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RESEARCH ARTICLE

Host Innate Immune Responses of Quails Infected with Avian Paramyxovirus-1 of Different Pathogenicity

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ABSTRACT

Newcastle disease virus (NDV) inflicts considerable financial damage to the poultry industry globally. Japanese quails act as natural reservoirs for NDV and play significant role in virus epidemiology. Previous studies have documented variable susceptibility levels to the NDV among Japanese quails; however, the underlying mechanisms are still not well understood. In this study, the innate immune responses of quails to velogenic and lentogenic NDV strains were characterized. Pronounced transcriptional responses were seen in lung and spleen shortly after viral infection, inducing genes related to innate antiviral and pro-inflammatory cytokines. In the NDV genotype VII 1.1 infected group, a notable increase in the expression levels of interleukin-6 (IL-6), interleukin-8 (IL-8), and interferon-gamma (IFN-γ) was observed at 3 days post-infection (dpi), prior to their peak expression in the spleen at 5 dpi. In contrast, the LaSota inoculated group exhibited different transcriptional responses, with a notable increase in lung on day 1 post-infection. The NDV genotype VII 1.1 exhibited an increased viral load in the spleen at 5 dpi. Meanwhile, the viral load of LaSota strain was considerably higher at 3 dpi. These findings imply that infections with NDV strains of varying virulence are linked to variable viral loads and unique cytokine expression patterns in different tissues, proposing that the host response could possess a role in the pathobiology of NDV in quails. As well, the results emphasize significant variations in the quail's innate immune responses, infection dynamics and disease progression following NDV infection with different virulence. Additional research is warranted to further elucidate the molecular mechanism involved in NDV infection in a wide range of avian species.

Introduction

Orthoavulavirus javaense, commonly known as the Newcastle disease virus (NDV) is an enveloped virus that has negative-sense single-stranded RNA genome infecting birds. It is classified within the genus *Orthoavulavirus*, sub-family *Avulavirinae* in the family *Paramyxoviridae* [1]. The virus possesses a non-segmented genome of 15 kb, which encodes six proteins—namely; hemagglutinin-neuraminidase, fusion, matrix, nucleoprotein, phosphoprotein, and polymerase. Notably, RNA editing of the phosphoprotein gene results in the production of additional proteins, V and W [2]. These proteins are involved in suppressing the host cell interferon response, thereby promoting the virus replication [3]. The hemagglutinin-neuraminidase and fusion proteins serve as the primary targets for the immune response against NDV [4]. The N and P, along with L, constitute an RNA polymerase complex essential for the viral RNA replication [5].

The NDV can infect a wide range of avian species, encompassing both domestic and free-living species. However, chickens remain the primary and most vulnerable host. The isolates of NDV belong to a single serotype; however, they exhibit a wide spectrum of virulence, ranging from lentogenic (avirulent), mesogenic (intermediate virulent), and velogenic (highly virulent), contingent upon the severity of the disease they induce in birds [6]. Furthermore, based on its pathogenicity, it can be categorized into five pathotypes; i) viscerotropic velogenic, which produces fatal hemorrhagic lesions in the visceral organs, ii) neurotropic velogenic that causes respiratory and neurological issues, iii) mesogenic, iv) lentogenic and v) asymptomatic enteric [7]. The very virulent virus strains responsible for Newcastle disease (ND) present a significant threat to various bird species and cause substantial financial losses for both commercial and/or pet birds. In contrast, the lentogenic or avirulent virus strains are typically asymptomatic in adult birds or causing mild respiratory distress. These strains are used commonly as live ND vaccines within the poultry industry [6]. In spite of the accessibility of vaccines for NDV, the disease outbreaks continue to occur regularly, even among vaccinated birds. Thereby, ND is one of the most detrimental diseases affecting the poultry sector [8, 9].

Quails believed to be a significant carrier of NDV [10] and could have a critical impact on transmitting the virus among poultry species kept in close proximity to or with quails [11-13]. The antibody-mediated response plays a crucial role in protection against NDV infection. The cell-mediated immune response, which occurs after antigen stimulation can be distinguished by specific cytokine profiles. Historically, most studies have predominantly focused on the adaptive humoral or cell-mediated immune response of chickens to NDV, particularly in light of vaccine development [14, 15]. However, recent findings proposed that the innate immune response significantly affected the variability observed in responses to NDV infection [16, 17]. The innate immune defense of the susceptible host serves as the initial

guard mechanism against pathogens. In the early stages of NDV infection in chickens, pro-inflammatory and anti-viral cytokines, chemokines, as well as types I and II interferon are integral to the host's innate immune response. Nonetheless, little is known regarding the innate immunity of quails in response to NDV infection. Though, prior research has indicated that infections with NDV of varying virulence level are linked to distinct patterns of cytokine expression in both spleen and peripheral blood of chickens. In addition, modulating the cytokine-induced responses might play a role in the pathogenesis of highly virulent strains in chickens [16, 18-20]. *In-vivo* investigations have demonstrated that NDV triggers robust immune responses in the lymph nodes, peripheral blood and spleen of infected chickens, with notable increase in the mRNA expression levels for pro-inflammatory cytokines and chemokines [16, 20-22].

