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*Short Communication* / **Kısa Bilimsel Çalışma**

# Diagnosis of infectious bursal disease by immunoperoxidase technique*

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**Summary:** The diagnosis of infectious bursal disease of naturally infected 21-day-old chickens was investigated with immunoperoxidase method. Viral antigens were observed in the macrophages and lymphocytes of the bursa of Fabricius. It was observed that the method was sensitive and specific and it could be used in the diagnosis of this disease.

Key words: Immunohistochemistry, infectious bursal disease, polyclonal antibody,

## İnfeksiyöz bursal hastalığın immunoperoksidaz tekniği ile tanısı

**Özet:** İnfeksiyöz bursal hastalığın tanısı, immunoperoksidaz metodu kullanılarak doğal olarak infekte 21 günlük civcivlerde incelendi. Viral antijenler bursa Fabricius'daki makrofaj ve lenfositlerde gözlendi. Çalışmada kullanılan metodun duyarlı ve spesifik olduğu ve hastalığın tanısında kullanılabileceği saptandı.

Anahtar sözcükler: İmmunohistokimya, infeksiyöz bursal hastalık, poliklonal antikor.

Infectious bursal disease (IBD) virus is the causative agent of a highly contagious disease of young chickens. The disease is widespread in chickens and has a great economic importance for both broiler and pullet growers (4,6,7). IBD is normally diagnosed in veterinary laboratory by the isolation of causative virus in eggs and/or cell cultures and demonstration of bursal lesions. However, these procedures can be relatively time consuming and some strains either can not adapt themselves or not show characteristic changes on embryos. Therefore, diagnosis of the disease might be difficult and demonstration of viral antigen by immunoperoxidase (IP) method useful for differential diagnosis and confirmation of histopathological changes (2,3,5).

Present study describes detection of IBD virus in the bursa of Fabricius (bF) within naturally infected 21 days old chickens by the indirect IP method using polyclonal antibodies.

Twenty one days old 30 chickens showing clinical signs of IBD virus infection were collected from commercial flocks. All chickens were euthanasied by cervical dislocation and systemically necropsied. Tissue specimens were collected from bF, spleen, liver, cecal tonsil, kidney, lung, heart and brain, than fixed in buffered formalin and processed for paraffin embedding. Two sections were cut from each block, one for IP method and the other for hematoxyline and eosin staining. The control group was sampled at the same intervals and included 30 specific pathogen free chickens.

For the IP method, formalin fixed paraffin embedded tissue sections from field cases and SPF chickens were deparaffinized and hydrated. Hydrated samples were reacted with 3% hydrogen peroxide in methanol for 30 minutes at room temperature to quench endogenous peroxidase activity and blocked with normal goat serum for 30 minutes. Serum was blotted and slides were incubated with polyclonal chicken anti serum to IBD virus (1/100) for 30 minutes in a humidified chamber. The slides were incubated with peroxidase labelled rabbit anti-chicken IgG (1/1000 sigma) for 30 minutes and reacted for 5 minutes in DAB, counterstained with hematoxyline.

Macroscopically, no significant gross lesions were observed in organs except bF. The basic finding was a slight increase in the weight of bF (bF mean weight was 3,1 gr and 2,8 gr, in naturally infected chickens and con-

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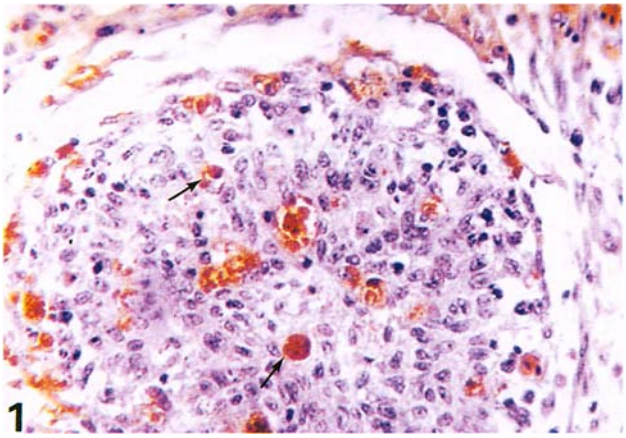

Figure 1. Immunopositive cells in the bursa of Fabricius (arrows). Biotin-streptavidin/DAB immunohistochemistry, counterstain haemotoxylin, x 240.
Şekil 1. Bursa Fabricius'ta immunopositif hücreler (oklar). Biotin-streptavidin/DAB immunohistokimya, hematoksilen karşıt boyama, x240.

trol group, respectively). Microscopically, the main pathological alterations were degenerative and multifocal necrotic changes in the lymphocytes belonging to the medullar zone of bF. In the interstitial tissue, acute inflammation characterised by heterophil accumulation and edema was observed. Cystic cavities were found in the medulla of some follicles. In control chickens, no macroscopic and microscopic lesions were seen bF or the other organs.

Immunohistochemically, IBD virus antigens were detected in lymphoid cells in the cortex and medulla of lymphoid follicles of the bF (Figure 1). Strong stain intensity and great numbers of positive cells were observed. In addition of these findings, antigens were found mainly in macrophages within all follicles and the interstitium. No antigen was observed in the other organs and the control chickens.

In the experimental studies, IBD virus antigens have been detected in macrophages within follicles, the interstitium and the lymphoid cells of bF (1,6,8). This antigen localisation was confirmed by our study. IBD virus antigens have also been observed in lymphocytes of spleen, thymus and cecal tonsil (8). However, in the present study, antigens could not been observed in these organs of naturally infected chickens. This may be due to the field cases of IBD virus have a high virulence and/or acute infections.

It has been recorded that the indirect IP method with polyclonal antibodies were used for detecting viral antigens in the bursae of chickens experimentally infected with IBD virus (6). These method findings were confirmed by the present study in naturally infected chickens.

The protocol used in this work was verified high sensitivity of the IP test. It can be performed very rapidly, a large number of specimens can be processed quickly. Moreover, subclinical infection of IBD may be diagnosed by this technique.

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