *et al.* (2000). 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. In contrast anemia on basis of red cell morphology was normocytic-normochromic in 64% (Figs 1a,1b), macrocytic-hypochromic in 23% (Fig.1c), and microcytic-normochromic in 6% of anemic cows. Mean myeloid to erythroid ratio (M:E ratio) was 0.60±0.05 in anemic cows showed predominance of erythroid over myeloid cells (Fig. 4a,4b) and 1.02±0.03 in non-anemic cows showed comparable number of myeloid and erythroid cells (Fig. 2a,2b). The normal myeloid: erythroid (M:E) ratio in cattle varies from 0.31–1.85 with a mean of 0.70 (Jain 1986). The M:E ratio is a valuable index to indicate intensification (low M:E ratio) or depression of erythropoiesis (high M:E ratio).

Prussian blue stained smears showed normal to moderately increased hemosiderin (+1 to +2) in 7 of 10 (Fig.5a, 5c) and low hemosiderin (+0.5 to +0.75) in remaining three anemic cows (Fig. 5b). Harvey (1981) reported that bone marrow from normal adult animal exhibited stainable iron (Hemosiderin) within macrophages and little or no stainable iron was found in animals with iron deficiency. Fairbanks and Beutler (1983) found that cytological examination of bone marrow were some times useful for evaluating disorders of iron metabolism based on a prussian

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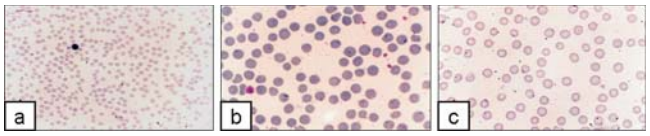
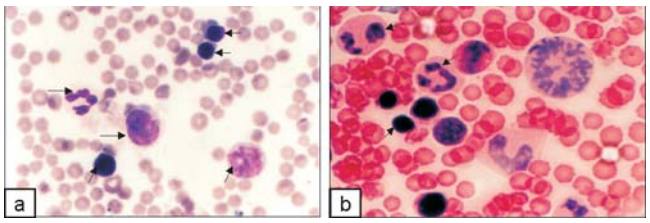
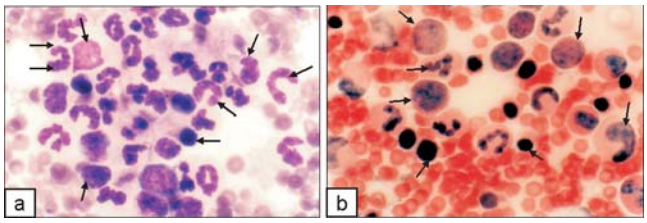


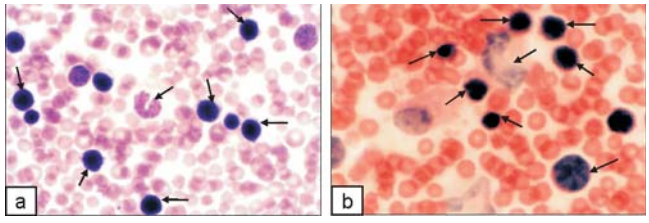

Figs 1a–1c. **a.** Peripheral blood smear of cow showing normocytic normochromic anemia (Giemsa×300). **b.** Peripheral blood smear of cow showing normocytic normochromic anemia (Giemsa×750). **c.** Peripheral blood smear of a cow showing macrocytic hypochromic anemia (Giemsa×750).




Figs 2a–2b. **a.** Bone marrow aspirate from healthy cow showing a comparable number of myeloid (bold arrow head) and erythroid (narrow arrow head) cells (Giemsa×750). **b.** Bone marrow aspirate from healthy cow showing comparable number of myeloid (bold arrow head) and erythroid (narrow arrow head) cells. A mitotic figure is also visible (HE×750).

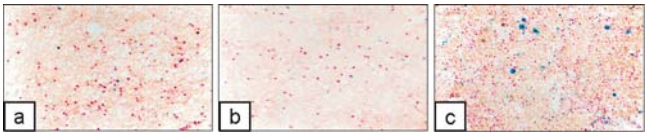



Figs 3a–3b. **a.** Bone marrow aspirate showing predominance of myeloid (bold arrow head) over erythroid (narrow arrow head) series of cells in an AFB shedding cow (Giemsa×750). **b.** Bone marrow aspirate showing predominance of myeloid (bold arrow head) over erythroid (narrow arrow head) series of cells in an AFB shedding cow (HE×750).

