Cytological Evaluation of Bone Marrow in Normal Laying Hens and those With Lymphoid Leukosis

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Abstract

The purpose of this study was to evaluate cytologically the bone marrow (and peripheral blood) of adult laying hens affected with lymphoid leukosis. Diagnosis of the neoplasm was made on the basis of clinical history, signs and symptoms and pathology. Only histologically confirmed cases were included in the study. Examination of blood smears revealed +2 heterophil toxicity and the presence of large numbers of reactive (blast – transformed) lymphocytes. Smears that were prepared from the bone marrow showed increased numbers of hemopoietic cells. The total erythrocyte count (TEC), hemoglobin percentage (Hb%) , hemoglobin concentration (Hb conc.), packed cell volume (PCV) and the mean corpuscular hemoglobin concentration (MCHC) values were significantly higher ($P<0.01$) in hens with lymphoid leukosis than in apparently normal hens. The mean corpuscular volume (MCV) and the mean corpuscular hemoglobin (MCH) were significantly lower ($P< 0.01$) in hens with lymphoid leukosis than in apparently normal hens. Results of the leukogram indicated that the total leukocyte count (TLC) and the percentage (%) of lymphocytes were significantly higher ($P < 0.01$) in hens with lymphoid leukosis than in apparently normal hens. From results of this study it was concluded that cytological evaluation of bone marrow may prove to be a simple , rapid , and useful tool in the diagnosis of lymphoid leukosis in laying hens.

Keywords : Laying Hens , Bone Marrow , Cytology, Tumor, Blood smear, Lymphoid leukosis.

Introduction

Examination of the bone marrow is considered a valuable diagnostic tool to diagnose nonregenerative anemias, heteropenias thrombocytopenias, pancytopenias, suspected leukemia, and other unexplained cellular changes in the peripheral blood (Campbell, 1995; Thrall et al., 2004). The proper evaluation of the hemopoietic system involves careful evaluation of the circulating blood hemogram obtained in the same day as the bone marrow (Campbell, 1995; Thrall et al., 2004). In poultry, the most economically important virus–induced neoplastic diseases are Marek's disease (caused by a herpesvirus) and the avian leukoses and reticuloendotheliosis (caused by retroviruses) (Fadly, 2003). A number of closely related avian retroviruses cause the leukosis / sarcoma (L/S) group of transmissible benign and malignant neoplasms of chickens (Fadly and Payne, 2003).

The most common of these neoplastic diseases seen in field flocks is lymphoid leukosis. Diagnosis of lymphoid leukosis depends on isolation and characterization of the etiologic virus, clinical signs, and pathobiology and epizootiology of the disease (Fadly and Payne, 2003). To the best of our knowledge no study has been done to evaluate the cytology of bone marrow in cases of lymphoid leukosis. The purpose of this study was to evaluate the cytology of the bone marrow and the peripheral blood hemogram of laying hens affected with lymphoid leukosis. It was thought that results of such study may prove to be useful in the early detection of the disease.

Materials and Methods

The Laying Hens:

Twenty apparently normal adult laying hens were bought from the local market and used as a control group. Fifteen adult laying hens affected with lymphoid leukosis were obtained from several poultry flocks after close inspection of these flocks for 3 months. Diagnosis of lymphoid leukosis was done according to clinical history, signs and symptoms, and the gross and microscopic pathology. Among the organs that were examined histopathologically were the liver, spleen, bursa of Fabricius, lungs, kidneys, heart, and reproductive organs. Only histologically confirmed cases were included in the study.

Collection of Blood Samples:

Blood samples (2ml) were collected through venipuncture of brachial vein into a micrcollection tube containing the anticoagulant EDTA (ethylenediamine-tertraacetic acid). To avoid hemolysis, the needle was removed from the syringe before transferring blood to the vial containing the anticoagulant. Additionally, the blood and the anticoagulant were mixed adequately by inverting the vial several times. The blood samples were processed shortly after collection and when this was not possible the samples were stored in a refrigerator at 4°C until use (Coles, 1980).

Bone Marrow Collection :

Marrow samples for cytologic evaluation was obtained through bone marrow aspiration and through direct sampling following necropsy. The source of bone marrow was the proximal tibiotarsus and a Jamshidi bone marrow biopsy - aspiration needle was used for marrow collection. The procedure involved application of a skin disinfectant, as for any surgical procedure and to facilitate passage of the needle through the skin, a small skin incision was made on the medial aspect of the proximal tibiotarsus just below femoral - tibiotarsal joint. The needle with stylet is placed against the bone and using gentle pressure and rotary movements the needle was advanced into the marrow cavity. The stylet was then removed and a 6 to 12 ml syringe was attached and the marrow was aspirated into the lumen of the needle by applying negative pressure to the syringe using the syringe plunger. After that the needle was removed from the syringe and the marrow was forced from the lumen onto a glass microscope slide. Another slide was palced atop of the marrow sample, and the marrow was allowed to spread between the two slides as they are pulled apart. Marrow samples were also obtained at necropsy where the proximal tibiotarsus bone was opened longitudinally and the marrow was exposed. Imprint films were made from the marrow samples and the samples were placed in holders and fixed in 10% neutral buffered formalin.

