Comparative transcriptome analysis of spleen of Newcastle Disease Virus (NDV) infected chicken and Japanese quail: A potential role of NF-κβ pathway activation in NDV resistance

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Abstract

Newcastle disease (ND) affects a few hundred avian species including chicken and several species of domestic and wild birds. The clinical outcome of Newcastle disease virus (NDV) infection ranges from mild to severe fatal disease depending on the NDV pathotype and the host species involved. Japanese quails serve as natural reservoirs of NDV and play important role in NDV epidemiology. While infection of chicken with velogenic NDV results in severe often fatal illness, the same infection in Japanese quails results in inapparent infection. The molecular basis of this contrasting clinical outcomes of NDV infection is not yet clearly known. We compared global gene expression in spleen of chicken and Japanese quails infected with lentogenic and velogenic NDVs. We found contrasting regulation of key genes associated with NF-κB pathway and T-cell activation between chicken and Japanese quails. Our data suggests association of NDV resistance in Japanese quails to activation of NF-κB pathway and T cell proliferation.

Keywords: Newcastle Disease Virus, chicken, Japanese quail, global gene expression, NGS

Introduction

Newcastle disease (ND) continues to be one of the most economically important viral diseases affecting poultry and many other avian species globally [1] . Newcastle disease virus (NDV) is a negative-sense, single-stranded RNA virus, also known as avian paramyxovirus- 1 (APMV-1), and has been classified in the genus Avulavirus of the family *Paramyxoviridae* [2]. It is transmitted by ingestion or inhalation, and infected birds can show gastrointestinal, respiratory and/or nervous signs, with mortality up to 100%, depending on the host species and the pathotype of the virus involved [3].

Predominantly, ND is exhibited in different forms such as lentogenic, mesogenic, velogenic and asymptomatic enteric [4]. In chickens, lentogenic NDV, such as the LaSota strain, causes mild or inapparent respiratory disease. Infection with mesogenic NDV strains leads to moderate respiratory and nervous signs. In contrast, velogenic NDV pathotypes, such as strain 2K3, cause severe gastrointestinal and neurological complications.

Global gene expression studies provide a comprehensive view of the host response to virus infection at molecular level. Various techniques such as cDNA microarray analysis [5], suppression subtractive hybridization (SSH) [6], and the specific Affymetrix exon chip assay [7] have been used for transcriptome analysis of NDV-infected chickens. However, very limited work has been carried out using RNA sequencing transcriptome analysis of samples from NDV-infected birds [8]. The NGS and RNA-Seq techniques have expanded our ability to better understand the extent and complexity of transcriptomes [9].

It is widely known that host response to viral infection plays a key role in the clinical outcome of infection. For example, distinct immune-related cytokine expression patterns have been found to be associated with velogenic and lentogenic NDV infections, and modulation of cytokine responses plays a key role in NDV pathogenesis in chickens [10]. In contrast to chickens, Japanese quails (*Coturnix japonica*) show mild or inapparent illness when infected with velogenic NDV strains [11]. As a result, they are considered to serve as natural reservoirs of NDV. However, the precise molecular basis for the differences in clinical outcome of NDV infection between chicken and Japanese quail is not known. In this study, differences in global gene expression profiles of chicken and Japanese quail infected with velogenic or lentogenic strains of NDV were investigated using next generation sequencing (NGS).

Methods

Experimental birds

Fertile specific pathogen free (SPF) hen's eggs were procured from M/s Venkateswara Hatcheries Pvt Ltd, Pune, India. Japanese quail eggs were procured from the Poultry Research Station (PRS), Madhavaram, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai, India. The embryonated chicken and Japanese quail eggs were hatched at the PRS-TANUVAS, and the chicks after hatch were maintained without vaccination in a barrier-controlled environment to prevent infection. The study was approved by the Institutional Animal Ethics Committee (IAEC) of TANUVAS (Approval number: 3028/DFBS/2014) and all experiments were performed according to institutional guidelines.