The significance of the antibody-mediated immune response in safeguarding against NDV infections is well recognized; however, the specifics of the innate immune response triggered by NDV exposure remain largely undefined. Notably, there has been a lack of research focusing on the host's innate immune responses of quails infected with NDVs of varying pathogenicity, which may influence the progression of the infection. Thus, the present study was executed to systematically explore the dynamic expression of inflammatory cytokines and chemokines in quails infected with highly virulent NDV or lentogenic LaSota strains. Additionally, it aims to offer basic data for understanding the balance of inflammatory cytokines following viral infection. To assess cytokine responses in quails upon infection with NDV genotype VII 1.1 or genotype II (LaSota strain), the expression of mRNA of pro-inflammatory cytokines, specifically interleukin 6 (*IL-6*), chemokine interleukin 8 (*IL-8*), and interferon-*gamma* (*IFN-γ*) in the lung and spleen tissues of quails were evaluated.

Material and Methods

Newcastle disease virus strains

A field strain of the virulent NDV, specifically the sub-genotype VII 1.1 strain identified as "NDV/Chicken/Egypt/ALEX/ZU-NM99/2019" and cataloged in GenBank under the accession number OP219680, has been made available through the generosity of Dr. Amal Eid, Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Zagazig University, Egypt. The virus was propagated and titrated using 10-days old embryonated chicken eggs (ECEs) and the resulting virus suspension was used with a dose of 10^6 embryo infective dose 50 (EID_{50}) per 0.1 mL. As well, the lentogenic LaSota strain of NDV, known as CEVAC NEW L LaSota Vaccine, was utilized. It comprises a live, freeze-dried ND virus (LaSota strain) of fowl embryo origin.

Propagation and titration of NDV in Embryonated Chicken Eggs

Ten-day-old ECEs were employed for the propagation and titration of NDV. A volume of 0.2 mL of NDV reference strain was inoculated into the allantoic sac of the ECEs obtained

from non-vaccinated chickens against NDV, Sharkia, Egypt, following the standard inoculation protocols established in a prior research [23]. The inoculated eggs were subsequently sealed with melted wax. Additionally, five fertile eggs were maintained uninoculated to serve as control negative ones. Both the control and inoculated ECEs were placed in an incubator at 37°C for a period of five days, with daily candling conducted. Following the incubation, the ECEs were cooled at 4°C for four hours prior to examination. Allantoic fluids were collected and analyzed through a rapid hemagglutination assay with 10% (v/v) washed chicken red blood cells (RBCs). The collected fluids were titrated by inoculating them into ten-day-old ECEs to ascertain the EID_{50}, as per the methodology outlined previously [24]. The viral stock was then diluted in sterile phosphate-buffered saline (PBS) to get a final concentration of 10^6 EID_{50} per 0.1 mL.

Experimental birds

Seventy unvaccinated Japanese quails (*Coturnix coturnix japonica*) were sourced from a commercial farm located in Dakahlia Governorate, Egypt at the age of 21 days. These quails were housed in disinfected cages within the experimental animal facilities at the Faculty of Veterinary Medicine, Zagazig University, where they were maintained under controlled environmental conditions. Prior to the infection, the quails underwent a 14 day acclimatization period to adapt to their new environment, reaching 35 days of age by that time.

Experimental design

To assess the levels of inflammatory cytokines following NDV infection, a group of 20 healthy quails, confirmed serologically negative for NDV, were inoculated oculonasally with 0.1 mL of viral fluid containing 10^6 EID_{50} of highly virulent NDV genotype VII1.1 (Group 1). A second group of quails (n=20) received an oculonasal inoculation of the NDV lentogenic strain LaSota (CEVAC NEW L LaSota Vaccine) at a dose of 10^6 EID_{50} in 0.1 mL (Group 2). Furthermore, a separate group of twenty-five sham-inoculated quails was maintained as a negative control (Group 3). All quails were monitored two or three times daily over a period of 14 days, during which clinical signs were meticulously recorded (Figure 1). Two quails were randomly selected from each group and euthanized at 1, 3, 5, 7 and 10 dpi for collection of tissues and postmortem examination. At 14 dpi, the remaining birds in all groups were euthanized and examined for PM lesion. The scoring system for lesions in NDV-infected tissues was adapted from previously established criteria [25]. In summary, lesions in the trachea, lung, proventriculus, cecal tonsils, spleen, and brain were categorized into four grades: 0 (normal), 1 (mild), 2 (moderate), and 3 (severe). All animal experiments were conducted following the guidelines of the Institutional Animal Care and Use Committee at the Faculty of Veterinary Medicine, Zagazig University, Egypt.

