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Microscopic Appearance of the Cloacal Bursa in Broiler Chickens During Infectious Bursal Disease in Algeria

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Abstract

Algeria has recently witnessed an unprecedented spread out of viral infections, among which Gumboro disease (IBD) ; Clinically characterized by dullness, depression, anorexia, white watery diarrhoea, ruffled feathers, soiled vent, severe prostration, vent picking and subnormal temperature followed by death ;Twenty-two 22 to 29 days-old broilers clinically suffering from Gumboro disease (IBD) were investigated histopathologicaly in order to evaluate the mechanism of IBD outbreak in vaccinated broiler chickens. The histological aspects of the bursa were observed using an optical microscope after being passed through different phases; Formalin fixing, ethanol dehydrating, paraffin soaking, microtome cutting, and finally Haematoxylin and eosin staining.

The Microscopic lesions of the cloacal bursa were mainly illustrated by severe depletion, degeneration and necrosis of lymphocytes in the medulla of many bursal follicles , accompanied by inflammation and fibrous connective proliferation with mild lymphocytic infiltration in the inter follicular areas.

Keywords: IBD ; Gumboro disease ; Microscopic appearance ; Broiler chickens ; Cloacal bursa.

INTRODUCTION

Algerian poultry production is getting highly important and much wider day by day in order to satisfy the rapidly-increasing demand for animal protein. However, it seems that the development of serious fatal diseases of different causes is still away of being got-rid of in poultry farms. These diseases strike negatively the national and global economy by the decrease in productivity they entail, the intervention costs of public and private veterinary staff, and sometimes the 100% losses [1].

One of these diseases is Gumboro disease or Infectious Bursal Disease, which is caused by a birnaviridae virus. This disease mainly affects birds at 3-6 weeks of age and has an affinity to the bursa of Fabricius where the virus infects actively differentiating and dividing B-lymphocytes [2, 3].

There are actually 03 well-characterized antigenic types; These are standard (often called classic), variant serotype 1 and serotype 2 viruses. Subtypes of these antigenic types have also been distinguished. The vvIBDV strains are antigenically similar to the standard serotype 1 [4, 5, 6, 7, 8], they were first reported in Europe [9]. Sequencing studies approved several amino acid changes in “VP2 hydrophilic peaks” that correlated with the antigenic changes identified in the variant viruses. [10, 11, 12].

The IBD is clinically characterized by dullness, depression, anorexia, white watery diarrhoea, ruffled feathers, soiled vent, , severe prostration, vent picking and subnormal temperature followed by death [13]. Despite the widespread use of vaccines, several sporadic outbreaks are still observed; In terms of public health, along with its contribution to malnutrition (by limiting access to animal proteins).

The aim of our study was to investigate and to highlight the histological appearance of the bursa of Fabricius in broiler chickens during Infectious bursal disease in Algeria.

MATERIAL AND METHODS

The study of the impact of Gumboro disease on the chickens cloacal bursa was conducted in 04 poultry farms located in the eastern area of Algeria (Annaba, Batna, Mila, and Skikda) These poultry farms contains approximately 5000 chicken flock per coop. All of these coops were vaccinated by CEVA® IBD L in the fourteenth day.

Twenty-two 22 - to 29 day-old broilers with Gumboro disease (IBD) were investigated histopathologicaly in order to evaluate the mechanism of IBD outbreak in vaccinated chickens . The broilers were vaccinated with IBD live vaccine (CEVA® IBD L) via drinking water ; By means of a combination of characteristic clinical symptoms and lesions found at post-mortem examination 95 broilers with pathognomonic lesions of IBD and 10 healthy birds were taken from this affected chicken coops to histopathological laboratory of Souk ahras veterinary institute.

Representative and appropriate bursa of Fabricius showing a typically post-mortem pathognomonic lesions were collected from chickens and fixed in 10% formalin for 24 hours, then underwent successive passes through different compartments, dehydrated in increasing concentration level of ethanol, then cleared in xylene and finally soaked in paraffin [14, 15, 16, 17].

The section were cut at the thickness of 3-4 µ and stained with H & E stain , in order to realise the histopathological studies [18, 19]. The bursa's histological structures were observed using an optical microscope (low and high magnification).