Virus infection study

An initial virus infection experiment was performed to establish the optimum time post-infection and tissue of choice for transcriptome analysis. Three-week-old SPF chickens and Japanese quails negative for NDV antibodies as determined by hemagglutination inhibition (HI) assay were each randomly assigned to three groups of 20 birds. The birds were infected with 10^7 50% egg infectious doses (EID_{50}) of the LaSota or 2K3 NDV strains or mock-infected with PBS by the oculo-nasal route (Supplementary Figure 1). All virus stocks were maintained at the department of Animal Biotechnology, Madras veterinary College and were propagated in 9 days old embryonated chicken eggs. All the birds were monitored regularly, and clinical signs and mortality, if any, were recorded. Two birds in each group were euthanized daily from day 1 until the tenth day post-infection (DPI). Spleen, trachea, proventriculus, cecal tonsils and brain were collected from euthanized and dead birds. Tissues were used for RNA extraction to detect NDV by RT-PCR using 'M' gene specific primers (forward: 5′-ATCACCACCTATGGATTC-3′; reverse: 5′-AGTATTAGTTGCACTCTT-3′). A second experiment was carried in which groups of six 3-week-old NDV-negative chickens and quail were infected as described for the first experiment. At 24hr post-inoculation, all birds were euthanized and spleens were harvested for RNA extraction (Supplementary Figure 1). Pooled RNA samples from each group were used for NGS analysis. Key genes identified were further validated by qRT-PCR using the original total RNA samples.

Library preparation and sequencing by NGS

Total RNA was isolated using TRIzol (Invitrogen) followed by treatment with DNase. Ribosomal RNA contamination was evaluated by RNA 6000 Pico Chip kit (Agilent, USA) using an Agilent 2100 BioAnalyzer.

Ribosomal RNA was depleted using a RiboMinus Transcriptome Isolation kit (Agilent, USA), and the remaining RNA was subjected to fragmentation to obtain approximately 200 bp length fragments, which were reverse transcribed into double-stranded cDNA. The cDNA was treated to generate blunt ends, then adapter sequences were ligated to both ends of the cDNA. High-throughput sequencing of the cDNA library was carried out on an Illumina HiSeq 2000 platform (Illumina, USA).

RNA-Seq quality control

Read quality check parameters such as base quality score distribution, sequence quality score distribution, average base content per read, GC distribution in the reads, PCR amplification issues, over-represented sequences, biasing of kmers, and read-length distribution were analyzed from the FASTQ file obtained. From the trimmed paired-end reads, contaminating sequences were removed, including non-polyA tailed RNAs, mitochondrial genome sequences, ribosomal RNAs, transfer RNAs, adapter sequences and others. The contamination removal step was performed for chicken samples using the help of Bowtie 2 (version 2.2.2), in-house Perl scripts and Picard tools (version 1.115). No contamination removal step was performed for Japanese quail samples as the transfer RNA and ribosomal RNA sequences were not available for Japanese quail.

The pre-processed reads of both chicken and Japanese quail origin were aligned to the *Gallus gallus* (chicken) reference genome and gene model downloaded from Ensembl database (ftp://ftp.ensembl.org/pub/release83/fasta/gallus_gallus/dna/Gallus_gallus.Galgal4.dna.toplevel.fa.gz). The alignment was performed using the Tophat program (version 2.1.0) with default parameters. The aligned reads were then used for estimating the expression of the genes and transcripts in fragments per kilobase of exon per million reads mapped (FPKM), using the Cufflinks tool suite (version 2.2.1).

Transcriptome analysis

Differential gene expression (DEG) analysis was performed using the Cuffdiff program of the Cufflink package with default settings to assess differences between uninfected control vs. infected chicken samples and uninfected control vs. infected Japanese quail samples. Upregulated and downregulated genes were identified as having differential gene expression values with $p \leq 0.05$. Functional analysis of all the upregulated and downregulated genes based on biological process functions was done manually by blasting the gene ID in the NCBI GenBank database, UniProt, and OMIM, and the functional information was gathered for all the genes. Genes involved in innate immune response,

apoptotic activity, and antiviral activity were selected for further comparison between chickens and Japanese quail. Transcriptome data is available at GEO database accession number GSE98296.

