



Diagnostic importance of cytochemical and cytoenzymic observations on the blood cells of pig*

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ABSTRACT

Cytochemical observations based on diagnostic importance of blood cells were carried out on 10 apparently healthy adult pigs. The eosinophils and neutrophils showed positive reactions in the form of pink granules in the cytoplasm when the blood smears were stained with the periodic-acid-Schiff's stain. The eosinophilic granules stained black when stained with Sudan black B, whereas neutrophil showed very weak to negative reaction with Sudan black B. The granules of basophils showed strong positive reaction and stained metachromatically with toluidine blue. All leukocytes showed negative reaction for acid phosphatase. The neutrophils showed moderately positive reaction in the form of brown granules, when stained for alkaline phosphatase. The granules of eosinophils and basophils showed strong positive reactions for peroxidase and non specific esterase. The neutrophils and eosinophils showed intense positive reactions in the form of blue granules when stained for beta-glucuronidase.

Key words: Blood cells, Cytoenzymic, Cytochemical, Pig

The cytochemistry of the blood cells is the important tool in the hands of clinical veterinary practitioners. The accurate identification of the cells and localization of various enzymes in them is of great importance in understanding body response to any kind of stress and diseases. The cytochemistry of blood cells is documented in buffalo calves (Singh 2000), black bear (Salakij *et al.* 2005) and macaque (Sakulwira *et al.* 2008). In spite of large quantum of literature available on domestic animals the reports on cytochemical and cytoenzymic studies on blood cells of pig are meager. Hence the present study was conducted because of importance and its application in various fields of veterinary sciences.

MATERIALS AND METHODS

Apparently healthy Large White Yorkshire pigs (10), 1- to 2- year- old, were used. The blood samples were collected from ear vein of pig and transferred to test tubes containing EDTA as an anticoagulant. The smears were prepared immediately on grease free slides. The blood films were

stained with the different staining procedures: 1% toluidine blue stain for acid mucopolysaccharide (Bover 1964), periodic acid Schiff's stain for glycogen (Jain 1986), Sudan black -B stain for lipid (Jain 1986), acid phosphatase enzyme (Bover 1964), alkaline phosphatase (Bover 1964), peroxidase (Bover 1964), esterases (Jain 1986) and beta-glucuronidase (Bover 1964). The stained blood smears were examined under oil immersion to record the cytochemical and cytoenzymic activity in different blood cells.

RESULTS AND DISCUSSION

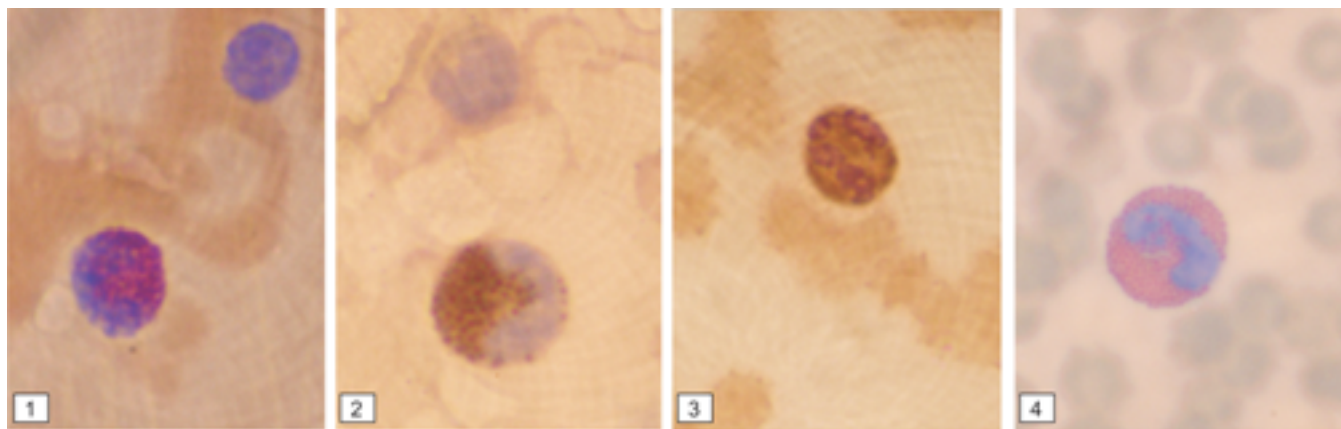
The basophilic granules showed strong positive reaction and stained metachromatically in the form of intense violet granules when the blood smears were stained with 1% toluidine blue. Other leukocytes did not show reactivity for mucopolysaccharides as observed by Singh *et al.* (1998) in camels, Menaka and Singh (2002b) in goats and Singh and Menaka (2004a) in horses. The metachromatic reaction indicated the presence of acid- mucopolysaccharides in the granules of basophils. Yokohama (2002) reported that the intensity of toluidine blue increased in chronic myeloproliferative disorders whereas decreased in myeloid leukemia.

