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Differential Expression of Mitogen-Activated Protein Kinase Kinase 3 *(MAP2K3)* and Interleukin12B *(IL12B)* in the Bursa of Fabricius of chicken infected with Infectious Bursal Disease Virus[#]

V.V.G. Nikhil Teja[1*], Sreeja R. Nair[1], P.M. Priya[1], R. Ambily[1] and Elizabeth Kurien[2]

[1]Department of Veterinary Microbiology, [2]Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651, Kerala Veterinary and Animal Sciences University, Kerala, India.

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Abstract

Infectious Bursal Disease (IBD) caused by Infectious Bursal Disease virus (IBDV), poses a significant threat to the poultry industry, causing immunosuppression and economic losses since its discovery in 1957. To understand the host response to IBDV infection, this study examined the modulation of MAP2K3 and IL12B in chickens infected with IBDV. A local IBDV isolate propagated in embryonated chicken eggs was inoculated into 18-day-old chicken. The mRNA transcription levels of MAP2K3 and IL12B in bursal tissue were quantified by real-time quantitative polymerase chain reaction on day two post-infection and compared with those of uninfected, age-matched control birds (sham inoculated). The study revealed increased expression of the MAP2K3 and IL12B genes, indicating their role in inflammatory response during IBDV infection. Thus, the present investigation sheds light on the involvement of MAP2K3 and IL12B in the inflammatory response to IBDV infection in chickens.

Keywords: *Infectious bursal disease virus*, *immunosuppression*, *MAP2K3, IL12B, pro-inflammatory cytokine gene expression*

Infectious bursal disease virus infection causes highly contagious and immunosuppressive disease in poultry. Despite effective vaccination, sporadic outbreaks of the disease highlight the need for a further insight into the immune response of the host for the development of effective control strategies (Akhila *et al*., 2022). The involvement of *MAP2K3* in MAPK signalling pathway indicates the activation of pathways related to inflammation and immune responses (Khatri and Sharma, 2006) whereas *IL12B* upregulation suggests an early attempt by the host to enhance the pro-inflammatory environment to mount an effective immune response (Farhanah *et al*., 2018). This in turn suggests the vital role of these genes in chicken's immune response. Hence the present study was undertaken to elucidate the differential expression of *MAP2K3* and *IL12B* by real-time quantitative polymerase chain reaction (qPCR) in chicken infected with IBDV.

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**Corresponding author: nikhilteja96@gmail.com, Ph. 8790253858*

The clinical isolate IBD/8/Tr was propagated in embryonated chicken eggs and used to infect three eighteen-day-old unvaccinated crossbred Gramasree chicken *via* oculonasal route at a titre of $1x10^4EID_{50}$/mL. Three birds of the same age group sham inoculated with Phosphate Buffered Saline formed the control group. The animal experiments were carried out as per the approved study protocol of the Institutional Animal Ethics Committee (Number-328/GO/ReBt-S/Re-L/01/CPCSEA). The bursal tissue collected from both infected and control birds after sacrificing them on the 2nd day post-inoculation (dpi) was subjected to gene expression analysis by qPCR targeting the *MAP2K3* and *IL12B* genes.

Total RNA extracted from the samples by TRIzol method. The RNA extracted from bursal tissue yielded a concentration of approximately 2600 ng/µL, with a mean OD value of 2.03 at 260/280. Reverse transcription was performed using the cDNA Synthesis Kit (Origin, India) according to the manufacturer's protocol and stored at -20°C. Primers were designed targeting *MAP2K3*, *IL12B* and *GAPDH* (internal control) by using Oligo Analyser tool (IDT) as detailed in table 1. The specificity for each primer set was tested by subjecting the PCR products in two percent agarose gel electrophoresis. The amplification was carried out in CFX Opus Real Time system (Bio-Rad, USA) with the following thermal cycling conditions of an initial denaturation at 95°C for two minutes followed by 40 cycles at 95°C for 15 seconds and 60°C for 30 seconds and the final amplification was verified by melt peak analysis from 65°C to 95°C with an increase of 0.5°C per 0.05 seconds to determine the specificity of the amplifications.