Verification of selected gene expression by quantitative reverse transcription PCR (qRT-PCR)

To validate expression of selected genes, 500 ng of total RNA was converted into cDNA using the High-Capacity cDNA Reverse Transcription Kit containing MultiScribe™ Reverse Transcriptase (Applied Biosystems, USA) according to the manufacturer's instructions. Quantitative PCR (qPCR) was performed using SYBR Green Master mix (TaKaRa, Japan) in an Eppendorf Mastercycler (Eppendorf, Germany). Primer sequence details of host-specific genes, chicken β-actin, and quail β-actin are listed in Table 1. The RT-qPCR data were analyzed using the ΔΔCt method and the expression was normalized to β-actin.

Transcriptome analysis

At least 21 million Illumina paired-end reads were generated for each infection-type group. Of these, an average of 87.9% of total reads per group were of high-quality with Q score ≥30. The average GC distribution of all the samples was found to range between 50 to 55%. The overall alignment results showed that 99.99% of reads were QC passed.

Statistical analysis

Standard error of mean (SEM) calculation and one-way ANOVA analysis followed by Tukey's multiple comparison test were performed using GraphPad Prism (Version 5.04).

Result

Contrasting clinical outcome of NDV infection between chicken and Japanese quail

No clinical signs were noticed during the observation period of 10 days in either chickens or Japanese quails infected with the lentogenic LaSota strain of NDV. However, chickens infected with the velogenic 2K3 strain exhibited dullness, respiratory distress and greenish diarrhea at 2 DPI with mortality on 4 DPI. Notably, Japanese quail infected with NDV 2K3 showed no clinical signs with no mortality. NDV nucleic acid could be detected in the spleen of all chicken and Japanese quail infected with LaSota and 2K3 (Table 2). However, viral nucleic acid was detected in the brain tissue of chicken and Japanese quail infected with 2K3 and not LaSota (Table 2).

Velogenic NDV causes differential regulation of many more genes than lentogenic NDV

Significantly, differentially expressed genes ($p<0.05$) in samples from virus-infected chickens and Japanese quails are furnished in Figure 1. Four genes were upregulated and 19 genes were downregulated in LaSota-infected chickens

compared with mock-infected controls. In contrast, 79 genes were upregulated and 4 genes were downregulated in 2K3-infected chickens compared with mock-infected controls. Five genes were upregulated and 18 genes were downregulated in LaSota-infected Japanese quail compared with mock-infected controls. In contrast, 54 genes were upregulated and 69 genes were downregulated in 2K3-infected Japanese quail compared with mock infected controls. Notably, major differences in gene expression profiles were observed between chicken and Japanese quail infected with the 2K3 strain but not with the LaSota strain of NDV.

Genes involved in key biological processes are differentially regulated by NDV infection

GO analysis was carried out on significantly (p-value ≤ 0.05) differentially expressed transcripts in samples from virus-infected chicken and quail compared with samples from mock-infected controls (Figure 2). Genes involved in key biological processes including immune response, transcriptional regulation, inflammation, G-protein coupled receptor signaling, and NF-κB signaling were differentially expressed due to NDV infection in both chicken and Japanese quail. Notably, chemokine-mediated signaling was only differentially regulated in 2K3-infected Japanese quail.

Contrasting transcriptional regulation of genes associated with apoptosis between chickens and Japanese quail infected with the velogenic 2K3 strain of NDV

Transcriptional regulation of host genes associated with antiviral responses, immune responses, and apoptosis was compared (Table 3). A number of genes associated with regulation of apoptosis were differentially regulated in 2K3-infected quail compared with mock-infected quail. Notably, transcriptional regulation of all these genes was not affected by 2K3 infection in chickens. However, a few additional apoptosis genes were upregulated by 2K3 infection in chickens that were unaffected in quail. Such differences were not noticed between LaSota-infected chickens and quail (Table 3).

Many more immune and host antiviral response genes were upregulated in 2K3-infected chicken compared with Japanese quail

Contrasting expression profiles of key immune and antiviral genes were observed between 2K3-infected chickens and quail (Table 4). Several genes involved in mediating host immune and antiviral responses were significantly upregulated in 2K3-infected chicken but not the quail (Table 4). In contrast, two of these genes and several others were significantly downregulated in Japanese quail infected with 2K3. Transcriptional regulation of these types of genes were not greatly affected in either chicken or Japanese quail infected with the LaSota strain.

