

**RESEARCH ARTICLE**

## Morphological Immunological and Genetic Studies in The Bursa of Fabricius in Nephropathogenic Infectious Bronchitis Virus -Induced Inflammation Via the MDA5/IPS-1 Signaling Axis

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**ARTICLE HISTORY (25-438)**

Received: May 06, 2025  
Revised: July 27, 2025  
Accepted: July 28, 2025  
Published online: September 18, 2025

**Key words:**

Bursa of Fabricius  
Immunity  
Inflammation  
MDA5/IPS-1  
NIBV

**ABSTRACT**

The objective of this study was to explore the mechanism by which Nephropathogenic Infectious Bronchitis Virus (NIBV) could induce chicken bursal inflammation and immune damage through the MDA5/IPS-1 signaling axis. 28 one-day old Hailan brown chicks were randomly divided into control group (Physiological saline) and diseased group (vaccination with NIBV SX9 10<sup>-5</sup> ELD<sub>50</sub>/0.2ml). Follow up experiments were conducted on 1,7,11,13, and 18 days post-infection (dpi) of virus through observation of changes in autopsy and virus copy number detection. Histopathological sections showed an increase in interstitial fibers, a reduction in follicles and an increase in necrotic lymphocytes. The levels of IgG and IgM in the diseased group's bursa of Fabricius showed a decreasing trend. At 1dpi, the mRNA levels of the MDA5/IPS-1 signaling axis and downstream I-IFN related regulatory genes (MDA5, IPS-1, TRAF3, TBK1, IRF7, IRF3) were significantly reduced, and then significantly increased. The mRNA levels of inflammatory cytokine related regulatory genes (TRAF6, IKK $\beta$ , NF- $\kappa$ B P65, NF- $\kappa$ B, P50) downstream of the MDA5/IPS-1 signaling axis significantly increased at multiple time points. Gene expression levels of pro-inflammatory cytokines (IL-6, IL-8, TNF- $\alpha$ , IFN- $\beta$ ) in the diseased group were significantly downregulated in the early stage of the attack, and then upregulated at later time points, while protein expression levels also increased at multiple time points. NIBV infection significantly activated the MDA5/IPS-1 signaling axis and down-streamed regulatory axis, causing the body to produce a large amount of pro-inflammatory factors, damaging the bursa of Fabricius tissue, and triggering immune disorders.

**To Cite This Article:** Li Z, Zhang B, Shen Y, Liu P, Shi Y, Cai G, Zheng Z, Gao X and Guo X 2025. Morphological immunological and genetic studies in the bursa of Fabricius in Nephropathogenic Infectious Bronchitis Virus -induced inflammation via the MDA5/IPS-1 signaling axis. Pak Vet J, 45(3): 1226-1234. <http://dx.doi.org/10.29261/pakvetj/2025.244>

**INTRODUCTION**

Infectious bronchitis virus (IBV) is a type of virus that mainly spreads through the respiratory tract and can cause an acute and severe avian infectious disease. Its fatality rate can reach up to 30%, and it causes incalculable losses to the poultry farming industry every year (Bing *et al.*, 2007; Kuang *et al.*, 2021a). IBV is widespread globally, with QX being the predominant serotype in China, accounting for 74.7% existence (Bande *et al.*, 2017a). NIBV is one of the main types of IB virus. The clinical symptoms of chickens infected with NIBV

include depression, cough, drooling, and diarrhea. (Huang *et al.*, 2017). The patho-anatomy of the diseased chicken is characterized by swelling and bleeding of the kidney, and urate crystals with chalky appearance covering the surface of kidneys. The gut is being damaged, leading to changes in microbial composition (Xu *et al.*, 2020; Chen *et al.*, 2022). Due to the numerous serotypes of NIBV and the lack of cross-protection among them, prevention and control have become a major challenge (Shu-Yi and Hui-Wen, 2017; Qin *et al.*, 2021). Our previous research has shown that NIBV infection could trigger inflammation through the TLR7/NF- $\kappa$ B signaling pathway and cause

kidney damage in chickens. (Li *et al.*, 2022). The bursa of Fabricius is one of the important immune organs in chickens as it secretes B lymphocytes, which help chickens resist the invasion of pathogenic factors and provides a solid support for chickens to defend against the invasion of pathogenic microorganisms. (Schat, 2022). However, research has shown that the reason for the decline in immune function is that the follicular sac structure of chickens is being attacked by viruses (Sharma *et al.*, 1994; Huang *et al.*, 2020). After NIBV infection in chickens, an increase in the viral load of NIBV in the bursa of Fabricius and changes in the expression levels of genes related to inflammation could be detected. (Kuang *et al.*, 2021a; Chen *et al.*, 2024). However, the mechanism of NIBV-induced damage to bursa of Fabricius remains unclear.

MDA5 is considered as an important pathogen recognition receptor in certain RNA viral infections. It cooperates with downstream IPS-1 to activate the immune function of the body, which is the mechanism by which it combats viral infections. (Faul *et al.*, 2010; Yu *et al.*, 2016). Therefore, activating the MDA5/IPS-1 signaling pathway may enable the body to recognize RNA viruses, thereby initiating the body's resistance against viral invasion (Fredericksen *et al.*, 2008). Tembusu virus (ATMUV) can affect the expression of IFN by regulating MDA5 and TLR3. In the cell experimental model, the expression of IFN is positively correlated with IPS-1 (Chen *et al.*, 2016). Inhibition of MDA5 can affect the decrease of I-IFN expression, leading to inflammation in the body. Related reports indicate that activation of the MDA5/IPS-1 pathway can also alleviate cadmium induced cytotoxicity during cadmium induced peripheral blood lymphocyte damage in chickens (Wanqiu *et al.*, 2017). Therefore, studying whether MDA5/IPS-1 is involved in NIBV infection and exploring its mechanism in infection may be a key factor to combat viral infections through immune mechanisms.

Therefore, this study established animal models at five key time periods of NIBV infection to search for the effects of NIBV infection on the MDA5/IPS-1 signaling axis and the mechanism of immune function in the damaged bursa of Fabricius.

## MATERIALS AND METHODS

**NIBV:** NIBV strain SX9 utilized in the present study was isolated and stored at a temperature of  $-80^{\circ}\text{C}$  within the Clinical Veterinary Laboratory, which falls under the remit of the College of Animal Science and Technology at Jiangxi Agricultural University. The accession number was MN707951.1.