The neutrophils and eosinophils showed positive reaction in the form of pink granules when the blood smears were stained with periodic acid Schiff's stain as observed by Singh and Menaka (2004b) in sheep. Jain (1968) reported positive

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Figs 1–4. **1.** Photomicrograph of blood smear showing basophil with metachromatically stained granules (a) and lymphocyte (b). Toluidine blue stain $\times 1000$; **2.** Photomicrograph of blood smear showing intense sudanophilia in eosinophil (a) and a lymphocyte (b) devoid of sudanophilia. Sudan black B $\times 1000$; **3.** Photomicrograph of blood smear showing brown coloured granules in neutrophil Alkaline phosphatase $\times 1000$; **4.** Photomicrograph of blood smear showing eosinophil with reddish brown granules. Peroxidase $\times 1000$

PAS activity in neutrophils of cow, horse and sheep. Singh *et al.* (1998) reported that the neutrophils of camel blood were strongly positive for PAS. Menaka and Singh (2002b) stated that the cytoplasmic granules of goat neutrophils showed diffuse reaction with PAS stain. Singh and Menaka (2004a) found that the neutrophil granules of horse showed strongly positive reaction with PAS. Jain (1986) reported increased PAS reactivity in myeloblastic leukemia in cats and horses and eosinophilic leukemia in cats. The pattern of cytoplasmic PAS reactivity can be used to characterize lymphocytic leukemias (Schwarze 1980).

The eosinophils showed strong positive reaction in the form of brownish-black granules whereas neutrophils showed very weak to negative reaction when the blood smears were stained with Sudan black B as observed by Singh (2000) in buffalo calves. Jain (1986) reported that neutrophils showed positive reactivity for Sudan black B in cow, horse, dog and sheep. Singh *et al.* (1998) observed that the neutrophils and eosinophils of camel blood were positive for Sudan black B. Menaka and Singh (2002b) stated that the cytoplasmic granules of goat neutrophils and eosinophils showed positive reactivity with Sudan black B. Singh and Menaka (2004a) noticed that the neutrophil granules of horse showed strongly positive reaction with Sudan black B. Salakij *et al.* (2005) stated that the eosinophils of black bear stained moderately positive with Sudan black B. The affinity of Sudan black B in neutrophils and eosinophils increased in myeloblastic leukemia without maturation, myeloblastic leukemia with maturation and monocytic leukemia whereas lymphoid malignancies lack positive reaction (Jain 1986).

The neutrophils, eosinophils, basophils, lymphocytes and monocytes all showed negative reactions when blood smears were stained for acid phosphatase. Singh *et al.* (1998) observed no activity in neutrophils of camels. However, Jain (1986) reported positive reactions in the lymphocytes of cows, horses, dogs and sheep. Facklam and Kociba (1985)

reported that neutrophils of dogs showed positive reactions for acid phosphatase enzymes. Acid phosphatase activity within the lymphocytes were correlated with various types of lymphoproliferative disorders (Schwarze 1980 and Savage *et al.* 1981). The highest levels of acid phosphatase were found in sickle cell disease or multiple myeloma or lysosomal disorders, such as Gaucher's disease which showed moderately increased levels (Moul 1998).

The neutrophils showed moderately positive reaction in the form of brown granules, when stained for alkaline phosphatase. Present finding was in agreement with the finding of Jain (1968) in cow, horse and sheep, Nigam and Singh (1972) in equine, and Martinez *et al.* (1996) in heifer. Singh *et al.* (1998) stated that the eosinophils of camel were strongly positive for alkaline phosphatase. Higher levels of leukocyte alkaline phosphatase were seen in polycythemia vera, essential thrombocytosis, primary myelofibrosis and the leukemoid reaction and lower levels were found in chronic myelogenous leukemia and paroxysmal nocturnal hemoglobinuria. The alkaline phosphatase activity of circulating neutrophils can facilitate differentiation of chronic myelogenous leukemia from leukemoid reactions or neutrophilic leucocytosis associated with non-malignant causes (Okum and Tanaka 1978).

The granules of eosinophils and basophil showed strong positive reaction for peroxidase as observed by Nigam and Singh (1972) in equine eosinophils and Singh (2000) in eosinophils of buffalo calves. Singh *et al.* (1998) found that the basophils in camels had negative reactions for peroxidase. Intensity of peroxidase activity increased in myeloblastic leukemia in dogs, horses and decreased in eosinophilic as well as basophilic leukemia in dogs and cats (Jain 1986). The negative peroxidase activities of lymphocytes help in differentiation of lymphocytic leukemia from granulocytic leukemia.

The granules of eosinophils and basophils were positive

for non specific esterase as observed by Salakij *et al.* (2005) in black bear. The non specific esterase reaction is used in diagnosis of myelomonocytic leukemia, monocytic leukemia, megakarioblastic leukemia, blast cell of essential thrombocythemia, and malignant histiocytosis (Jain 1986).

The neutrophils and eosinophils showed intense positive reaction in the form of blue granules when stained for beta-glucuronidase. These findings were in agreement with the findings of Singh (2000) in neutrophils of buffalo calves and Salakij *et al.* (2005) in eosinophils of black bear. Deficiencies in β -glucuronidase result in the non recessive inherited metabolic disease known as Sly syndrome or mucopolysaccharidosis. Beta-glucuronidase is used in veterinary medicine primarily to detect focal staining in T lymphocytes and acute undifferentiated leukemia (Jain 1986).

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