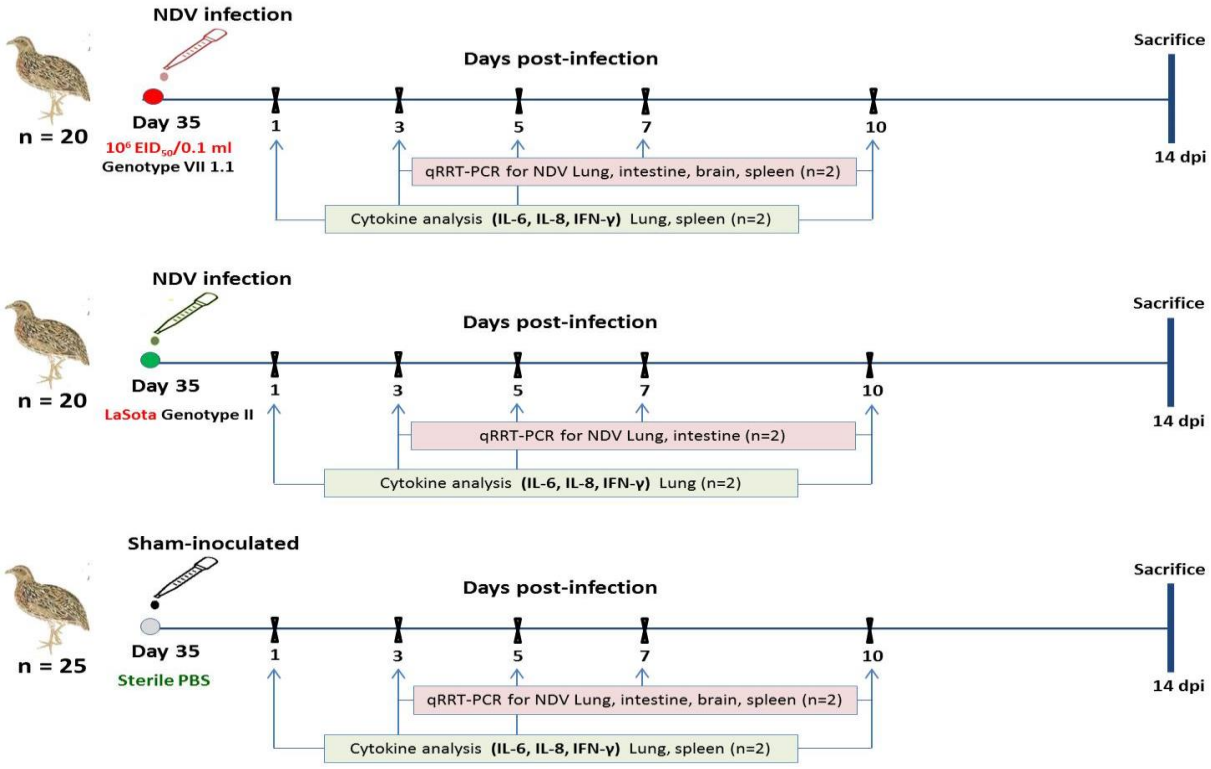

Figure 1: Schematic illustration of the experimental timeline for Japanese quails infected with either velogenic NDV genotype VII 1.1 or lentogenic LaSota NDV genotype II for assessment of cytokine gene expression profiles and viral loads in quail's tissues.
EID_{50}: Embryo infective dose 50; qRRT-PCR: Quantitative reverse transcriptase real-time PCR; NDV: Newcastle disease virus *IL-6*: Interleukin 6; *IL-8*: Interleukin 8; *IFN-γ:* Interferon-*gamma*; PBS: Phosphate buffer saline.

Quantitative reverse transcriptase real-time PCR (qRRT-PCR) for cytokine analysis

Total RNA was isolated from quail's tissues (lungs and spleen) following the protocol provided by RNeasy® Plus Mini kit (Qiagen, Valencia, CA). The obtained viral RNA was utilized for qRRT-PCR to assess gene expression levels of *IL-6*, *IL-8* and *IFN-γ*, using specific primer sets (Table 1), as described elsewhere [26, 27]. The β-actin was utilized as an internal control for mRNA normalization. The qRRT-PCR was conducted using GoTaq 1-Step RT-qPCR System (Promega, USA) and StepOne real time PCR system (Thermo Fisher Scientific, USA). A negative control was included along the experimental samples. The reaction was conducted in a total volume of 25 µL. The qRRT-PCR thermal profile commenced with an initial reverse transcription step at 37°C for 15 minutes, followed by a primary denaturation step at 95°C for 10 minutes. The PCR cycling conditions included 40 cycles of (i) denaturation for 10 seconds at 95°C, (ii) annealing for 30 seconds at 60°C, and (iii) extension for 10 seconds at 72°C. The relative expression levels of the target genes were determined using the 2-ΔΔCt

method. The fold changes were determined by comparing the expression level in the infected group to those in the uninfected group [28].