A select number of genes that could be functionally relevant to NDV pathogenesis have been identified

Based on the comparative analysis of transcriptomes of NDV-infected chicken and quail, a select number of genes have been identified that could be important in NDV pathogenesis (Figure 3). A notable contrasting transcriptional regulation of immune responsive gene 1 (IRG1) and interleukin 4-induced gene-1 (IL4I1) was observed. Expression of both *IRG1* and *IL4I1* was upregulated in chicken but downregulated in quail infected with the 2K3 strain.

Using the short-listed genes of potential relevance, two major host pathways that could be important in NDV pathogenesis were identified, NF-κB signaling and T-cell proliferation. The expression levels of these key genes were further validated by RT-qPCR, which corroborated the observations made by NGS (Figure 4).

Discussion

Differences in clinical outcome between chickens and Japanese quails infected with a lentogenic or velogenic NDV strain [11] were confirmed. As expected, the lentogenic LaSota strain infection did not cause notable clinical signs in either chicken or Japanese quail. However, the velogenic 2K3 strain caused significant clinical signs in chicken at 2 DPI and culminated in 100% mortality by day 5.

Viral nucleic acid and antigen was consistently found in the spleen but not in the brain of chickens and Japanese quail infected with the LaSota strain from 24 hpi until 10 DPI. Similarly, NDV nucleic acid and antigen was found in spleens of velogenic 2K3 infected chicken and Japanese quail from 24hpi. In contrast, after infection with the velogenic 2K3 strain, virus was also found in the brain of chickens by 2 DPI and of quail by 3 DPI. This suggests a difference in the tissue tropism between the two pathotypes and that there is a delay in virus spread to the brain in quail compared to chickens. Notably, despite the presence of virus in the spleen and brain, the quail did not show any significant clinical signs throughout the study.

Many genes were differentially regulated by 2K3 infection compared to LaSota infection in both chickens and Japanese quails. This suggests that the infection by velogenic strains that cause severe disease is associated with greater host gene dysregulation compared to infection by lentogenic strains that cause mild disease. It is widely known that dysregulation of host immune genes contributes to the severity and the outcome of viral infections such as avian influenza in chickens [12, 13].

Differences in the transcriptional regulation of several genes involved in apoptosis were found between the hosts and viruses. For example, basic transcription factor 3 (BTF3), was downregulated in chicken and Japanese quail infected

with the LaSota strain but not the 2K3 strain. BTF3 encodes an essential subunit of the nascent polypeptide-associated complex, suppressing apoptosis [14] and acts as a transcriptional regulator of several genes that suppress apoptosis. This contrasts with infectious bronchitis virus (IBV) infection in chicken, where greater levels of apoptosis are associated with increased pathogenicity of IBV strains in renal and tracheal tissues [15]. Heterogeneous nuclear ribonucleoprotein K (hnRNPK), which is associated with suppression of hepatitis C virus particle production in mammalian cells [16], was downregulated in chicken and Japanese quail infected with the LaSota but not the 2K3 strain.

Many more immune-related genes were upregulated in 2K3-infected chickens that were not affected in Japanese quail. This observation is in accordance with an earlier observation of greater immune gene activation in highly pathogenic avian influenza (HPAI) H5N1 infected chicken compared with ducks [13].

Notable differences were found in the transcriptional regulation of genes associated with NF-κB signaling and T-cell proliferation between 2K3-infected chickens and Japanese quails. Immune responsive gene 1 (IRG1), was up-regulated in chicken but was downregulated in quail after 2K3 infection. Notably the transcriptional regulation of IRG1 is much more subtle in both chicken and Japanese quail following LaSota infection. It is known that endogenous expression of IRG1 plays a pivotal role in regulating the NF-κB pathway which involved in the antiviral mechanism against the NDV infection [17]. IRG1 is a negative regulator of NF-κB pathway [18], through stimulating tumor necrosis factor alpha-induced protein 3, which in turn inhibits NF-κB signaling. In mammals, IRG1 is important in the maintenance of reduced inflammatory cytokine production, plays a major role in the immune response pathway and is one of the highest induced genes in macrophages under pro-inflammatory conditions [19]. IRG1 is also known to play a key role in the pathogenesis of many viral infections. For example, IRG1 is highly upregulated in the lungs of mice infected with influenza A virus that causes increased inflammation [20]. Suppression of IRG1 improves immune lung injury recovery after RSV infection by reducing ROS production [21]