**Experimental animals and sample collection:** Three hundred (300) 1-day-old Hailan brown laying hens used in this experiment were provided by the Nanchang Livestock Research Institute in Jiangxi Province. All experimental procedures and protocols were meticulously conducted in accordance with the national regulatory framework concerning the ethical treatment and welfare of animals. Furthermore, the proposed plan for conducting the experiment on animals was formally endorsed by the Animal Ethics Committee of Jiangxi

Agricultural University (JXAULL-2021-31). Chicks were randomly divided into two groups and while considering the mortality rate of NIBV, 100 chickens were placed in the control (Con) group while 200 chickens were allotted to the diseased (Dis) group. To avoid cross infection, they were placed in different rooms and vaccinated according to the protocol (the experimental group did not receive the IBV vaccine). On the 28<sup>th</sup> day, the Con group was dripped with physiological saline solution through nasal route, while the Dis group was nasal dripped with SX9 virus solution. Afterwards, the condition of the chickens were observed daily, and samples of bursa tissue were collected at 1, 3, 5, 7, 9, 11, 13, 15, and 18-days post-infection (dpi) during the experiment. Take a photo and record the weight of the bursa of Fabricius. The tissue samples were placed in 2ml cryovials and stored in a  $-80^{\circ}\text{C}$  freezer for subsequent experiments. The other part is placed in a 10% concentration formalin solution for subsequent H&E staining and sectioning.

**Histopathological examination:** Tissue samples were subjected to fixation with 4% paraformaldehyde for a period of 24 hours. Following this, the samples were dehydrated through a series of graded ethanol solutions and subsequently embedded in paraffin. For this study, sections measuring  $4\mu\text{m}$  in thickness were deparaffinized. The sections were then rehydrated and were subsequently stained with hematoxylin for 5 minutes. Following this procedure, the samples were subjected to differentiation in 1% acid alcohol. Prior to dehydration and mounting, the samples were subjected to eosin counterstaining for a duration of one minute. The nuclei appeared blue, while cytoplasm and collagen fibres showed pink-to-red staining under microscopy.

**Determination of immunoglobulin IgG and IgM:** IgG and IgM were determined using commercially available kits (Nanjing Jiancheng, China) as per instructions provided by the manufacturer and the OD values were measured under a spectrophotometer.

**Total RNA extraction and Qpcr:** Total RNA was extracted from chicken bursa using a reagent kit (Takara, Beijing, China), according to the instructions provided by the manufacturer and then reverse transcribed the extracted RNA into cDNA.

The genetic information was obtained from NCBI, primer design was performed using Primer Express3.0 software and the optimal sequence was selected for RT-PCR (the primer sequences are shown in Table 1). According to the instructions of the RT-PCR kit (Beijing TransGen Biotech, China), the CT values of the Con and Dis groups were normalized to GAPDH levels using the delta delta threshold cycle ( $\Delta\Delta\text{CT}$ ) method, the relative expression levels were calculated and plotted using Graphpad prism 8.

**Viral load:** A standard curve was established using the NIBV positive plasmid previously constructed in our laboratory and quantitatively measured the copy number of viral load in the kidneys of chicks at different infection times using a fluorescence quantitative PCR instrument.

**Table 1:** The primer sequences.

Gene Name	Primers Sequences (5'-3')
MDA5	F:5'-TGGTCACATACAGCTCCAAGA-3' R:5'-ACGAGGAGATCAGCGTGTG-3'
IPS-1	F:5'-GGGATTTGAGTGCTGCTCCT-3' R:5'-GTCTCCACCAACCTCACTGG-3'
TRAF6	F:5'-ATGGAAGCCAAGCCAGAGTT-3' R:5'-ACAGCGCACCAGAAGGGTAT-3'
IKK-β	F:5'-GCAGCAGGATGAGGAAAGTCT-3' R:5'-CGTACGATACCGACTTCATCTG-3'
NF-κB-p65	F:5'-CACATGGTGGTGACCGCCAATAG--3' R:5'-GTGCCATCGTATGTAGTGCTGTCC-3'
TRAF3	F:5'-GAGGAGTGAGCGAGTGATAGACAGT-3' R:5'-AGTCACTCTGTTCTGGAGGGATTC-3'
IRF7	F:5'-ACCACATGCAGACAGACTGACACT-3' R:5'-GGAGTGGATGCAAATGCTGCTCTT-3'
IRF3	F:5'-TACACTGAGGACTTGCTGGAGGT-3' R:5'-AAGATGGTGGTCTCCTGATCC-3'
TBK1	F:5'-GGTTTGCCAGAATCGGAGT-3' R:5'-TGTAATACTCCTCTGTGCCGT-3'
IL-6	F:5'-TTCACCGTGTGCGAGAACAGC-3' R:5'-CAGCCGTCTCCTCCGTAC-3'
IL-8	F:5'-GCAAGGTAGGACGCTGGTAA-3' R:5'-GCGTCAGCTTACATCTTGA-3'
TNF-α	F:5'-TGATCGTGACACGTCTCTGC-3' R:5'-CAACCAGCTATGCACCCAG-3'
GAPDH	F:5'-ATGACATCAAGAAGGTGGTG-3' R:5'-CATACCAGGAAATGAGCTTG-3'

**Extraction of total protein and Western blotting:** Bursa of Fabricius tissue was ground and mixed thoroughly with a certain amount of RIPA lysis buffer containing PSMF and centrifuged (Beijing Soleibao Biotechnology Co., Ltd., China). The entire process was carried out on ice. BCA standard curve was established to measure the total protein concentration in the supernatant, and unified this concentration for subsequent experiments. The uniform Concentration was used for protein immunoblotting. After SDS-PAGE, the protein was transferred onto a PVDF membrane and sealed with 5% skim milk (dissolved in PBS) for about 2hr. Finally, the grayscale values of the protein bands obtained were measured using Image J software, and the ratios were analyzed and plotted in a graph.

#### Data analysis and processing

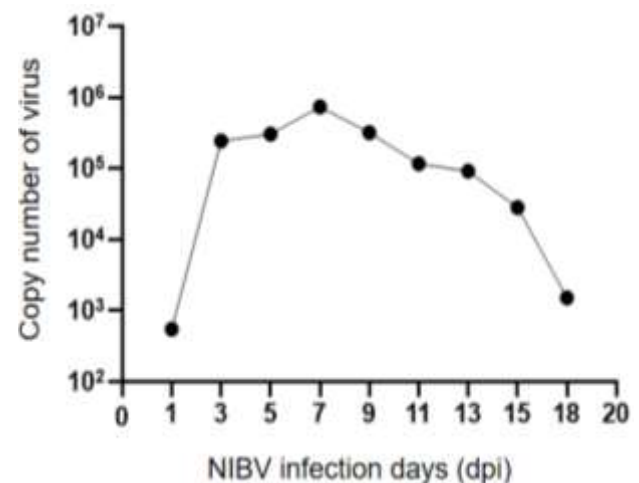
At least 6 independent samples were used for data analysis in each group of this experiment. Independent sample t-test was performed using SPSS26.0, and the calculated results were visualized using GraphPad Prism8.0 software. While  $P < 0.05$  was marked with “\*” to indicate significant difference and  $P < 0.01$  was marked with “\*\*”, indicating a significant difference.