Detection of NDV load in tissues

The extraction of RNA was performed in accordance of the guidelines provided by the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA). The qRRT-PCR was performed following the protocols outlined in the Qiagen OneStep RT-PCR Kit (Qiagen, Valencia, CA) as per the manufacturer's specifications. The PCR was conducted in a total volume of 25 µL in sterile 0.2-mL PCR tubes. The process utilized a specific set of primers and probe (Table 1) to amplify and detect a highly conserved sequence within viral RNAs of the NDV M gene [29]. The thermal profile for RT-PCR involved an initial cycle at 50^{o}C for 30 minutes, followed by a subsequent cycle at 95 ^{o}C for 15 minutes. The PCR cycling protocol comprised 40 cycles, which included (i) 94 ^{o}C for 15 seconds, (ii) 52 ^{o}C for 30 seconds, and (ii) 72 ^{o}C for 10 seconds. The cycle thresholds (Ct) values were established after the calculation of the standard curve. The EID_{50} of virulent NDV genotype VII 1.1 strain from the samples was determined based on the Ct values, utilizing a standard curve created from known RNA concentrations of the reference virus. The detection limit for each qRRT-PCR run was documented using the standard curve. The results were reported as EID_{50}/mL equivalents.

Table 1: Oligonucleotide primers and probes used in this study

Target gene	Primer sequence (5`-3`)	Application	Reference
β-actin	F: CACCACAGCCGAGAGAGAAAT R: TGACCATCAGGGAGTTCATAGC	Housekeeping gene	**[26]**
IL-6	F: AAATCCCTCCTCGCCAATCT R: CCCTCACGGTCTTCTCCATAAA	Cytokine analysis	**[26]**
IL-8	F: ATGAACGGCAAGCTTGGAGCT R: GCCATAAGTGCCTTTACGATCAG	Cytokine analysis	**[27]**
IFN-γ	F: AAGTCATAGCGGCACATCAAAC R: CTGGAATCTCATGTCGTTCATCG	Cytokine analysis	**[26]**
M	F: AGTGATGTGCTCGGACCTTC R: CCTGAGGAGAGGCATTTGCTA Probe:[FAM]TTCTCTAGCAGTGGGACAGC CTGC[TAMRA]	One step real time RT-PCR for detection and quantitation of NDV	**[29]**

Statistical analysis

The statistical analyses were achieved using GraphPad Prism version 8.00 for Windows, developed by GraphPad Software, San Diego, CA, USA (www.graphpad.com). Each quail RNA sample was tested in duplicate. ANOVA was used to assess variations in the expression level of cytokine genes among quails infected with NDVs of varying pathogenicity. Two-tailed Student's t-test was used to compare NDV virus titer data. Standard deviations were computed using the relative expression ratios of three replicates for each gene measured, and p-values < 0.05 were regarded as statistically significant.

Results

Velogenic and lentogenic NDV experimental infection lesion scores

In our previous study [13], the pathogenicity of the velogenic and lentogenic NDV strains in Japanese quails was investigated. Quails infected with the velogenic NDV began to exhibit mild symptoms of ND (conjunctivitis, nasal discharges and/or slight depression) 72 h post-infection. The most frequently observed clinical signs were nervous signs (at 8 dpi), accounting for 35.7%, followed by respiratory signs (25%) and finally the digestive signs (11%). In LaSota-infected group, mild lacrimal and nasal discharges were seen at 2 dpi. To further assess the virulence of these two viral strains in quails, the lesion scores following experimental NDV infection were calculated. The lesions were observed in the trachea, lungs, proventriculus, cecal tonsils, spleen and brain. Particularly, lesions in the brain and cecal tonsils seemed more pronounced and severe, with a score of 0.4±0.68 and 0.35±0.74, respectively. Conversely, the lowest score (0.1±0.31) was documented in proventriculus and spleen. In quails infected with lentogenic NDV strain, the only observed lesion was mild hemorrhages in trachea, which received a score of 0.05±0.22 (Figure 2). The sham-inoculated negative control group remained normal throughout the duration of the experiment. No mortalities were recorded in any of the experimental groups.