Interleukin 4-induced gene-1 (IL4I1) was up-regulated in chicken but was downregulated in Japanese quail after 2K3 infection. IL4I1 [22, 23] is primarily expressed in professional antigen-presenting cells, such as macrophages and dendritic cells, and downregulates Th1-mediated inflammation by inhibiting T cell proliferation [24]. Expression of IL4I1 is restricted to lymphoid tissues and it is highly expressed in spleen and lymph nodes [25, 26].

To further confirm the role of NF-κB pathway and T-cell activation, expression levels of Annexin A1 (ANXA1), a negative regulator of T cell proliferation [27] and blocker of NF-κB binding to DNA [28], was investigated. RT-qPCR analysis revealed that expression of ANXA1 was upregulated in chickens and downregulated in Japanese quail infected with the 2K3 strain.

Conclusion

In conclusion, our results provide a proof of concept that, differences in pathogenicity of the velogeneic 2K3 strain of NDV in chickens and Japanese quails appear to be associated with activation of NF-κB signaling and T-cell proliferation. Further functional studies are necessary to establish the functional association between the activation of NF-κB pathway and NDV susceptibility and resistance.

Declarations

Ethics approval and consent to participate

Animal infection study was approved by the Institutional Animal Ethics Committee (IAEC) of TANUVAS (approval number: 3028/DFBS/2014) and all experiments were performed according to institutional guidelines.

Consent for publication

"Not applicable"

Availability of data and materials

Transcriptome data is available at GEO database accession number GSE98296.

Competing interests

The authors declare that they have no competing interests

Conflict of interest

All the authors declare that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Authors' contributions

S.V.K and K.K conceived the study. K.K, V.K and M.K.P.S designed the experiments and M.K.P.S carried out the experiments. R.H.N, M.K and J.D analyzed the data, V.K, K.K, J.D and S.V.K interpreted the data. M.K.P.S, K.K, R.H.N and S.V.K prepared the manuscript and all authors have read and approved the manuscript.

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Table 1: List of oligonucleotide primers for quantitative Real time PCR used in this study

Primer	Sequence (5' to 3')
IRG1-F	ACAGTRGTTGGTACAATGGG
IRG1-R	ACTCAAACRAAGCCCCTCC
IL4I1-F	AGACRGATGACAATGCCTGG
IL4I1-R	CCAGACCATGAACTGCAAGG
RSAD2-F	GAGGAGAACCATTTCTTCAGG
RSAD2-R	CGGTGGTTCAAGAAGTATGGTG
ANXA1-F	ACAACCAGGAGCAGGAATGT
ANXA1-R	ATCCYTCRGCTGATGTTTC
c-βactin-F	ATTGTCCACCGCAAATGCTTC
c-βactin-R	AAATAAAGCCATGCCAATCTCGTC
q-βactin-F	ACCCCAAAGCCAACAGA
q-βactin-R	CCAGAGTCCATCACAATACC

Table 2. Detection of NDV by qRT-PCR and IHC in virus infected chicken and Japanese quail tissues

Species	Organs	NDV-LaSota (DPI)										NDV-2K3 (DPI)									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
SPF chicken	Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	N	N	N	N	N
	Brain	-	-	-	-	-	-	-	-	-	-	-	+	+	+	N	N	N	N	N	N
Japanese quail	Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Brain	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-

N- No birds sampled at this time because all birds died.