RESULTS AND DISCUSSION

The bursa is composed of longitudinal folds, each containing many follicles, Histological examination of cloacal bursa sections from clinically healthy chickens, stained with hematoxylin and eosin (H&E), revealed the normal structure and architecture of the bursa. The plicae (fold) contain tighly packed lymphoid follicles separated by connective tissue (ct) and are covered on the luminal suface by pseudo-stratified columnar epithelium. The bursal lobule or bursal lymphoid follicle has a cortex (c) and medulla (m) that are separated by a basement membrane, a layer of stellate reticular epithelial cells and blood capillaries, a follicle is seen at higher magnification ,fine connective tissue (ct) separates the central follicle from those on the other side. The cortex is composed mainly of small lymphocytes; the medullary lymphocytes are less tightly packed and are intermingled with macrophages and reticular epithelial cells. A thin layer (e) of large capillaries and undifferentiated epithelium that is continuous with the epithelium on the plical surface separate the cortex from the medulla. (Fig 1)

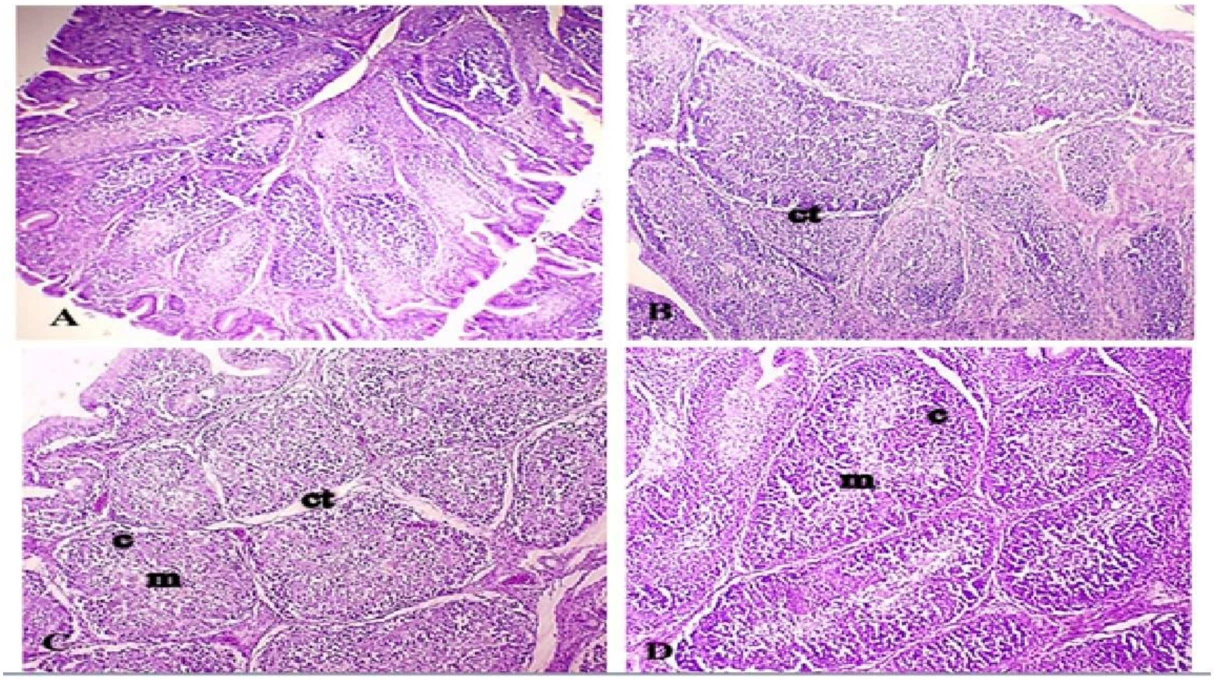

Fig 1. Histological section of healthy broiler chicken cloacal bursa tissue.

A: The cloacal bursa is composed of many folds or plicae, one of the folds is shown (H&E).
B, C: The plicae contains tightly packed lymphoid follicles separated by a connective tissue (ct), and covered on the luminal surface by pseudostratified columnar epithelium (H&E × 400).
D: Bursal lymphoid follicle with a dark cortex (c) and clear medulla (m) (H&E × 400).

The pathological stress discussed in this study is Gumboro disease, the bursa is the targeted organ (exactly the B lymphocytes at an immature stage); the trophic aspect mentioned an enlargement of the liver in some carcasses. In almost all IBD-affected broilers, the post-mortem lesions were noticed in bursa of Fabricius. Haemorrhages on thigh and pectoral muscles, oedematous bursa , congestion and haemorrhages in bursal follicles were also noticed during post-mortem examination of broiler chickens ; Nicolas Eterradossi and Yehia M. Saif mentioned that the increase in bursal weight seen was caused by severe edema, hyperemia, and marked accumulation of heterophils [20].(Fig 2).