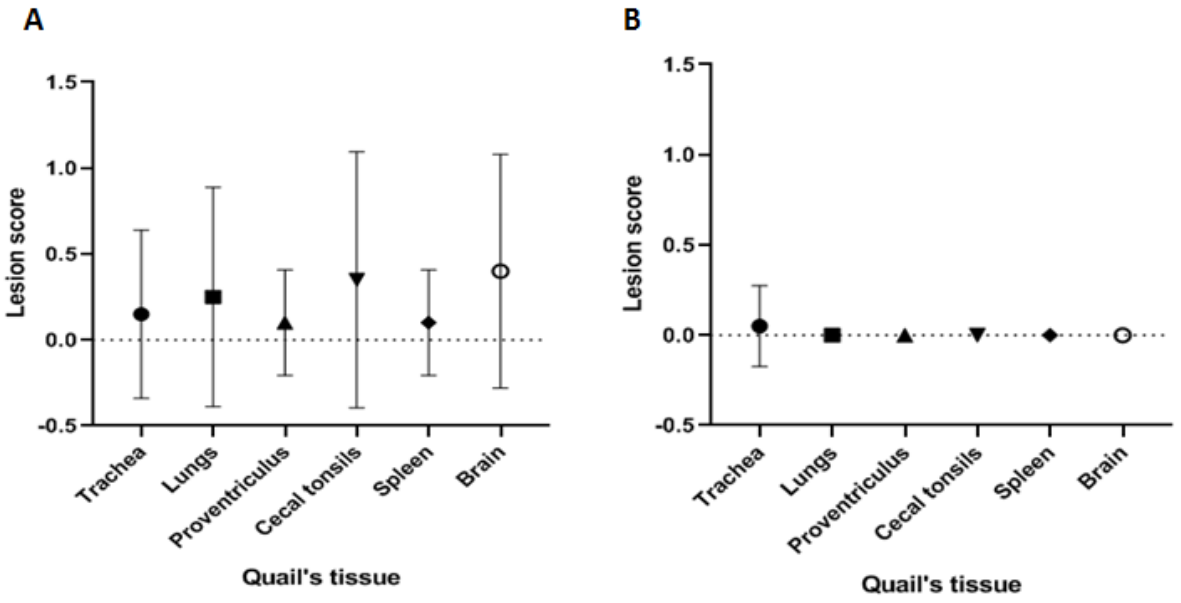

Figure 2: Postmortem lesion score in tissues of quails experimentally infected quails with NDVs of different pathogenicity; velogenic NDV genotype VII 1.1 (A) or lentogenic LaSota NDV genotype II (B). Lesions were scored on a scale ranging from 0 (no lesions) to 3 (severe). Data is presented as the mean values ± standard deviation (n = 20).

Expression patterns of immune-related genes in NDV-infected quails

A quantitative assay was employed to measure the expression profiles of immune-related genes in the spleen of quails infected with genotype VII 1.1 NDV strain at 1, 3, 5 and 10 dpi. Additionally, the assay was conducted on the lungs of quails infected with both genotypes VII 1.1 and II NDV strains at 1, 3 and 5 dpi. Both genotypes generally resulted in notable increase in the levels of pro-inflammatory cytokines, chemokines and Th1 cytokines in the spleens of infected birds. The overall gene expression profiles, displaying the average expression of all analyzed genes, along with the corresponding values are depicted in (Figures 3 and 4). The analysis of the mRNA expression revealed up-regulation of *IL-6*, *IL-8*, and *INF-γ*, upon infection, as compared to the sham-infected negative control group.

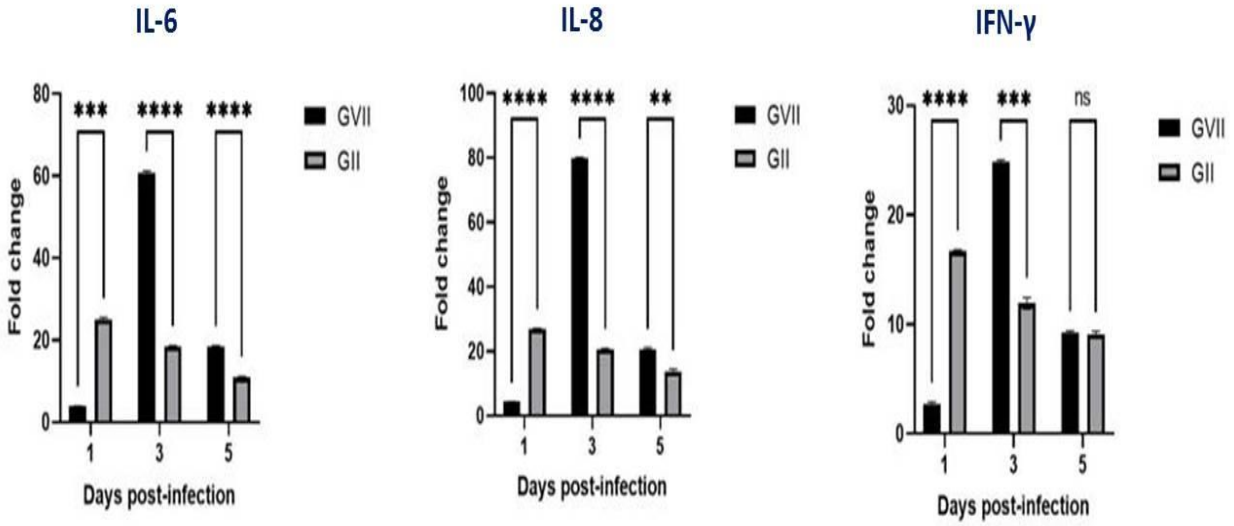

Figure 3: Differential *IL-6*, *IL-8*, and *IFN-γ* mRNA expression in lungs of quails infected with velogenic and lentogenic NDV at 1, 3 and 5 dpi. A comparison between the two groups (GVII and GII) was performed using two-way ANOVA with Šídák's multiple comparisons test, allowing for concurrent representation of each time point. The data are displayed as fold changes in cytokine mRNA levels ± Standard deviation relative to those observed in sham-inoculated negative controls; ns means non-significant, ** denotes $p \leq 0.01$ *** indicates $p \leq 0.001$ and **** stand for $p \leq 0.0001$. Gene expression was assessed by qRRT-PCR and normalized against the β-actin gene expression. Fold change was determined using the $-\Delta\Delta$ *Ct* method.