## RESULTS

**Viral load:** At 1dpi of virus attack, a viral load of  $10^3$  was detected in the bursa of Fabricius (Fig. 1). At 3dpi and 5dpi, it continued to rise and reached its highest value of  $10^6$  at 7dpi. Afterwards, it began to decrease and returned to  $10^3$  at 18dpi. Based on the trend of virus load changes, experiments were conducted at 1dpi, 7dpi, 11dpi, 13dpi, and 18dpi.

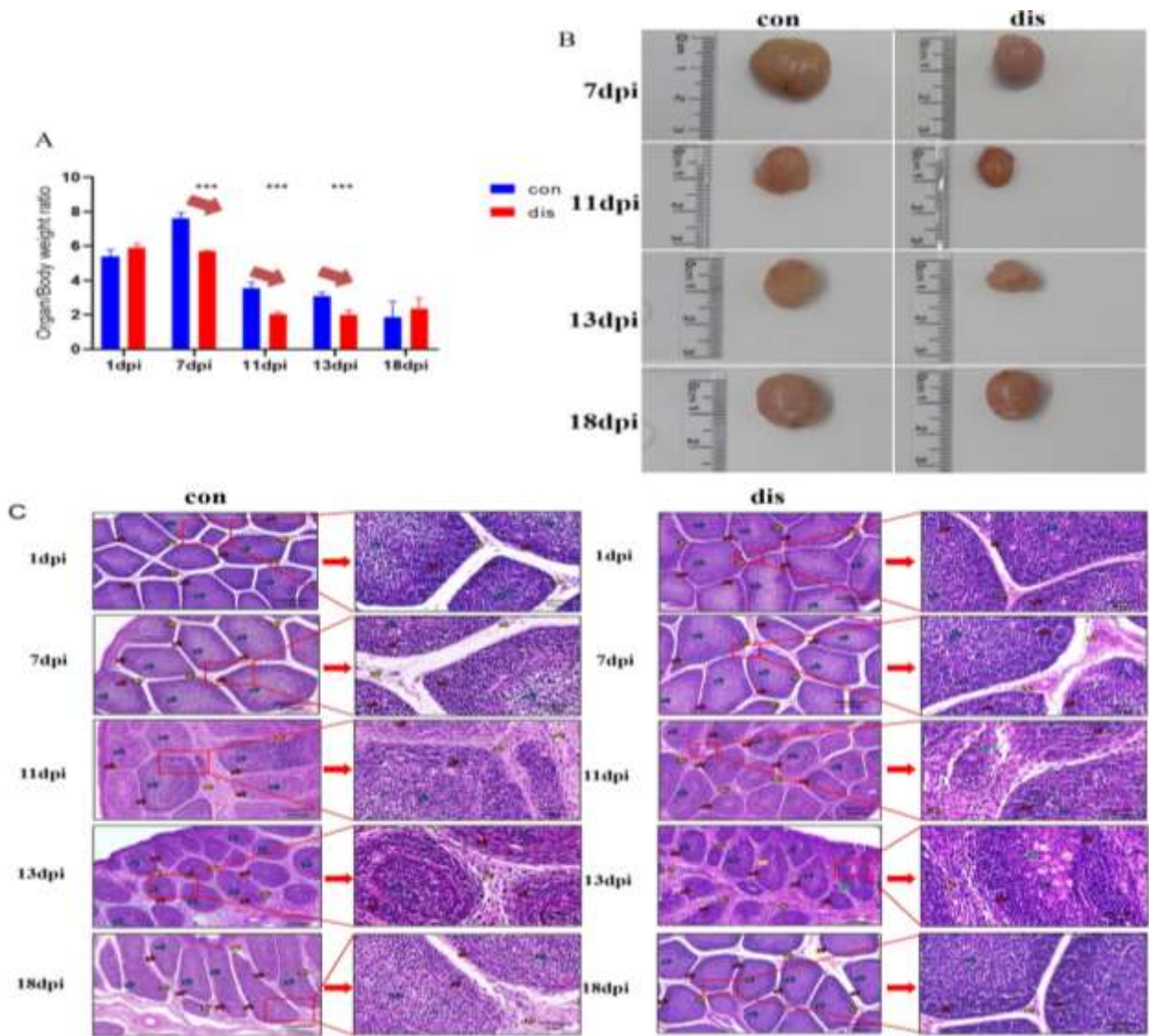
**Pathoanatomical and examination:** As shown in Fig. 2A-B, at 7dpi, 11dpi, and 13dpi after poisoning, the organ index of the Dis group significantly decreased ( $P < 0.01$ ). As shown in Fig. 2C, at the 1dpi and 7dpi the follicular structure of the bursa of Fabricius was relatively intact and

the lymphocytes are tightly arranged in chicks of Con groups. The epithelial cell layer between the cortex and medulla inside the follicle was clear, while the interstitial fibers of the bursa of Fabricius gradually increased at 11dpi, 13dpi, and 18dpi, while the follicles of the bursa of Fabricius shrank. At 7 dpi and 13 dpi, the pathological group showed thinning of the mucosal epithelium, shrank follicles along with incomplete follicular structure. The epithelial cell layer between the cortex and medulla in the lymphoid follicles was blurred, making it difficult to distinguish between the cortex and medulla. The medulla area was infiltrated by lymphocytes, with an increase in necrotic lymphocytes. The disappearance of lymphocytes caused partial vacuolization of the medulla. At 18 dpi, there was no significant difference in the size and structure of the bursa of Fabricius between the pathological group and the normal group.