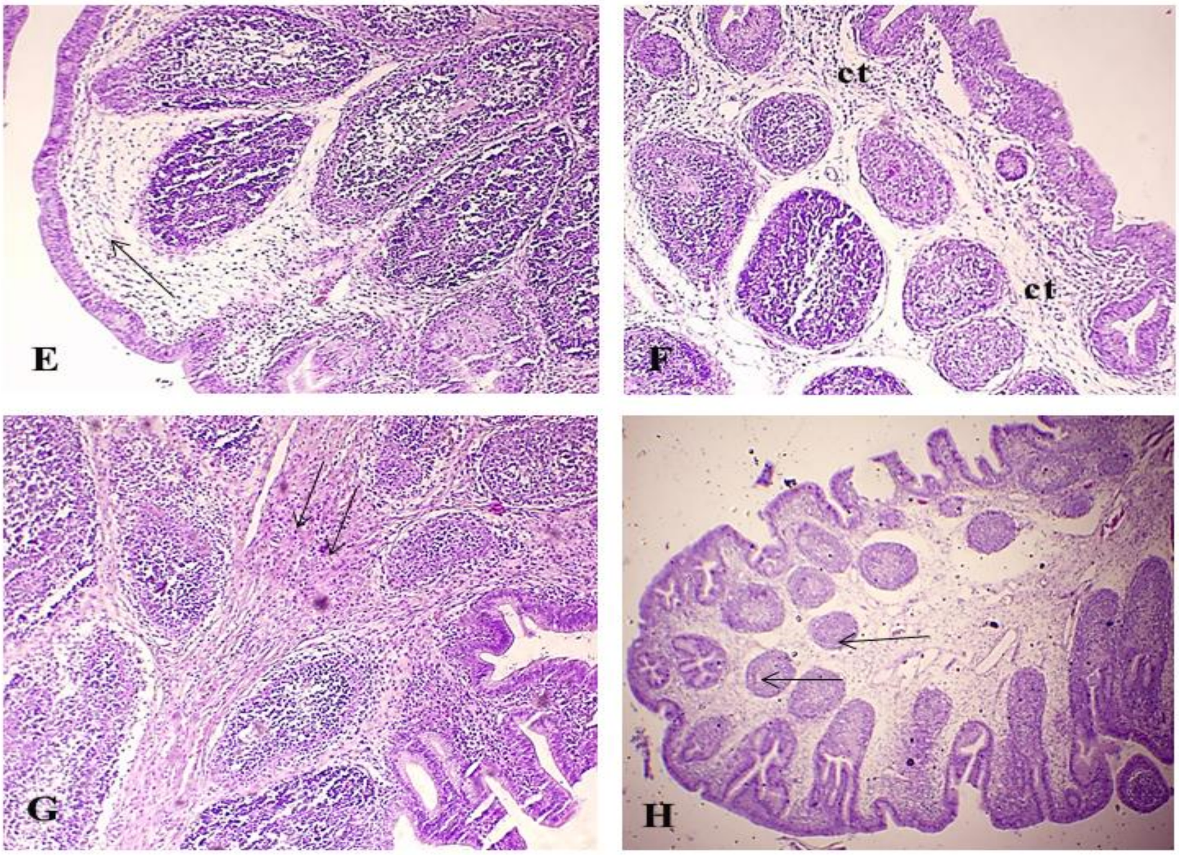

Fig2. Photomicroraphs showing histopathological lesions in the cloacal bursa in Gumboro disease affected broiler chickens.
E,F: Depletion of lymphocytes in bursal follicles and fibrous connective tissue (ct) proliferation with mild lymphocytic infiltration in interfollicular areas ,the interfollicular tissue is oedematous and inflamed (arrow) (H&E × 100)
G: Congestion and marked haemorrhages (arrow) in bursa of fabricius (H&E × 400)
H: Atrophy of bursal follicles (arrow) (H&E × 40).