In the lungs, on the first day post-infection, the LaSota NDV strain induced a statistically significant elevation in *IL-6* ($p \leq 0.001$) and IL-8 and IFN-**γ** transcripts ($p \leq 0.0001$). By day 3 post-infection, the oculonasal inoculation with the velogenic NDV strain resulted in a statistically significant peak in *IL-6* and *IL-8* mRNA levels ($p \leq 0.0001$) when compared to LaSota-infected quails. The fold changes in mRNA transcripts

in relation to sham-inoculated negative control samples were 60.8 and 79.7 for *IL-6* and *IL-8*, respectively (Figure 3). In the spleen, there was significant up-regulation of mRNA expression of *IL-6*, *IL-8* and *INF-γ* at 3 and 5 dpi in quails infected with velogenic NDV, in comparison to the levels recorded at 1 and 10 dpi ($p \leq 0.0001$). Splenic *IL-8* expression in the velogenic NDV-infected quails was found to be the highest, (99.2 fold change) at 3 dpi (117.6 fold change) at 5 dpi (Figure 4).

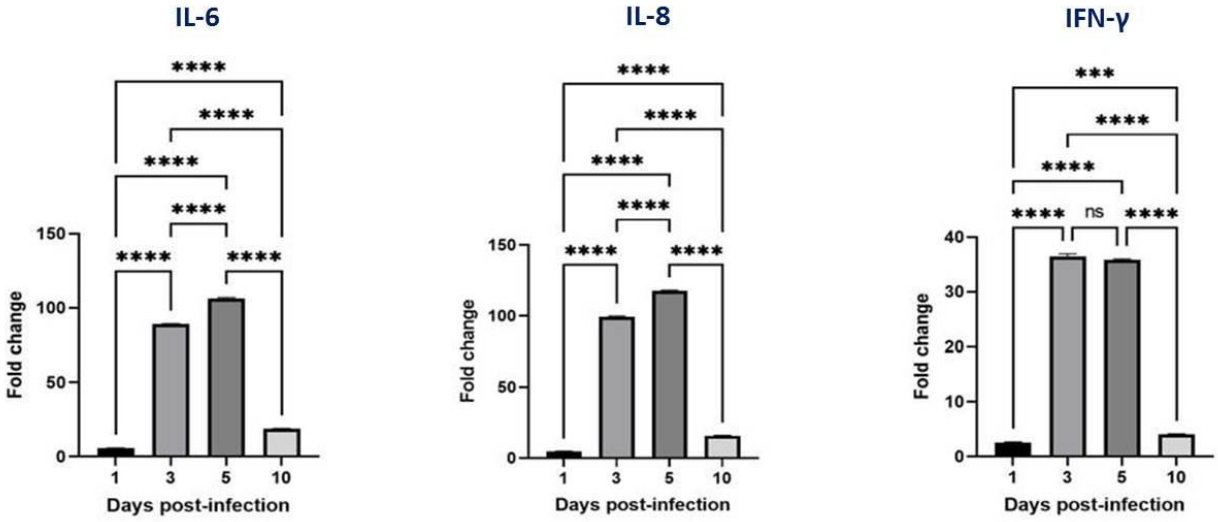

Figure 4: Differential *IL-6*, *IL-8*, and *IFN-γ* mRNA expression in the spleens of quails infected with NDV/Chicken/Egypt/ALEX/ZU-NM99/2019 at 1, 3, 5 and 10 dpi. . The data are displayed as fold changes in cytokine mRNA levels ± Standard deviation relative to those observed in sham-inoculated negative controls (Tukey`s multiple comparison one-way ANOVA); ns means non-significant, *** indicates $p \leq 0.001$ and **** denotes $p \leq 0.0001$. Gene expression was assessed by qRRT-PCR and normalized against the β-actin gene expression. Fold change was determined by the $-\Delta\Delta Ct$ method.

Detection of NDV in quail's tissues by qRRT-PCR:

The NDV load was noticed by qRRT-PCR in the tissues of infected quails at 3, 5, 7 and 10 dpi. No viral RNA was found in the tissues of sham-inoculated control quails. In contrast, the viral RNA was detected in all quails at 3 and 5 dpi for both genotypes. The quantity of the virus in the spleen of velogenic NDV-infected quails was significantly higher ($P < 0.05$) at 5 dpi. The virus titer in the spleen ranged from 3.1±0.3 to 4.8±0.6 $\log_{10}$/g tissue was recorded. Generally, viral load of LaSota strain in lungs was higher at 3 dpi than at 5 and 7 dpi.

Discussion

Newcastle disease is one of the most critical diseases in both poultry and wild bird populations, primarily due to its high levels of morbidity and mortality, as well as its global prevalence, and threat of considerable economic losses to avian industries [6]. Significant research

efforts have been directed towards the development of innovative vaccines and therapeutics; however, the dynamics of NDV interaction with its host remain incompletely elucidated. It is well established that the host's response to viral infections significantly influences the clinical outcomes. For instance, distinct patterns of immune-related cytokine expression have been observed to be correlated to lentogenic and velogenic NDV infections, with the modulation of cytokine responses being pivotal in the pathogenesis of NDV in chickens [19]. Conversely to chickens, Japanese quails showed mild or asymptomatic disease when infected with velogenic strains of NDV [30, 31]. Accordingly, they are considered to act as natural reservoirs of NDV. Nevertheless, the precise molecular mechanisms that account for the differing clinical outcomes of NDV infections among chickens and Japanese quails remain unclear. In the present study, variances in gene expression profiles and viral loads in tissues of Japanese quails infected with velogenic or lentogenic strains of NDV were investigated.