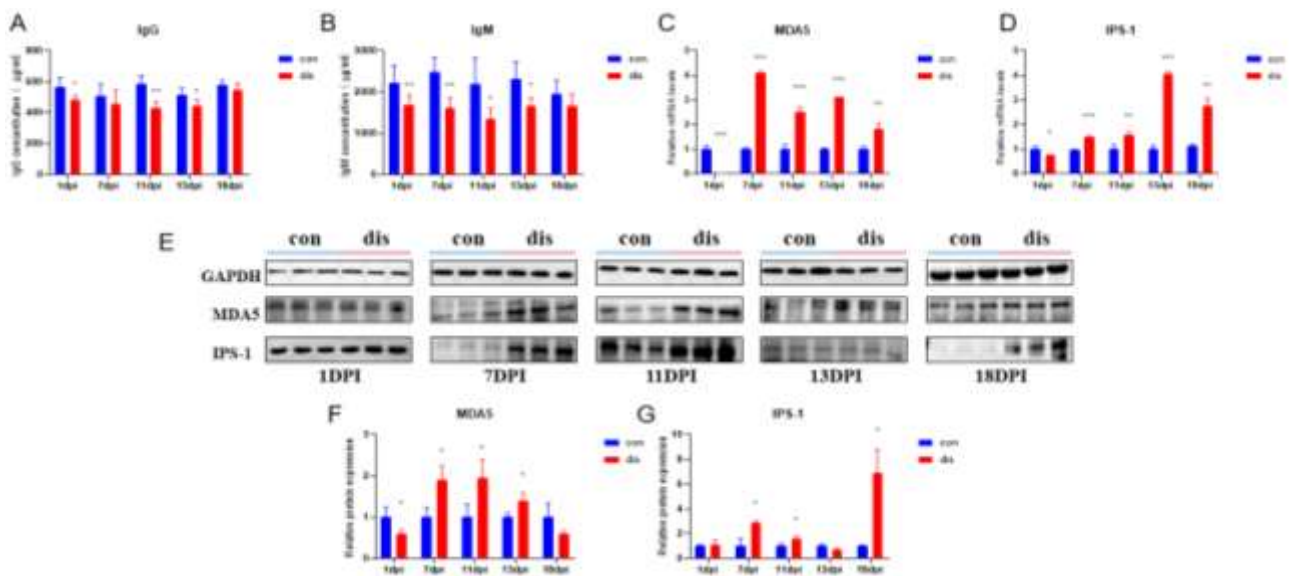


**Fig. 1:** The trend of viral load in the bursa of Fabricius over time.

**The effect of NIBV on the immune function of the bursa of Fabricius and the MDA5/IPS-1 signaling axis:** As shown in Fig. 3A and 3B, the levels of IgG and IgM in the bursae of Fabricius of the Dis group decreased significantly at 1 dpi, 7 dpi and 11 dpi. Moreover, we investigated the changes in mRNA expression levels of the MDA5/IPS-1 signalling axis in the bursa of Fabricius during NIBV infection. We detected changes in the mRNA and protein levels of the MDA5 and IPS-1 genes at different stages. As shown in Fig. 3(C-D), the mRNA levels of the MDA5 and IPS-1 genes in the Dis group were significantly lower than in the Con group at 1dpi ( $P < 0.05$ ) and significantly higher from 7dpi to 18dpi ( $P < 0.01$ ). The protein expression trends of MDA5 and IPS-1 were consistent with the mRNA expression levels. As shown in Fig. 3E-G, the MDA5 protein expression level in the Dis group decreased in the early stage of infection ( $P < 0.05$ ) and increased in the middle and late stages ( $P < 0.05$ ). IPS-1 protein expression increased in the middle and late stages of infection ( $P < 0.05$ ). Fig. 3H shows the changes in fluorescence expression of the MDA5 protein in bursa of Fabricius tissue. The fluorescence intensity of the MDA5 protein decreased in the Dis group on the first day of NIBV infection, increased significantly at 7 dpi, 11 dpi and 13 dpi, and showed no significant change at 18 dpi.



**Fig. 2:** The impact of NIBV infection on the morphology and structure of the bursa of Fabricius. (A). Organ index. (B). Observation of the morphology of the bursa of Fabricius. (C). Sections of bursa of Fabricius tissue from the control and disease groups. The red arrow shows the cortex, the blue arrow shows the medulla, the yellow arrow shows the stroma and the green arrow shows follicle lesions.



**Fig. 3:** The effect of NIBV on immune function in the bursa of Fabricius, as well as on the expression levels of mRNA and proteins of immune-related genes. (A-B) Changes in the expression levels of IgG and IgM in the bursa of Fabricius were observed. (C-D) mRNA expression levels of genes in the MDA5/IPS-1 signalling pathway. (E) A Western blot was used to detect the expression level of the target protein. (F-G) Protein expression levels of MDA5/IPS-1 signalling pathway-related genes.

**The effect of NIBV on factors related to bursal inflammation:** As shown in Fig. 5 (A-D), TRAF6, p65, and p50 in the Dis group increased over multiple time periods, while IKK- $\beta$  decreased at 1dpi and also showed an upward trend at 7, 11, 13, and 18dpi. Compared to the Con group, the expression levels of TRAF6 protein and p65 protein in the Dis group increased at 1, 7, 11, and 13dpi, while the expression levels of IKK- $\beta$  protein increased throughout the entire time period, with a more pronounced

trend. The expression changes of p65 protein in the bursa of Fabricius after vaccination are shown in Fig. 6. The fluorescence signal of p65 was significantly upregulated at multiple time periods after infection.

**Effect of NIBV infection on I-IFN-related pathways:** As shown in Fig. 7 (A-D), the TRAF3 gene in the Dis group decreased at 1 dpi, increased at 7 dpi, and showed no significant changes at other times. The IRF7 and IRF3 of

