

SEQUENTIAL HISTOPATHOLOGICAL CHANGES IN EXPERIMENTAL REPRODUCTION OF CHICKEN INFECTIOUS ANEMIA

N. Tanuja, B. Sreedevi* and Ch. Srilatha

Department of Veterinary Epidemiology and Preventive Medicine
College of Veterinary Science, Tirupati – 517 502, Andhra Pradesh
E-mail: bollinisreedevi@rediffmail.com (**Corresponding author*)

Abstract: An outbreak suspected of Chicken Infectious Anaemia (CIA) was observed in one of the organized poultry farms in Tirupati. Tissue suspensions of bone marrow, thymus and bursa were prepared and used for experimental reproduction of the disease in one-day-old chicks. The sequential histopathological studies revealed characteristic changes in bone marrow, thymus, bursa, spleen and liver of experimentally infected chicks. Severe degenerative changes were observed in bone marrow followed by thymus with depletion of lymphoid follicles suggesting the severe anaemia and immunosuppression caused by the disease.

Keywords: Chicken infectious anaemia, experimental infection, histopathology

INTRODUCTION

Chicken infectious anemia virus is an economically important pathogen that has been commonly identified in chicken populations worldwide since it was first discovered in Japan in 1979 from contaminated vaccines. CIAV can cause the atrophy of bone marrow hematopoietic tissue and lymphatic tissues like thymus in young chickens, leading to anemia and immune suppression. Chicken Infectious Anaemia is manifested both in clinical and subclinical forms and can be spread by both horizontal and vertical transmission. Chicken Infectious Anemia is one of the emerging diseases of young chicken in Indian poultry farms (Tanuja et al., 2008, Wani et al., 2014). It has been responsible for considerable health problems and economic losses to the poultry industry. The present study was undertaken up to record the sequential histopathological changes in different organs in experimental infection with chicken infectious anaemia virus day old chicken.

MATERIALS AND METHODS

Preparation of Inoculum: An outbreak suspected of chicken infectious anemia was observed in one of the organized layer poultry farm (flock size of about 23,000) in Tirupati. The birds showed signs of weakness, emaciation and paleness of carcass. Based on clinical and hematological findings, the disease was suspected as chicken infectious anemia (Tanuja

et al., 2007). Hence, tissues like bone marrow, thymus, bursa, liver and spleen were collected in 50% glycerol saline and the same were used for experimental reproduction of the disease in one - day old chicks.

The pooled clinical field samples i.e. liver, spleen, bone marrow, thymus and bursa (weighing 2 gms) were taken in sterilized mortar and triturated using pestle by adding sterile glass wool. The material was made into a homogenous 20% suspension by adding phosphate buffered saline, centrifuged and the supernatant was filtered and used as inoculum.

Chicks: Day old white leghorn male layer chicks from a single hatch were procured from M/s Balaji Hatcheries Pvt. Ltd., Chittoor. The chicks were maintained in the experimental animal house of the Department of Pathology, College of Veterinary Science, Tirupati with standard managemental and feeding practices throughout the period of experiment. The control chicks were maintained in the Department of Epidemiology and Preventive medicine, College of Veterinary Science, Tirupati under similar conditions.

Experimental Design: 210-day-old chicks were divided into three groups, each containing 70 chicks. Group I chicks were inoculated with 0.1ml of inoculum and 0.005mg of betamethasone. Group II chicks were inoculated with 0.1ml of inoculum alone. Group III chicks were inoculated with 0.1ml of distilled water, and treated as controls.

During this experiment, six birds from each group were sacrificed on 2nd, 4th, 6th, 8th, 10th, 12th, 14th, 18th, 21st, 28th and 30th days post inoculation and observed for gross pathological changes. Tissues like bone marrow, thymus, bursa Fabricius, liver and spleen were also collected and fixed in 10% neutral buffered formalin for histopathological studies. They were dehydrated in different - grades of alcohols, cleared with xylene, infiltrated and embedded in paraffin. Embedded tissues were sectioned at four to six micrometers thickness. The sections were stained with hematoxylin and eosin (H & E staining). Tissue sections were coded before microscopic evaluation to eliminate examiner bias. The different changes noticed in the tissues were recorded.