Considering their crucial roles in regulating the immune responses, cytokine and chemokine gene expression levels are widely utilized as indicators of the host's immune responses to viral infections [32]. The modulation of specific cell populations, as macrophages and T-cells during an infection can influence cytokine expression, consequently affecting various components of immune function [33]. Despite the implementation of vaccination, genotype VII NDV remains the most frequently isolated strain from vaccinated birds across numerous regions globally. Hence, it is essential to further investigate the significance of viral load and the varying cytokine patterns that emerge following infection with virulent NDV strains of genotype VII, particularly in relation to their impact on vaccine-induced immune responses. The two primary components of adaptive immunity, governed by the balance of Th1 and Th2 cytokines [34]. This study focused on profiling the mRNA expression level of cytokines linked to Th1-like responses. Interferon-gamma (*IFN-γ*), classified as a type II interferon, is known to stimulate specific immune responses, particularly the cell-mediated immunity [19, 35]. The infection with both NDV genotypes resulted in pronounced pro-inflammatory and Th1 responses which might significantly contribute to the virus's pathogenesis. The expression of lung IFN-γ was significantly higher in the LaSota-infected group than velogenic NDV-infected group at 1 dpi ($P \leq 0.0001$). Conversely, at three dpi, the velogenic NDV-infected group exhibited significantly elevated levels ($P \leq 0.001$). The findings indicated that infection with NDV led to induction of *IFN-γ* in lung and spleen tissues of quails. Likewise, previous studies revealed that the mRNA expression of *IFN-γ* was increased in the spleens of chickens infected with NDV and in NDV-infected peripheral blood mononuclear cells *in-vitro* [19, 34, 36, 37]. The acute viral infection was characterized by heightened expression of *IFN-γ* in the tissues of quails, underscoring its significance in combating NDV infection. Raised levels of IFNs typically exert an inhibitory effect on NDV replication. The previous findings indicated that mortality induced by virulent NDV was preceded by ferociously secreted IFN-γ. Consequently, the protective role of

IFN-γ was further confirmed by introducing chicken IFN-γ into the virulent NDV ZJ1 strain. The findings exposed a protective effect against virulent NDV infection *in-vivo*, although this effect was not observed *in-vitro* [38]. The application of chicken IFN-γ to chicken embryonic fibroblasts (CEFs) provoked an antiviral atmosphere characterized by the presence of interferon-stimulated genes [39]. It is important to highlight that the levels of IFN expression do not always determine the pathogenicity of the strains. The expression of IFNs is specific to the type of cell; for example, primary chicken intestinal epithelial cells (IECs) yield a more robust IFN response when infected with the Herts/33 NDV strain compared to CEFs [40].

Along with IFN-γ, the innate host response to viral infections encompasses the release of proinflammatory cytokines, including IL-6, as well as chemokines such as IL-8 in various avian species, including chickens [16, 27], ducks [41], and geese [42]. In this context, the NDV infection resulted in an evident rise in *IL-6* expression in the lungs and spleens of infected quails at 3 dpi and 5 dpi. Meanwhile, the *IL-8* expression was also up-regulated in the lungs and spleens. These findings align with the previous studies targeting the NDV infection in chickens [27, 43]. Besides, the result displayed that the expression of different cytokines in lungs was higher at 1 dpi following LaSota infection compared to those observed with velogenic NDV infection. The virulent genotype VII GD strain was found to enhance the levels of IL-6 mRNA in the lungs and Harderian glands of one-day-old chicks at 48 h post-infection [27]. In contrast, the velogenic F48E9 strain resulted in only a slight increase in IL-6 expression in both CEFs and bursa of Fabricius of chickens, while the lentogenic LaSota strain didn't affect IL-6 levels [39, 44, 45]. Here, the LaSota NDV strain led to a statistically significant rise in both IL-6 and IL-8 transcripts at 24 h post-infection. These levels exhibit considerable variability depending on the viral strains, cell types, and animal species involved. The innate immune signaling in birds is a highly varied, multifaceted, interconnected, and precise process that relies on numerous factors [39]