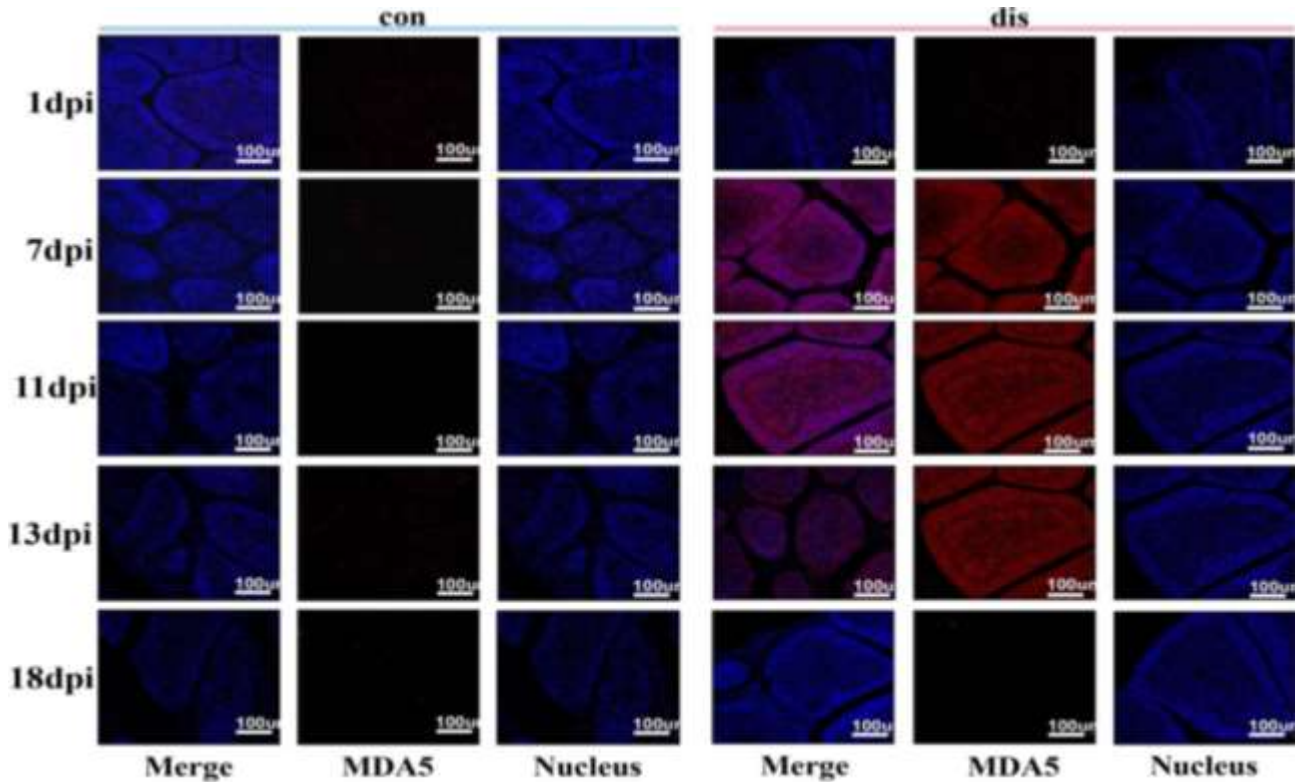


Fig. 4: Immunofluorescence staining was used to detect the positive expression of MDA5 in the bursa of Fabricius.

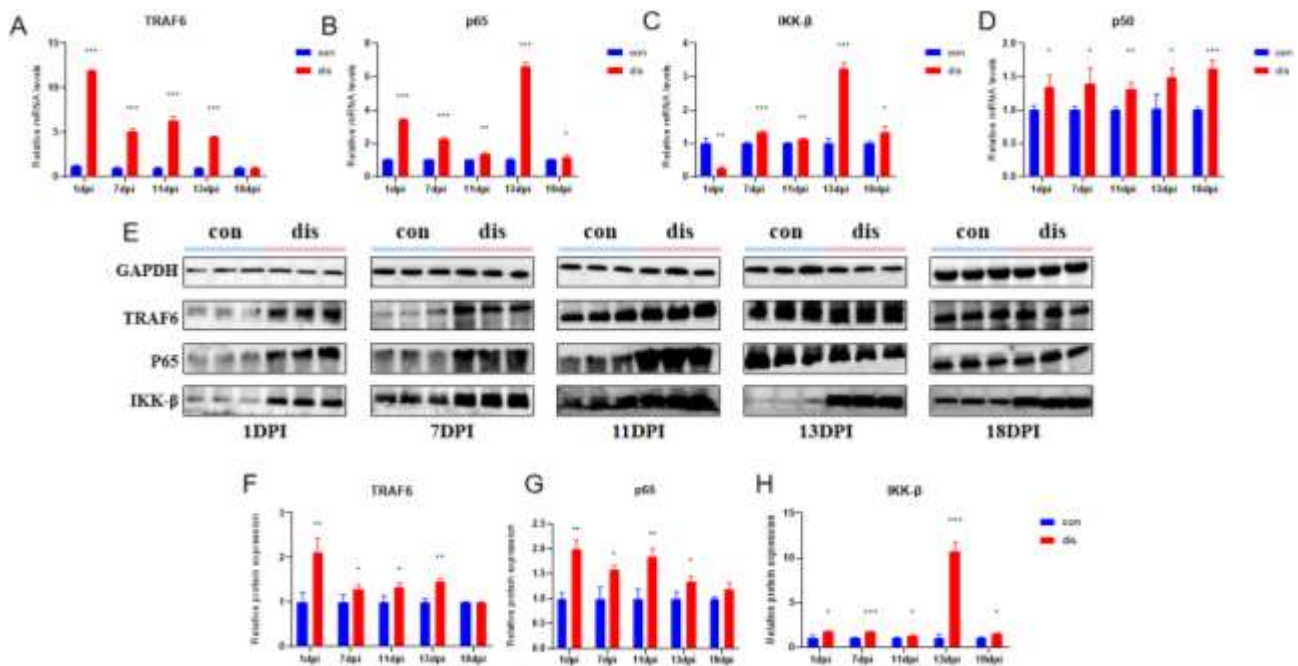


Fig. 5: The effect of NIBV infection on the mRNA and protein expression levels of the MDA5/NF- $\kappa$ B signalling pathway is investigated. (A–D) show the mRNA expression levels of genes related to the MDA5/NF- $\kappa$ B signalling pathway. (E) The expression level of the target protein was detected using a Western blot. (F–H) Quantitative analysis of protein expression levels related to the MDA5/NF- $\kappa$ B signalling pathway.