The Microscopic changes and lesions in the cloacal bursa appear in 72 hours post infection ; They were mainly illustrated by : depletion , degeneration , and necrosis of numerous lymphocytes in the medulla in many bursal follicles accompanied by fibrous connective proliferation with mild lymphocytic infiltration in inter follicular areas [21].One of the Atrophic changes at this microscopic level, which were in consonance with gross observations, is The cystic changes in which there was formation of cystic spaces observed in bursal follicles.Similar pathological findings were reported by Hoque et *al*. (2001) [22], Rudd et *al*. (2001) [23], Islam et *al*. (2008) [24] and Singh et *al*. (2015) [25].

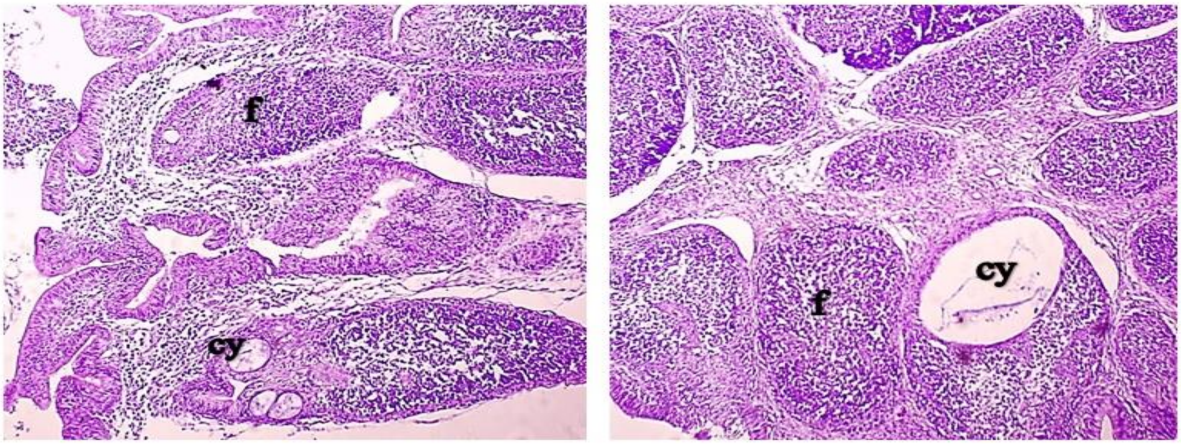

Fig 3. Cystic areas with accumulated necrotic debris in bursal follicle (f) and fibrous connective proliferation with mild lymphocytic infiltration (H&E × 100).
I, J: Virulent strains of the virus injure most of the follicles .a proteineous fluid is present in the medullary area of several follicles and in one a cyst (cy) has formed (H&E × 100).

The reversibility of these histological lesions of the cloacal bursae depends on the importance of the destruction of the reticulo-histiocytic system [26]. In chickens, B cells develop and differentiate in the bursa of Fabricius (BF), Each follicle is filled with B cells, and as in the thymus, the lymphocytes are organised into peripheral cortex and central medulla. Besides B cells, the cloacal bursa contains some T cells, macrophages, dendritic cells, plasma cells and reticulocytes. The presence of T cells and plasma cells show that although the cloacal bursa is a primary lymphoid organ, it can also trap antigen and undertake some limited antibody release, probably as a self-protection measure. The bursa of Fabricius produces a few hormones like bursin, a tripeptide that has a regulatory role in B cells differentiation and development [27, 28, 29, 30, 31].

Two different types of bursal follicles were then observed : the first ones are large functional reconstituted follicles, most likely reconstituted from endogenous bursal cells that survived virus infection ; The others are tiny, poorly developed follicles lacking a noticeable cortex and medulla. These small follicles are unable to produce functional peripheral B cells, this hypothesis is confirmed by the lack of active antibody responses in chickens lacking large reconstituted follicles [32].

CONCLUSION

From the present study, it can be concluded that outbreaks of IBD occur in broiler chicken flocks in many Algerian states. The Gumboro disease can be diagnosed on the basis of clinical signs, gross lesions and histopathological studies. Microscopically there was a lot of anomalies; follicles depletion and palor with an intrafollicular loss of cells and inflammation, oedematous interfollicular tissue which cause an increase in bursal weight accompanied sometimes with cystic changes in which there was formation of cystic spaces observed in bursal follicles, in addition the virulent infection produced haemorrhages in some cases. It is primordial to follow biosecurity measures and vaccinate chickens regularly with appropriate strain of vaccine. Finally, regular surveillance and identifications of field strains would help in making effective protecting strategies. These findings provided important insights into the microscopic progression of this disease..

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