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ISOLATION OF INFECTIOUS BURSAL DISEASE VIRUS AND IMMUNO HISTOCHEMSTRY OF CD4$^+$ AND CD8$^+$ FOR INFECTED IRAQI CHICKENS

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ABSTRACT : In this study, sixty samples (bursa of fabricous) were collected from different areas in Iraq. The age of chicks ranged (25-35) days. Most samples were collected from broilers. Real-Time reverse transcriptase polymerase chain reaction (RT-PCR) technique was conducted for collected samples to detect Infectious bursal disease virus (IBDV) virus by amplification of VP2 gene. Nineteen samples out of sixty were positive using Real-Time RT-PCR technique.After preparation of positive samples, these samples were inoculated in chicken embryos by charioallantoic membrane (CAM) method inoculation for two passages, Two passages of charioallantoic membrane was propagated in chicken embryo fibroblast cell culture to detect the cytopathic effect of virus on cell culture. The cytopathic effects (CPE) were rounded cells with holes and degeneration of infected cells compared with non-infected cells. The results of histopathological examinationwere conducted for infected bursa of fabricius. The histopathological examination were indicated many changes including inter and intra hemorrhage, mononuclear leukocytes infiltration and necrosis, peripheral hetrophil infiltration, epithelial hyperplasia, vacuolar degeneration, fibroplasias, lymphoid depletions and medullary cystic formation. These changes were comprised to non-infected bursa of fabricius. As a result of the immunohistochemistry of CD4$^+$and CD8$^+$ revealed the significant increase in CD4$^+$ and CD8$^+$ expression in bursal tissue than in uninfected bursal tissueand noticed the CD4$^+$ at the boundary of follicle but the CD8$^+$ were noticed through the follicle of bursa of fabricius.

Key words : Infectious bursal disease virus, RT-real time PCR, isolation, histopathology of bursa of fabricius, immunohistochestry.

INTRODUCTION

Infectious bursal disease (IBD) known as Gumboro disease, is a highly contagious disease in young chickens also is caused by infectious bursal disease virus (IBDV).The virus leads to severe immunosuppression due to loss the function of Bursa of Fabricius (BF) (Caston *et al,* 2008) therefore leads to an increased susceptibility to other pathogens which resulting in greater mortality (Bublot *et al,* 2007; Mahgoub, 2012). IBDV belongs to the genus *Avibirnavirus* of the *Birnaviridae* family and its viral genome is bisegmented of double stranded RNA (Dobos *et al,* 1995).

The IBDV has two serotypes including serotype1 and serotype 2 (Rekha *et al,* 2014). The serotype 1 of IBDVis pathogenic to chickens (Oladele *et al,* 2009) and classified as classical IBDV (cIBDV), variant IBDV (vIBDV), attenuated IBDV (atIBDV) and very virulent IBDV (vvIBDV)(Juneja *et al,* 2008). But the serotype 2 is avirulent (nonpathogenic strain) for chickens (Muller *et al,* 2003). As well as,IBDV has VP2 protein on their surface which requires a cellular receptor to penetrate target cells to cause infection. The cellular receptor mainly distribution on the target cells and the tissue specificity thereby the site of pathological changes are associated with infection (Haywood, 1994). Chicken B lymphocytes are the primary target for pathogenic stains of serotype 1 of IBDV.Therefore the infection causes a functional loss of the BFwhich leads to severe immunodepression (Rekha *et al,* 2014).

Although virulent serotype strains of IBDV are replicate efficiently in lymphoid cells of the BF in chickens. As with they are widely inoculation in embryonated chicken egg by charioallantoic membrane route (Mutinda *et al,* 2015). As well as propagated in chicken embryo fibroblasts cell culture(CEFCC)(Upadhyay *et al,* 2019). Also the immunohistochesitry staining with polyclonal or monoclonal antibodies in tissues is highly specific but its sensitivity is lower than molecular detection and it non specific follicular depletion that observed microscopically all strains of IBDV (Mutinda *et al,* 2015).

This study was aimed to detect local IBDV in Iraq by molecular diagnosis and conventional technique