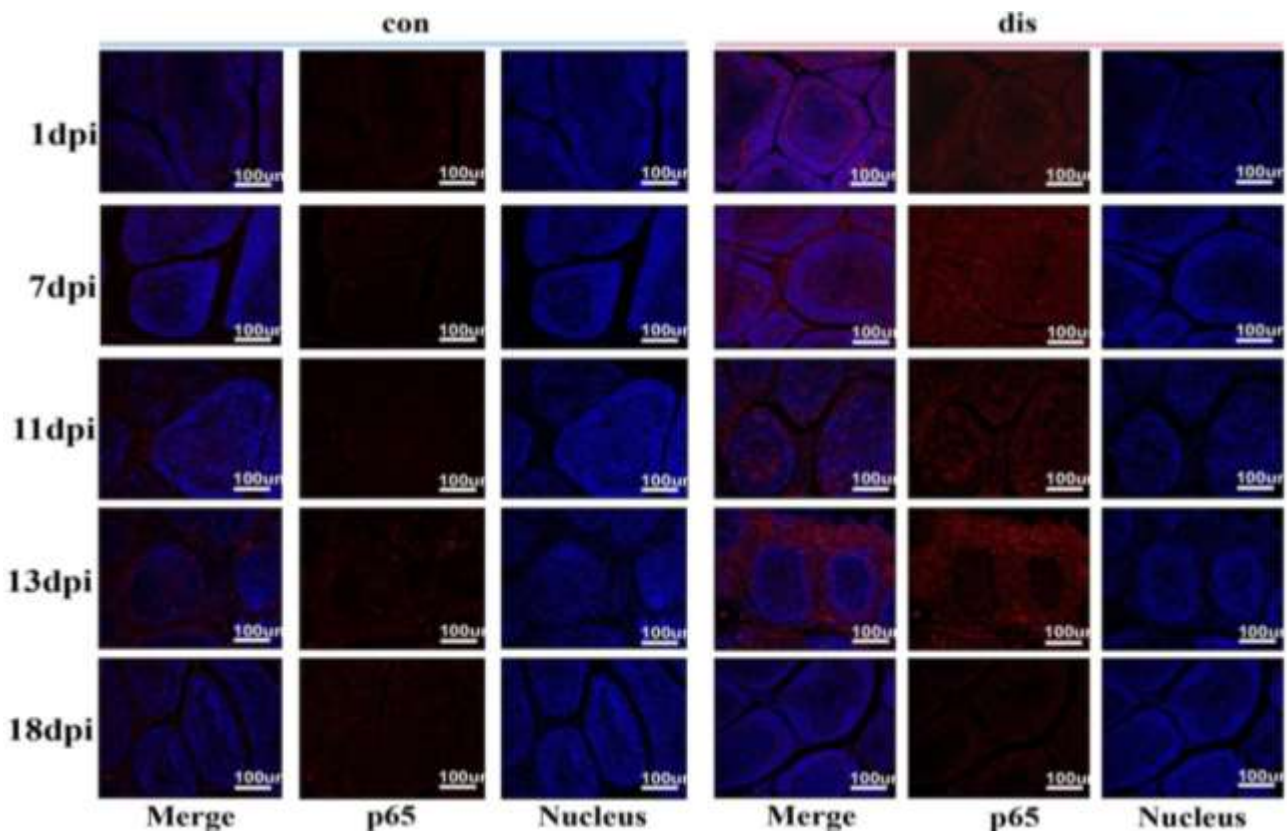


Fig. 6: Immunofluorescence staining was used to detect the positive expression of p65 in the bursa of Fabricius.

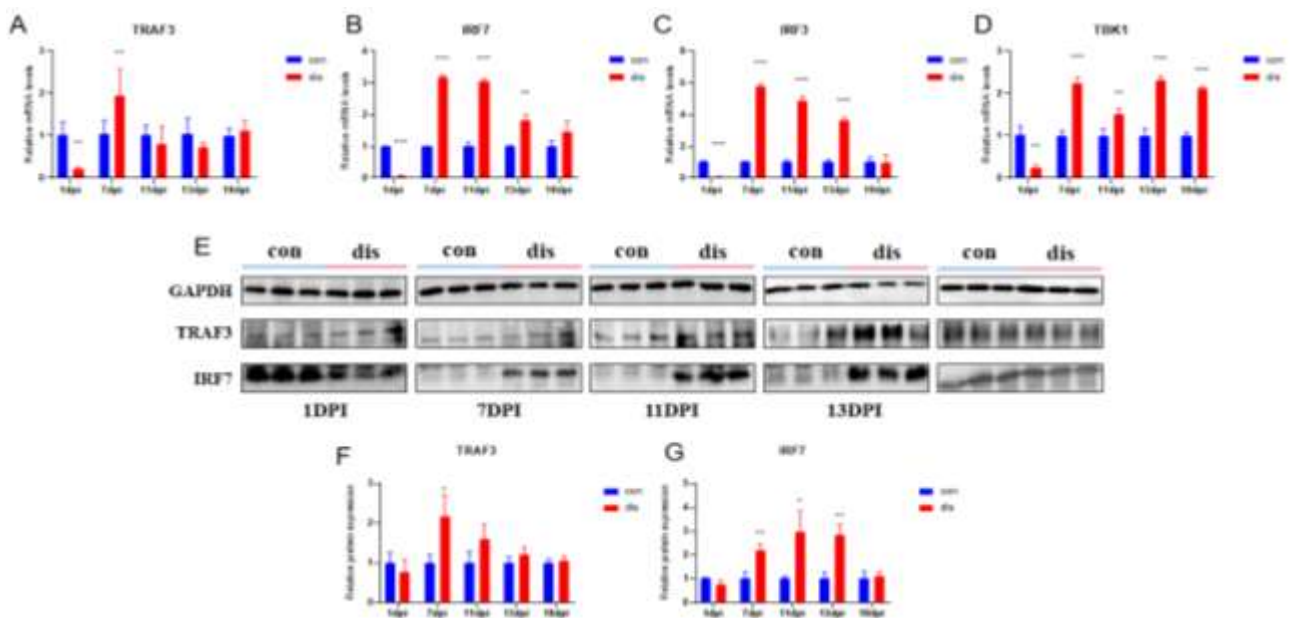
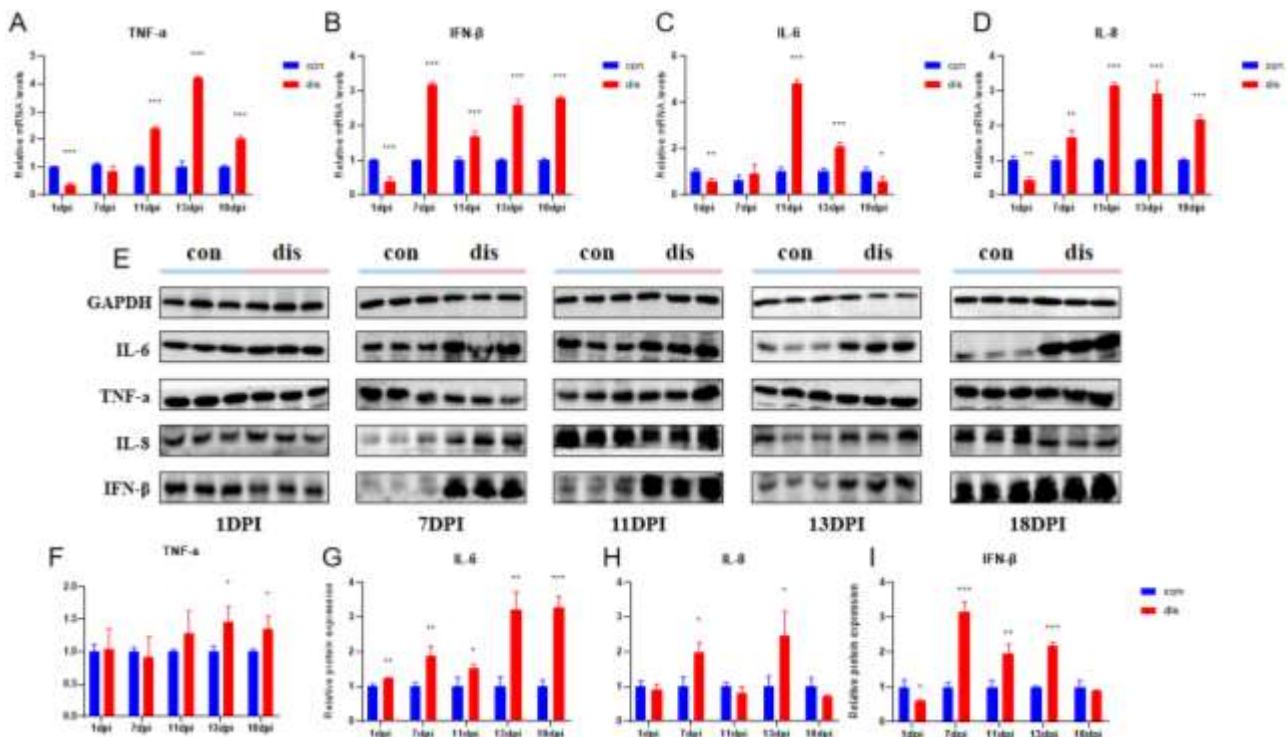