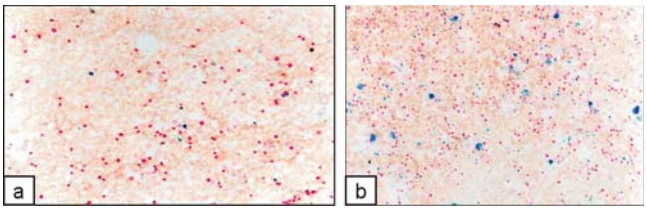



Figs 4a–4b. **a.** Bone marrow aspirate showing predominance of erythroid (narrow arrow head) over myeloid (bold arrow head) series of cells in an anemic cow (Giemsa×750). **b.** Bone marrow aspirate showing predominance of erythroid (narrow arrow head) over myeloid (bold arrow head) series of cells in an anemic cow (HE×750).

blue reaction. In chronic inflammatory conditions utilization of iron from the rapid pool is severely impaired and iron remain sequestered in macrophages (Smith 1997). 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




Figs 5a–5c. **a.** Bone marrow aspirate smear showing a scanty distribution of iron stores (pool) in a healthy cow (Gomeri×150). **b.** Bone marrow aspirate smear showing low iron stores in another anemic cow (Gomeri×150). **c.** Bone marrow aspirate smear showing moderately increased iron stores in an anemic cow (Gomeri×150).




Figs 6a–6b. **a.** Bone marrow aspirate smear showing a scanty distribution of iron stores (pool) in a healthy cow (Gomeri×150). **b.** Bone marrow aspirate smear showing an increase in iron stores in an AFB shedding anemic cow (Gomeri×150).

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 concluded 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 M:E ratio was 1.58±0.02 in AFB shedding cows, showed predominance of myeloid over erythroid cells (Fig. 3a, 3b) and 1.02±0.03 in non-anemic, showed comparable number of myeloid and erythroid cells (Fig. 2a,2b). In hypoplastic anemia, due to chronic inflammation, the anemia is usually mild and bone marrow finding typically included myeloid hyperplasia, increased iron stores and plasmacytosis in addition to mild erythroid hypoplasia (Constable 1999). Prussian blue staining indicated high hemosiderin reserves (+ 2.5 to + 4) in 8 cows (Fig. 3a) and normal (+1 to– +2) in remaining two AFB shedding cows. The M:E ratio of AFB shedding cows were above 1.0 and higher than non-anemic (1.02) cows, bone marrow examination revealed that erythropoiesis was depressed in AFB shedding compared to non-anemic and idiopathic anemic cows. It therefore followed that anemia was non-regenerative in AFB shedding cows. Morris and Johnston (2001) reported paratuberculosis as one of common cause of anemia in cattle, but not by Radostits *et al.* (2000). Recently association of anemia and

fecal shedding of AFB had been confirmed in anemic crossbred cows of Punjab (Heigopal 2003). Thus, it is also followed from the study that anemia in AFB shedding cows was of hypoproliferative type. These findings supported the opinion of Morris and Johnston (2001) that anemia in paratuberculosis was due to inadequate erythropoiesis.

## SUMMARY

In the study on chronic anemia in crossbred cattle of Punjab, discrepancy was seen in morphological classification of anemia as determined from calculated red cell indices and from peripheral blood smear examination. 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. In contrast anemia on basis of red cell morphology was normocytic-normochromic in 64%, macrocytic-hypochromic in 23%, microcytic-hypochromic in 7% and microcytic-normochromic in 6% of anemic cows. Therefore, a comparative study was conducted to evaluate the bone marrow aspirate of ten anemic, acid-fast bacilli shedding cows with five healthy controls to confirm type and nature of anemia and also to access iron status. Mean myeloid to erythroid ratio (M:E ratio) was 0.60 0.05 in anemic cows, 1.58±0.02 in AFB shedding cows and 1.02 0.03 in non-anemic healthy cows. The studies suggested that normocytic-normochromic anemia in crossbred cows was regenerative with adequate bone marrow iron store whereas erythropoiesis was depressed in AFB shedding compared to non-anemic cows. It therefore followed that anemia was non-regenerative in AFB shedder cows with heavy bone marrow iron reserves.

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