Table 3. Genes involved in apoptosis that are differentially regulated in NDV infected chicken and Japanese quail

	chicken 2K3	quail 2K3	chicken LaSota	quail LaSota
NBN	2.10			
FAM188A	2.83			
BCAP29	1.91			
ANG-1	1.85			
TRIAP1		∞		
YKT6		-∞		
WIF1		-∞		
TM4SF1		-∞		
SIRT4		-∞		
RYR3		-2.20		
BTF3			-∞	-∞
HINT1			-∞	
TMEM14A				-∞
RPL39		2.96		
Ex-FABP		-2.72		
CAV2		-∞		
ESR1		-∞		
FABP3		-2.41		
FABP4		-∞		
Wpkci			-∞	-∞

Fold-Change Values of Transcripts Showing Up- or Down-Regulation for Genes Involved in Apoptosis

down-regulated	-∞	indicates no transcripts in virus-infected sample
up-regulated	∞	indicates no transcripts in control sample

Table 4. Genes involved in immune response that are differentially regulated in NDV infected chicken and Japanese quail

		chicken 2K3	quail 2K3	chicken LaSota	quail LaSota
immune response	IL4I1	4.36	-∞		
	ISG12(2)	4.18	3.99		2.88
	IFI27L2	3.99			
	IRG1	3.55	-2.25		
	XDH	2.11			
	CCLI10	3.04			
	C1S	1.92			
	CXCL13L2	2.94			
	IL18	2.80			
	LECT2	2.88			
	SOCS1	3.08			
	SOCS3	2.87			
	STAT1	2.67			
	TRANK1	2.77			
	AP4S1		-∞		
	CHIA		-∞		
	STC1		-∞		
	PTPRR		-2.73		
antiviral	IFIH1	3.06			
	IFIT5	4.58	3.34		
	DDX60	3.69	3.07		
	unknown protein, IFITM family	3.37	3.35		
	DHX58	2.92			
	LY6E	2.82			
	EIF2AK2	2.08			
	TLR3	2.72			
	MITD1	2.77			
	MOV10	2.18			
	PHF11	2.14			
	CMPK2	3.63			
	RSAD2	4.64			
	Mx	3.72			
	OAS*A	3.55			
	CCL19	3.07			
	RPL13		2.82		
	EPSTI1	2.19	2.67		
	FAM46C		-1.91		
	U4		-3.45		
	PLA1A		-2.17		
	ANXA1		-1.81		
	HNRPK			-4.55	-2.42
	PTGER2				-∞

Fold-Change Values of Transcripts Showing Up- or Down-Regulation for Genes Involved in Response to Viral Infection

down-regulated	-∞	indicates no transcripts in virus-infected sample
up-regulated	∞	indicates no transcripts in control sample

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Figure legends:

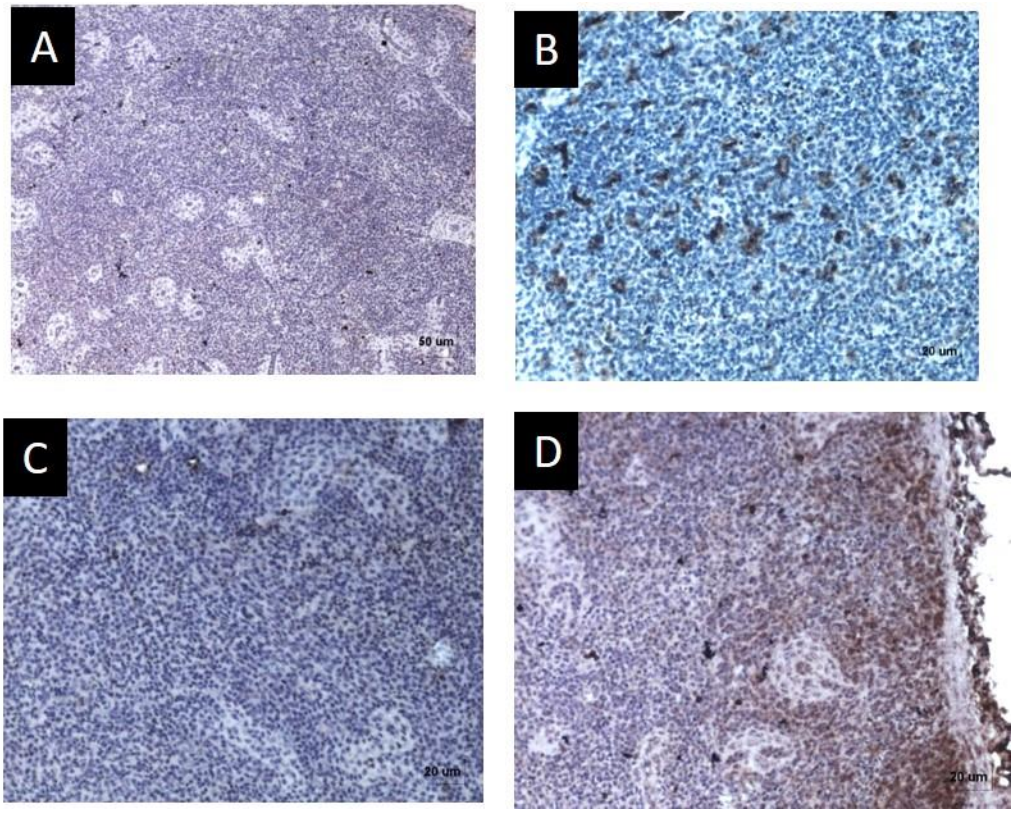
Figure 1: **Number of differentially expressed genes in virus-infected chicken and Japanese quail compared with controls.** Differential gene expression analysis of transcripts from spleen of chicken and Japanese quail infected with velogenic 2K3 or lentogenic LaSota strains of NDV was performed. Bar graph shows total number of downregulated (shaded) or upregulated (unshaded) transcripts.