Fig. 7: What happens when a person has NIBV infection and how it affects the way the body makes and uses mRNA and protein. (A–D) show the mRNA expression levels of genes related to the I-IFN regulatory pathway. (E) A Western blot was used to detect the expression level of the target protein. (F–G) The expression levels of proteins associated with the I-IFN regulatory pathway.

the Dis group decrease at 1dpi, and increase at 7dpi, 11dpi, and 13dpi. However, TBK1 in the Dis group decreased at 1dpi and increased during other time periods. Compared to the Con group, the TRF3 protein in the Dis group in Fig. 7F and G increased at 7dpi, while the IRF7 protein increased at 7dpi, 11dpi, and 13dpi, with no significant changes observed at other times.

**The impact of NIBV infection on pro-inflammatory factors:** As demonstrated in Fig. 8 (A–D), the expression

levels of TNF- $\alpha$ , IFN- $\beta$ , IL-6, and IL-8 genes in the Dis group were lower than those in the Con group during the initial stage of infection ( $P < 0.01$ ), and continued to increase in the subsequent period. As demonstrated in Fig. 8 (F–I), the infection caused by NIBV resulted in increased expression of the protein levels of TNF- $\alpha$ , IL-6, and IL-8. The IFN- $\beta$  protein levels in the Dis group were found to be lower than those in the Con group on day 1 post-inoculation (1dpi), yet higher than the Dis group decreased on 1dpi, while it increased on days 7, 11, and 13 post-inoculation.



**Fig. 8:** The effect of NIBV infection on the expression levels of mRNA and protein related to inflammatory cytokines. (A–D) mRNA expression levels of genes related to inflammatory factors. (E) The expression level of the target protein was detected using a Western blot. (F–I) Expression levels of inflammatory cytokine-related proteins.

## DISCUSSION

NIBV, as one of the main infectious diseases in chickens, has always been an important factor restricting the development of the poultry industry (Shu-Yi and Hui-Wen, 2017). NIBV infection can cause respiratory, urinary, and immune reactions (Bande *et al.*, 2017b; Kuang *et al.*, 2021b). As one of the main immune organs in poultry, the bursa of Fabricius plays a unique and important role in combating infectious diseases (Schat, 2022). This article mainly discusses the mechanism of NIBV infection induced chicken bursal inflammation and its impact on immune function, which is conducive to the search for potential treatment approaches for NIBV.

According to the experimental results, on the first day after being infected with the NIBV virus, the gene expression related to the MDA5/IPS-1 signaling pathway was inhibited. As the NIBV infection progressed to the middle stage, the MDA5/IPS-1 signaling pathway gradually became activated, and the content of inflammatory factors also showed an increase. These new experimental findings help us gain a deeper understanding of how NIBV infection affects the host's innate antiviral immune response and damages immune organs, providing important landmark for subsequent research on the immune mechanism of NIBV. Based on the appearance data, organ coefficient and weight data of the bursa of Fabricius obtained from this experiment, it could be concluded that NIBV infection had caused certain damage to the development and organ structure of the bursa of Fabricius. Meanwhile, in the HE staining sections, structural damages such as thinning of the mucosal epithelium and atrophy of the follicles could be observed. Our previous research in the laboratory has shown that NIBV infection can be mediated by the PINK1/Parkin axis for mitochondrial

autophagy, which damages the normal morphology and structure of the spleen and thereby affects the immune function of chickens. (Tian *et al.*, 2023). We measured the viral load, it increased between 1-7dpi and then showed a downward trend. This indicates that NIBV infection can replicate extensively in the bursa of Fabricius and subsequently be cleared or controlled by immune system (Bai *et al.*, 2022).

This experiment found that on the first day after virus infection, the mRNA level of MDA5 decreased significantly ( $P < 0.05$ ), while it increased significantly at the 7, 11, and 13dpi ( $P < 0.05$ ). The protein of MDA5 also showed similar trend. This demonstrates that the dynamic regulation of MDA5 gene expression by NIBV infection may be related to the immune response after viral invasion. In addition, the mRNA level of IPS-1 also changed significantly after NIBV infection. Similar to MDA5, IPS-1mRNA decreased on the first day of infection, but increased on days 7, 11, and 18 after infection. This demonstrates that NIBV infection can affect the expression levels of MDA5 and IPS-1 genes, thereby influencing the activation of the immune response signaling pathways. There is evidence suggesting that IBV infection to chicken macrophages leads to a first decrease and then increase in MDA5 gene expression. IBV activates the antiviral natural immune axis of macrophages. Immune suppression may occur during the early stage of IBV infection (Sun *et al.*, 2021). These conclusions drawn by previous researchers are consistent with our experimental results, indicating that NIBV evades the innate antiviral immune system during the initial stage of infection and activates the MDA5/IPS-1 signaling axis after a certain period of infection, thereby promoting the expression of downstream genes of the signaling axis.