RESULTS AND DISCUSSION

The sequential histopathological changes were studied in different organs viz., bone marrow, thymus, bursa, liver and spleen in experimentally affected chicken in different groups. The chicks in group I which were inoculated with the positive material along with the betamethasone showed the following changes.

Bone marrow: There were no histological changes detected in the bone marrow upto 4 days PI. At 6 days PI, in most of the inoculated birds, large degenerated cells were present in the

periphery of many tissues. At 8 days PI; there was diffuse, moderate cellular depletion of bone marrow. Between 10-16 days PI; there was severe depletion of erythroid and myeloid cells in the bone marrow, which were occupied by adipose tissue (Fig.1). Between 20-28 days PI, the marrow was hyper cellular with evidence of extensive hemopoetic activity and many immature erythrocytes were present. Between 28-31 days post infection, abundant mature erythrocytes were present in sinuses.

Thymus: There were no histological changes in thymus before 4 days PI. At 4th day PI, there was enlargement of lymphoblasts focally in the outer cortex of the thymus in inoculated birds. At 5-6 days PI; there was mild lymphocyte depletion of the cortex in some birds. At 7-8 days PI, there was marked lymphocyte depletion of the cortex, only a few islands of mature lymphocytes were observed.

Between 10-16 days PI, there was severe lymphoid depletion of the cortex and the cortex was more closely resembled the medulla (Fig.2). Very few mature lymphocytes were present. At 18th and 20th day PI; there was evidence of high mitotic activity in the outer cortex. Progressively more lymphocytes were present in the cortex between 18 to 26 days PI. Thereafter thymus structure appeared normal.

Bursa: There was no histological change up to 6-8 days of post infection (PI). At 8 days PI, mild depletion of lymphoid follicles was observed. At 12 days PI, there was thinning of cortex, lymphocytolysis and diffuse depletion of lymphoid follicles (Fig.3). In addition to these changes many epithelial crypts were found in the bursal plicae, presence of cysts in lining epithelium, both interfollicular and intrafollicular cystic spaces were observed. Between 14-18 days PI, medullary lymphocytosis was prominent.

Between 20-24 days PI, prominent washed out appearance of medullary portion of the bursa and depletion in lining epithelium and lymphoid follicles were observed. At 24th day PI, there was high mitotic activity in the cortex and medulla. Progressively more lymphocytes were found between 24 to 28 days PI. Thereafter it appeared to be normal.

Spleen: There was no histological change up to 8 days PI. At 8th day PI, there was mild lymphoid depletion in affected chicks. Between 10-14 days post infection, there was moderate to severe lymphoid depletion of spleen in inoculated birds. Between 14-22 days post infection, splenic hemorrhages and mild lymphoid depletion was detected in affected chicks. Between 24-28 days PI; there was regeneration of lymphoid follicles with mild hemorrhages in the affected chicks. Later the histological appearance of the spleen was found to be normal.

Liver: There was no histological change up to 8 days PI in infected chicks. At 8 days PI, sinusoidal hemorrhages and mild degenerative changes were detected in affected chicks. At 12 days PI, sinusoidal dilatation was detected. Between 14-18 days PI, mild degenerative changes with slight hemorrhages were observed.

Between 18-22 days PI; diffuse sinusoidal hemorrhages were detected in inoculated chicks. Between 22-28 days PI, regenerative changes with mild hemorrhages were noticed. By 28-30 days PI, the histological appearance of liver was found to be normal.

In-group II infected chicks, which were inoculated with the clinical material alone also revealed similar histopathological changes in different organs but with lesser intensity. In group I the lesions were comparatively more severe than in group II and the reason might be due to the immunosuppressive effect of the betamethasone. Bulow *et al.* (1987) also reported that chemical immuno-suppression by betamethasone aggravates the signs and lesions of CIA. The control chicks in group III revealed normal appearance of different organs in histopathology.