Figure 2: **Gene ontology analysis of differentially regulated genes following virus infection.** Gene ontology analysis was performed on differentially-expressed gene transcripts from spleen of chicken and Japanese quail infected with velogenic 2K3 or lentogenic LaSota strains of NDV. The top ten biological processes of all gene ontology designations assigned to differentially expressed transcripts were recorded, and the percent of this total is plotted on bar graphs for both downregulated (red) and upregulated (green) transcripts.

Figure 3: **Differential expression profiles of selected genes in NDV-infected chicken and Japanese quail.** Differential gene expression analysis of transcripts from spleen of chicken and Japanese quail infected with velogenic 2K3 or lentogenic LaSota strains of NDV was performed. Genes with transcripts encoding known products and displaying up- or downregulation in more than one treatment group are shown. Relative expression values compared with uninfected animals of the same species are displayed. Shading represents level of gene expression, with bright red indicating downregulation, bright green indicating upregulation, and darker shades indicating lower degrees of up- or downregulation.

Figure 4: **Expression profiles of key genes that are potentially important in the pathogenesis of ND.** Quantitative PCR was performed on cDNA generated from RNA extracted from chickens or Japanese quail infected with the velogenic 2K3 or lentogenic LaSota strains of NDV. Expression levels were normalized against chicken or quail β-actin. Error bars indicate SEM from three replicates. * represents $p<0.05$ between chicken and Japanese quail which received the same NDV strain.

400 Figure 1



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403 Figure 2

	chicken 2K3		chicken LaSota		quail 2K3		quail LaSota	
BIOLOGICAL PROCESS	down	up	down	up	down	up	down	up
immune response	11%	11%	11%	11%	11%	11%	11%	11%
regulation of transcription, DNA-templated	5%	5%	6%	6%	6%	6%	6%	6%
inflammatory response	5%	5%	5%	5%	6%	6%	5%	5%
translation	5%	5%	5%	5%	4%	5%	5%	5%
antigen processing and presentation of peptide antigen via MHC class I	4%	4%	5%	5%	5%	5%	4%	4%
positive regulation of transcription from RNA polymerase II promoter	4%	4%	3%	3%			4%	4%
positive regulation of I-kappaB kinase/NF-kappaB signaling	4%	4%	4%	4%	3%	3%	4%	4%
innate immune response	4%	4%	4%	4%	3%	3%	4%	4%
G-protein coupled receptor signaling pathway	4%	4%	4%	4%	4%	4%	4%	4%
regulation of cell proliferation	4%	4%	4%	4%	4%	4%	4%	4%
chemokine-mediated signaling pathway					3%	3%		