MDA5 recognizes the replication intermediate of NIBV in the cytoplasm and ubiquitinates TRAF6 through IPS-1. TRAF6, as an upstream gene of NF- $\kappa$ B, is involved in regulating immune response and inflammatory response (Liu *et al.*, 2025) and plays an important role in the various pattern recognition receptor signaling axis (Yoon *et al.*, 2018). In this study, the TRAF6 gene downstream of the MDA5/IPS-1 signaling axis was significantly upregulated in the early stage of infection (1dpi) ( $P < 0.05$ ), and the upregulation increased at subsequent time points after infection (7, 11, and 13dpi), which may be related to the immune response and inflammatory response after viral infection ( $P < 0.05$ ). The dynamic changes of IKK- $\beta$  gene after infection exhibit different characteristics from TRAF6. In the early stage after infection (1dpi), there was a downward trend, while at subsequent time points (7, 11, 13, and 18dpi), there was an upward trend. IKK- $\beta$  is crucial for activating the NF- $\kappa$ B signaling axis because it is one of the subunits of the IKK complex. Alterations in its expression have been demonstrated to exert an influence on the activity of NF- $\kappa$ B and activation of inflammatory factors. The expressions of NF- $\kappa$ B P65 and NF- $\kappa$ B P50 are also regulated by the infection. At several time points after viral infection, the expression of these two genes exhibited an upregulation trend ( $P < 0.05$ ). The TRAF6 protein and P65 protein exhibited an upregulation trend at varying time points following infection ( $P < 0.05$ ), while the expression of IKK- $\beta$  demonstrated fluctuations at different time points, which is consistent with the changes in their mRNA levels. The results of immunofluorescence detection further corroborated this finding. The results of this experiment show that NIBV infection activates the MDA5/NF- $\kappa$ B signaling axis, thereby raising the expression of downstream inflammatory cytokines.

TRAF3 is a member of the TRAF family and is also a positive regulator for IRF3 activation and type I interferon induction. (Lin *et al.*, 2023). In RNA virus infections, TRAF3-induced generation of type I interferons is crucial (Lin *et al.*, 2021). The research results demonstrate that the levels of gene and protein expression of TRAF3, in addition to the levels of gene expression of IRF7, IRF3 and TBK1. Afterwards, a rebound was observed, with a subsequent significant increase on the 7, 11, 13, and 18 dpi. This may reflect the impact of viral infection on immune regulation. This change may also reflect the activation of immune regulatory pathways and the intensification of inflammatory responses after infection. The expression changes of TBK1 may be influenced by the developmental course of the immune response (Chen *et al.*, 2022). At this point, we can make the following inference that NIBV infection may evade innate antiviral immune mechanisms and promote virus replication in the early stages by inhibiting the activation of I-IFN expression regulatory axis related genes induced by MDA5, and this pathway may be activated in the later stages.

In this study, gene expression levels of TNF- $\alpha$  and IFN- $\alpha$  genes underwent a significant decrease at 1dpi following viral infection ( $P < 0.05$ ), and a significant increase was observed at 11, 13, and 18dpi ( $P < 0.05$ ). This phenomenon may be attributed to the immune system's ability to reduce severe inflammatory responses at the early stage of viral infection, thereby promoting the subsequent

clearance of the virus by increasing the production of inflammatory factors. (Kuang *et al.*, 2021a). The mRNA levels of the IL-6 and IL-8 genes underwent a significant decrease at 1dpi following viral attack ( $P < 0.05$ ), yet these levels gradually increased during subsequent infection. This finding suggests that, in the later stages of infection, the immune system may combat the virus by increasing the production of these two inflammatory factors. The decrease in IFN- $\beta$  at 1dpi is due to early inhibition of I-IFN regulatory genes such as MDA5, IRF3, and IRF7 after NIBV attack. These results indicate that the production of inflammatory cytokines during NIBV infection is influenced by complex regulatory mechanisms, leading to damage to the bursa of Fabricius.

**Conclusions:** In summary, in terms of morphology, NIBV infection leads to a reduction in the volume of the chicken's bursa of Fabricius and slows down growth. In terms of pathological anatomy, the interstitial fibrosis of the bursa of Fabricius increases, the follicles shrink, the mucosal epithelium becomes thinner, and the medulla part of the lymph follicles becomes vacuolated, making it difficult to distinguish between the cortex and medulla. In terms of genetics, our study suggests that NIBV infection inhibits the expression of MDA5/IPS-1 and I-IFN related regulatory genes in the 1dpi, and promotes their expression in the middle and later stages. In addition, from the perspective of immunology, NIBV infection activates the classic MDA5/NF- $\kappa$ B signaling axis in the bursa of Fabricius, promotes the release of downstream inflammatory factors, induces inflammation of the bursa of Fabricius, and damages the normal function of the immune system.

**Declaration on the welfare and ethics of experimental animals:** We strictly follow national animal ethics and welfare regulations in all our experimental procedures and protocols. The Animal Ethics Committee of Jiangxi Agricultural University has approved this experimental protocol (approval number JXAULL-2021-31). The School of Animal Science and Technology at Jiangxi Agricultural University is responsible for the disinfection of the laboratory animal room where all chicks were raised according to laboratory feeding standards.

**Disclosures:** No.

**Acknowledgments:** We highly acknowledge College of Animal Science and Technology at Jiangxi Agricultural University for providing assistance and support for this experiment.

**Financial support statement:** This study was supported by Key R&D plan of Jiangxi Province (20224BBF62003), National Natural Science Foundation of China awards to Xiaoquan Guo (32360906 and 32072935), National Key Research and Development Program (2023YFD1801100), Key Project of Jiangxi Provincial Natural Science Foundation (20232ACB205013), Jiangxi Modern Agriculture and Poultry Industry Technology System Project (JXARS).

**Credit authorship contribution statement:** Zhengqing Li: Writing-Initial draft, Data organization and analysis,

Visualization, Methodology, Situation analysis. Bingqing Zhang: Writing-Commenting, Reviewing, Editing, Investigating, Project management. Yufan Shen: Resources, Verification, Editing. PingLiu: Methodology, Conceptualization. Yan Shi: Formal analysis, Data, Curating. Gaofeng Cai: Investigation and conceptualization. Zhanhong Zheng: Editing and supervision. Xiaona Gao: Writing - Review and editing, Supervision. Xiaoquan Guo: Funding acquisition, Resources, Review, Supervision, Editing, Project management, Methodology.

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