Table 1. Genes, primers and size of amplicons for primers used in real-time PCR

Gene	Sequence of primer	Product size (bp)
MAP2K3	F: CTGGAGCATCTGCACAGTAAAC; R: CAAGTAGCCACTGATCCCAAAG	117
IL12B	F: AGACCCACCTCAATGTCAGTAT; R: GACCCTGAAAGTCAAAGGGAAG	106
GAPDH	F: GCCATTCCTCCACCTTTGATG; R: CACGGTTGCTGTATCCAAACTC	102

The analysis revealed an upregulation in the expression of the genes *MAP2K3* and *IL12B* on second dpi. The data were represented as mean ± standard error of the mean (Table 2) following statistical analysis using an independent T-Test in the software SPSS (V 24). Following normalisation with the reference gene *GAPDH*, the fold change values were determined, indicating a 51.87-fold change for *MAP2K3* and 14.49-fold increase for *IL12B* in the infected group (Fig. 1). Liu *et al*. (2010) observed elevated *IL12B* expression following infection with very virulent IBDV on the third dpi. Farhanah *et al*. (2018), also observed upregulation of *IL12B* when infected with IBDV.

The gene *MAP2K3* is a protein kinase that is involved in the p38 mitogen-activated protein (MAP) kinase signalling pathway. Activated MAP2K3 leads to phosphorylation of p38 MAP kinase, which in turn promotes the production of pro-inflammatory cytokines such as interleukin-1 (IL-1), tumour necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) (Khatri and Sharma, 2006). These cytokines play an important role in organising the innate immune response by recruiting immune cells to the site of infection and activating antiviral effector mechanisms. The IL-12B is a subunit of IL-12, a pro-inflammatory cytokine that plays a crucial role in promoting Th1 cell responses. The IL-12B with another subunit, IL-12A initiate the differentiation of naïve T cells into T-helper 1 cells (Ullrich *et al*., 2020). T cells have been confirmed to play an important role in clearing IBDV by mounting

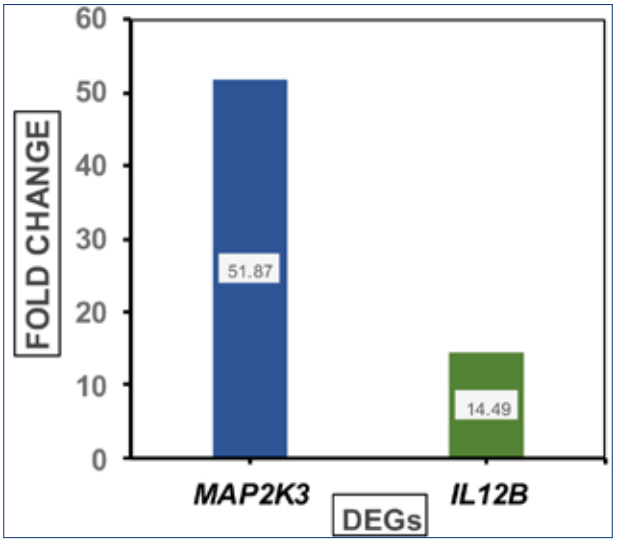

Fig.1. Fold change expression of *MAP2K3* and *IL12B*

Table 2. Relative expression of the genes selected

Gene	Sample	Mean Ct ± SE	ΔCt ± SE	ΔΔCt ± SE	Fold Change	P-value
MAP2K3	NT1 (Control)	30.94±0.11	12.83±0.47[a]	-5.62±0.57	51.87	0.00
	NT2 (Infected)	26.16±0.11	7.21±0.09[b]			
IL12B	NT1	27.54±0.07	9.43±0.38[a]	-3.83±0.36	14.49	0.00
	NT2	24.55±0.57	5.60±0.48[b]			
GAPDH	NT1	18.11±0.38				
	NT2	18.95±0.09				

an effective antiviral response. Thus, the involvement of *MAP2K3* and *IL12B* in signalling pathways and immune response makes them as potential therapeutic targets and their increased expression suggests their role in the inflammatory response during IBDV infection.

Summary

The study revealed significant upregulation of *MAP2K3* and *IL12B* in the bursae of chicken infected with IBDV. The observed upregulation of both the genes highlights the fact that the host is responding to the infection. This differential expression studies thus sheds light on the role of these genes in mediating inflammatory responses and immune modulation in the early stages of IBDV infection.

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Conflict of interest

The authors declare that they have no conflict of interest.

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