All these histological changes detected in different tissues viz. bone marrow, thymus, bursa, spleen and liver were in agreement with results of Yuasa *et al.* (1979) and Taniguchi *et al.* (1983) who reported that, hypoplasia and subsequently aplasia occurred all over the bone marrow 8 days after inoculation and the bone marrow returned to normal condition 32 days after inoculation or later. In the thymus of affected birds, the depletion of cortical lymphocytes was observed after 8 days PI. They concluded that, in chicks inoculated with CAA, bone marrow, thymus and bursa were affected at higher frequency followed by mild changes in spleen and liver. Similar histopathological changes were also reported by Jeurissen *et al.* 1992, Smyth *et al.* 1993 and Saini *et al.*, 2009.

In the present study it is concluded that, the incidence of microscopic lesions was high in bone marrow and lymphoid organs in the chicks infected with chicken anaemia virus. The degeneration and atrophy of primary lymphoid organs such as thymus and bursa might be one of the reasons for the immuno-suppression induced by CIAV.

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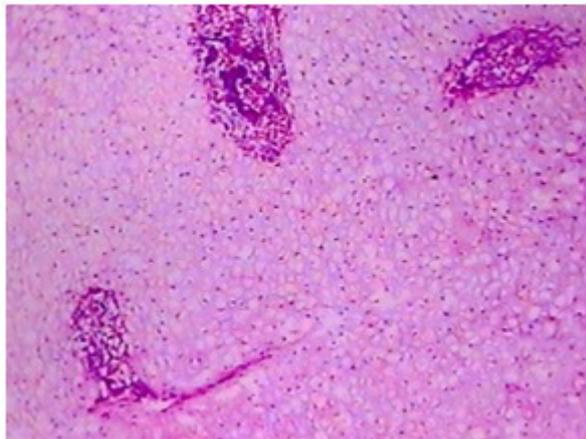


Fig 1: Histopathology of bone marrow of infected chicks showing hypoplasia of bone marrow with focal aggregation of mononuclear cells; H & E x70

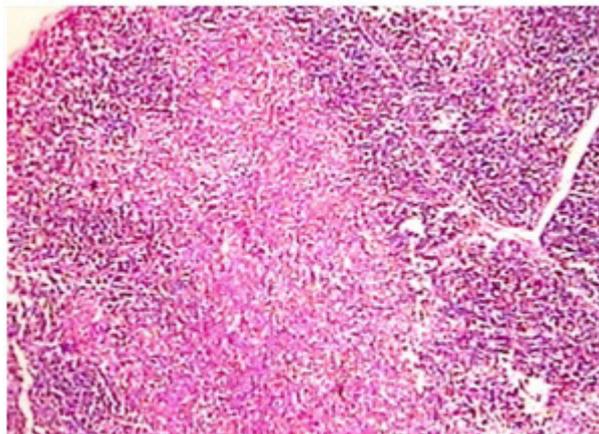


Fig 2: Histopathology of thymus of infected chicks showing depletion of thymic lymphocytes and lack of differentiation between cortex and medulla; H & E x70

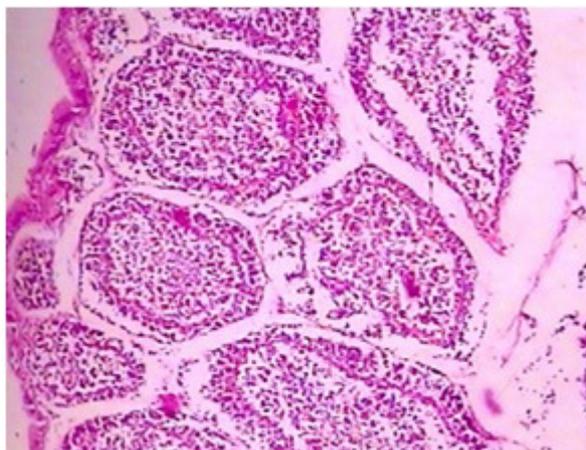


Fig 3: Histopathology of bursa Fabricius of infected chicks showing depletion of bursal follicles with prominence of corticomedullary junction; H & E x70