Hematological and Cytological Stains :

Blood smears , marrow smears, and imprint films of bone marrow were stained with Giemsa , Wright's and the new methylene blue stains (Campbell, 1995).

Histological Examination :

Following fixation of the marrow samples in 10% neutral–formalin for 48 hours the samples were washed, dehydrated, cleared in xylol , embedded in paraffin wax , sectioned at 4-5 mm thickness , stained with hematoxylin and eosin and examined with a light microscope.

Statistical Analysis :

Variations in the means of each trait was analysed using the student's – t – test at a p value of < 0.01 .

Results

Examination of blood smears prepared from the peripheral blood of hens with lymphoid leukosis revealed the presence of a few (5-10%) toxic heterophils and the degree of heterophil toxicity was a + 2 toxicity on a scale of + 1 to + 4. In this degree the heterophils have deeper cytoplasmic basophilia and partial degranulation. Another finding was the presence of large numbers of reactive (blast–transformed) lymphocytes. These cells were characterized by having heavily clumped nuclear chromatin and deeply basophilic cytoplasm. Examination of smears prepared from the marrow of hens with lymphoid leukosis indicated an increased numbers of hemopoietic cells on behalf of the adipocytes.

A comparison between the hemogram of apparently normal laying hens (n=20) and that of laying hens with lymphoid leukosis (n=15) is presented in table (1). From data presented in the table it was clear that the total erythrocyte count, percentage of hemoglobin , hemoglobin concentration, the packed cell volume , and the mean corpuscular hemoglobin concentration values were significantly higher ($p<0.01$) in hens with lymphoid leukosis than in apparently normal hens. The mean corpuscular volume and the mean corpuscular hemoglobin values were significantly lower ($p < 0.01$) in hens with lymphoid leukosis than in apparently normal hens. A comparison

Table-1. A comparison between the hemogram and leukogram in apparentty normal hens (n=20) and hens with lymphoid leukosis (n=15)

Groups of Hens	TEC (erythrocyte/ microliter)	Hb (gm/dl)	PCV(%)	MCV (fl)	MCH (pg)	MCHC (gm/dl)
Apparently Normal Hens	3,465,050 ± 225,792a	7.77 ± 0.21a	28.7 ±0.36a	96.58 ± 7.37a	26.76±1.74a	28.52 ± 0.84 a
Hens with lymphoid leukosis	8,035,000 ± 242,000 b	10.71± 0.08b	35.0 ± 0.3b	46.19 ± 1.39b	13.98 ± 0.43b	30.73 ± 0.38b
Groups of Hens	**TLC (leukocyte /microliter)**	**Heterophils (%)**	**Lymphocytes(%)**	**Basophils (%)**	**Monocytes (%)**	**Eosinophils (%)**
Apparently Normal Hens	22,773 ± 3440a	27.4 ± 1.54a	60.05 ± 1.38a	1.75 ± 0.29a	3.10 ± 0.52a	2.15 ± 0.21 a
Hens with lymphoid leukosis	55,341± 1513 b	19.33 ±0.86b	80.20 ± 0.83b	0.00 ± 0.00b	0.40 ± 0.19 b	0.00 ± 0.00 b

Different letters in the vertical columns mean presence of statistically significant difference (P<0.01).

between leukogram of apparently normal hens and those with lymphoid leukosis is also presented in table (1). From these data it became clear that the total leukocyte count and the percentage of lymphocytes were significantly higher ($p<0.01$) in hens with lymphoid leukosis than in apparently normal hens. The percentages of heterophils , basophils , monocytes , and eosinophils were significantly lower ($p<0.01$) in hens with lymphoid leukosis than in apparently normal hens.

Discussion

This study aimed to evaluate the bone marrow of laying hens affected with lymphoid leukosis in an attempt to find a useful, simple , and rapid method of diagnosis of the disease and to elucidate the changes that occur in the bone marrow and the peripheral blood of affected birds . In the present study ,a few + 2 toxic heterophils were found in the peripheral blood of hens with lymphoid leukosis. Toxic heterophils have associated with severe systemic illnesses such as septicemia, viremia, chlamydiosis, mycotic infections , and severe tissue necrosis (Thrall et al , 2004). Another finding was the occurrence of increased numbers of reactive lymphocytes. These cells have been mentioned to result from transformation of lymphocytes in the presence of antigenic stimulation and their presence in the peripheral blood has been said to be indicative of immune response (Compbell , 1995) . It has been also reported that these cells rarely predominate in frank leukemic cases in birds (Fadly and Payne, 2003). In the present study , examination of the marrow smears revealed an increase in the number of hemopoietic cells. In Marek's disease, variable bone marrow changes have been described including the occurrence of multiple tumor nodules or aplasia or changes were not observed (Witter and Schat, 2003).

In general, both the hemogram and the leukogram were significantly higher ($P < 0.01$) in hens with lymphoid leukosis than in apparently normal hens . Lymphocytosis was found to be the cause of the higher count of leukocytes. Lymphocytosis can occur with antigenic stimulation and with lymphocytic leukemia (e.g.,avian leukosis) (Thrall et al., 2004). In some cases of lymphocytic leukemia immature lymphocytes may be present in the blood film. It has been found that a marked lymphocytosis in which most lymphocytes appear as small, mature lymphocytes with scalloped cytoplasmic margins is associated with lymphoid neoplasia (Campbell , 1995).

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