Although, cytokines are recognized as fundamental inducers of specific immune responses preventing viral replication upon virus invasion, their overproduction might result in a cytokine storm, thereby exacerbating the harmful effects of inflammation on the host [46]. It was noted that the lesion score in lungs were 0.25±0.64, while the spleen recorded the lowest score (0.1±0.31). The virulence of NDV is influenced by several factors, including the specific tissues or organs it targets, its capacity to evade host's immune defenses, and its replication efficiency. The LaSota strain induces high levels of cytokines on day 1 post-infection; however, high viral RNA levels were recorded at 3 dpi. Obviously, the velogenic NDV was also able to replicate in spleen, resulting in significantly high viral load by 5 dpi. A greater viral load was perceived to correlate with an earlier peak in the expression of proinflammatory cytokines in the spleens of velogenic NDV-infected quails at 3 dpi and reached their peak at 5 dpi. A similar trend was previously documented in IBS002 infected spleen at 3 dpi, where the replication rate of the virus significantly impacted the expression of pro-

inflammatory cytokines [20]. Earlier studies indicated that two strains of NDV, namely JS3/05 and JS5/05 exhibited enhanced replication capabilities, and elicited more robust immune responses in chicken splenocytes at 6, 12, and 24 hpi *in-vitro* [18] or in the thymus, spleen, and bursa of chickens at 24, 48, and 72 hpi *in-vivo* [21]. These findings demonstrated that the pronounced immune response associated with velogenic NDV strain, along with JS3/05 and JS5/05 strains, may be correlated with elevated viral loads in infected cells or tissues during the initial phase of infection. Nonetheless, further research is required to explore the underlying molecular mechanisms. These findings suggest that elevated cytokine levels induced by velogenic NDV may be linked to accelerated viral replication, potentially allowing the virus to circumvent the antiviral effects of these cytokines. Furthermore, our findings, along with those of Hu *et al.* and Zhang *et al.* [18, 27] indicated that elevated *IFN-γ* expression correlated with a higher viral load in comparison to F48E9. Cytokines play a crucial role in eliciting a specific immune response that can thwart viral invasion. IFN-γ facilitates the release of nitric oxide, which leads to the recruitment of additional neutrophils and macrophages. As well, *IL-6* is instrumental in regulating the inflammatory and immune responses that are essential for the activation, proliferation, and differentiation of T lymphocytes [47].

Conclusion

This study revealed notable variations in the mRNA expression levels associated with pro-inflammatory responses, Th1 cytokines and chemokines in quails infected with two NDV genotypes exhibiting varying pathogenicity. The responses associated with the highly virulent NDV strain were found to be linked to the viral load. The patterns and magnitude of gene expression were comparable across the two genotypes. Genotype VII exhibited high viral load at an earlier time points, eliciting a more cytokine response in comparison to genotype II, LaSota strain. Ongoing research is focused on further investigating the differences in the load of virus and cytokine expression patterns in other genotype VII NDV strains, as well as their significance in the context of immune responses triggered by vaccines against virulent NDV challenges.

Conflict of Interest

All authors declare that they have no conflicts of interest.

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الملخص العربى

الإستجابات المناعية الفطرية فى السمان المصاب بفيروس باراميكسوفيروس الطيور-١ ذي الإمراضية المختلفة

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يتسبب فيروس مرض نيوكاسل فى خسائر اقتصادية فادحة بقطاع الدواجن عالميًا. يُعدّ السمان الياباني مستودعًا طبيعيًا لفيروس نيوكاسل، ويلعب دورًا هامًا في وبائياته. لقد أثبتت الدراسات السابقة مستويات حساسية متفاوتة لدى السمان الياباني للإصابة بفيروس النيوكاسل؛ إلا أن الآليات الكامنة وراء ذلك لا تزال غير مفهومة جيدًا. في هذه الدراسة، تم وصف الإستجابة المناعية الفطرية لدى السمان لسلالات فيروس نيوكاسل ذات التكاثر السريع والتكاثر البطيء. لوحظ وجود استجابة واضحة في الرئتين والطحال بعد فترة قصيرة من الإصابة بالفيروس، مما أدى إلى تحفيز جينات مرتبطة بالإستجابات الفطرية المضادة للفيروسات والمحفزة للالتهابات. في مجموعة النمط الجيني VII 1.1 لفيروس مرض النيوكاسل، لوحظ زيادة في التعبير عن الإنترلوكين-٦ (IL-6) والإنترلوكين-٨ (IL-8) والإنترفيرون-جاما (IFN-γ) بعد ٣ أيام من الإصابة، وذلك قبل الوصول إلى أعلى نسبة في الطحال بعد ٥ أيام من الإصابة. على العكس، أظهرت مجموعة LaSota استجابة مختلفة للسيتوكين، مع زيادة ملحوظة في الرئة في اليوم الأول بعد الإصابة. أظهر النمط الجيني VII 1.1 وجود أعلى عيارية للفيروس في الطحال بعد ٥ أيام من الإصابة. في الوقت نفسه، كانت عيارية الفيروس للـ LaSota أعلى بكثير بعد ٣ أيام من الإصابة. تشير هذه النتائج إلى أن العدوى بعترات فيروس مرض النيوكاسل ذات الضراوة المتفاوتة ترتبط بوجود عيارية مختلفة للفيروس ونمط فريد للتعبيرعن السيتوكين في الأنسجة، مما يشير إلى أن استجابة العائل يمكن أن تلعب دورًا في علم الأمراض الخاص بفيروس مرض النيوكاسل فى السمان. كما تُؤكد النتائج وجود اختلافات في الإستجابات المناعية الفطرية، وتطور المرض بعد الإصابة بفيروس مرض النيوكاسل. يتطلب إجراء المزيد من البحوث لتوضيح المسارات الجزيئية المرتبطة بعدوى فيروس مرض النيوكاسل.