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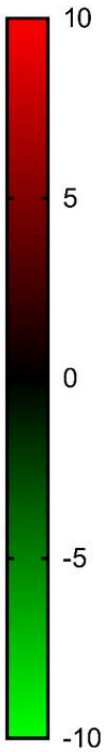
406 Figure 3

ENSEMBL designation	gene name	Lentogenic (LaSota) strain		Velogenic (2K3) strain		gene type
		chicken	Japanese quail	chicken	Japanese quail	
ENSGALG00000026592	micro RNA			-5.52	6.06	miRNA
ENSGALG00000018386	mitochondria rRNA			-2.64	4.47	MtRNA
ENSGALG00000016919	IRG1			3.55	-2.25	protein-coding
ENSGALG00000000081	IL4I1			4.36	-10.00	protein-coding
ENSGALG00000023622	Avidin		2.32	4.65	-10.00	protein-coding
ENSGALG00000029094	Metazoa_SRP	-4.02	4.14			SRP RNA
ENSGALG00000016964	EPSTI1			2.19	2.67	protein-coding
ENSGALG00000006384	IFIT5			4.58	3.34	protein-coding
ENSGALG00000013575	ISG12(2)		2.88	4.18	3.99	protein-coding
ENSGALG00000009639	DDX60			3.69	3.07	protein-coding
ENSGALG00000025389	SNORD99		10.00		10.00	snoRNA
ENSGALG00000027425	H2A-VIII		10.00		10.00	protein-coding
ENSGALG00000025698	5.8S rRNA			-6.25	-1.94	rRNA
ENSGALG00000017818	SNORD15		-10.00		-10.00	snoRNA
ENSGALG00000012396	PTGER2		-10.00		-10.00	protein-coding
ENSGALG00000027571	Histone H2B		-5.41		-5.23	protein-coding
ENSGALG00000000395	BTF3	-10.00	-10.00			protein-coding
ENSGALG00000022679	Wpkci	-10.00	-10.00			protein-coding
ENSGALG00000013312	NIPBL	-10.00	-3.54			protein-coding
ENSGALG00000014366	hnRNPK	-4.55	-2.42			protein-coding



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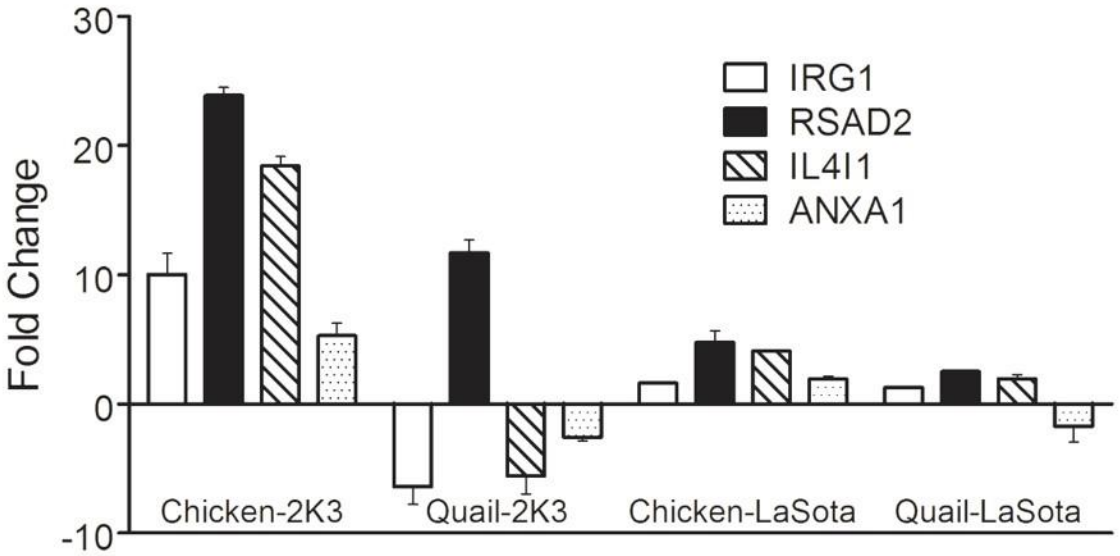
409 Figure 4



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