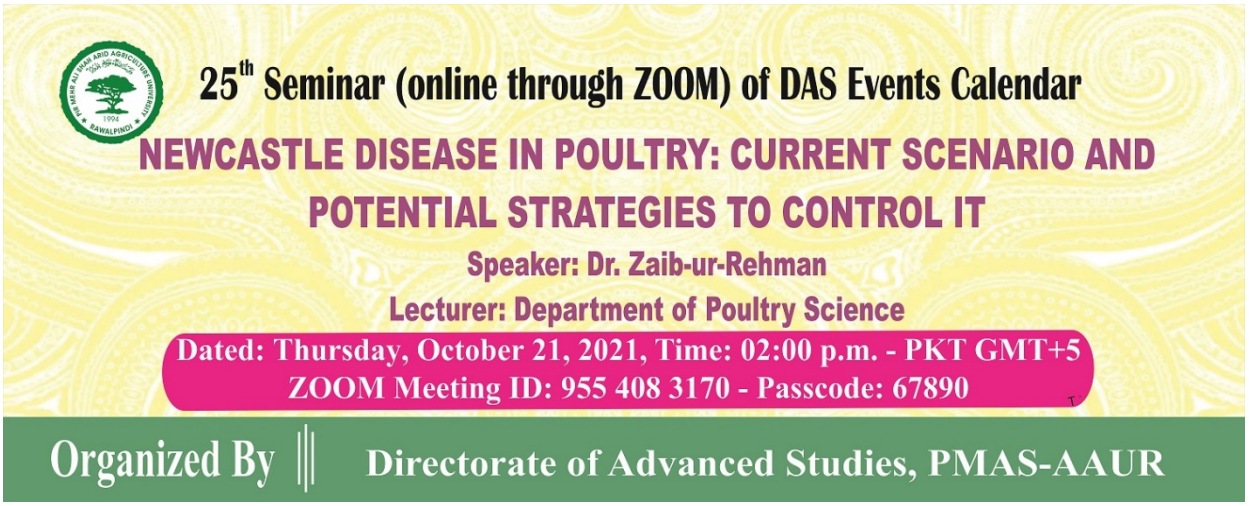
DIRECTORATE OF ADVANCED STUDIES
EVENT CATALOGUE
2021

25TH SEMINAR OF DAS EVENTS CALENDAR – 2021

NEWCASTLE DISEASE IN POULTRY, CURRENT SCENARIO AND POTENTIAL STRATEGIES TO CONTROL IT



ACTIVITIES

Background

- **Why Newcastle disease virus is important?**
- Virulent NDV cause highly infectious disease in poultry
- Mainly affect digestive, respiratory and nervous system
- It causes 100 % mortality in specific pathogen free chickens
- But in vaccinated chickens mortality varies depending on
  - Antibody titer
  - Biosecurity
  - Birds health status
  - Breeds/genetics
- What are the results of ND outbreak in commercial (vaccinated) flocks
  - Stunted growth
  - Decrease egg production
  - Mortality (0-30%)

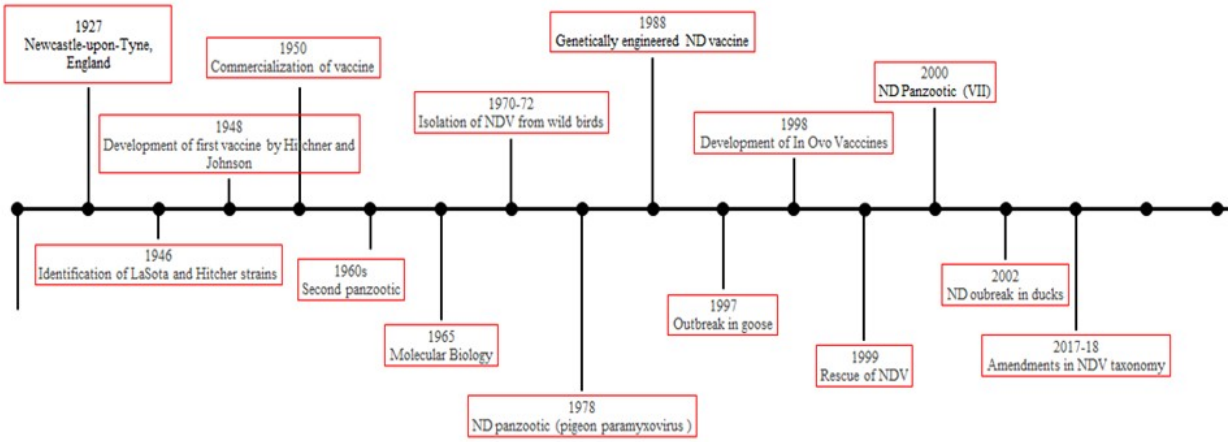
Background

Newcastle Disease

- Newcastle disease (ND) is highly contagious and one of the most devastating diseases
- *Caused by Avian avulaviruses serotype* 1
- Infections of poultry with virulent NDV (vNDV)= ND
- All APMV-1 strains are categorized as
  - velogenic (high mortality; mean death time (MDT) <60 hr),
  - mesogenic (respiratory signs, occasionally nervous signs; MDT 60–90 hr),
  - lentogenic (subclinical to mild respiratory infects; MDT >90 hr)
  - asymptomatic enteric (unapparent infection)

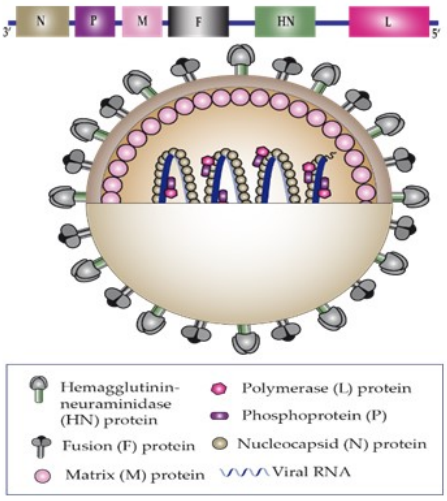
(Rehman et al., 2018, Veterinary Research, 49. 94)

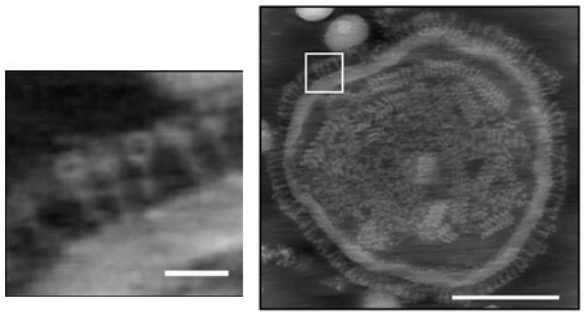
History



Newcastle disease virus

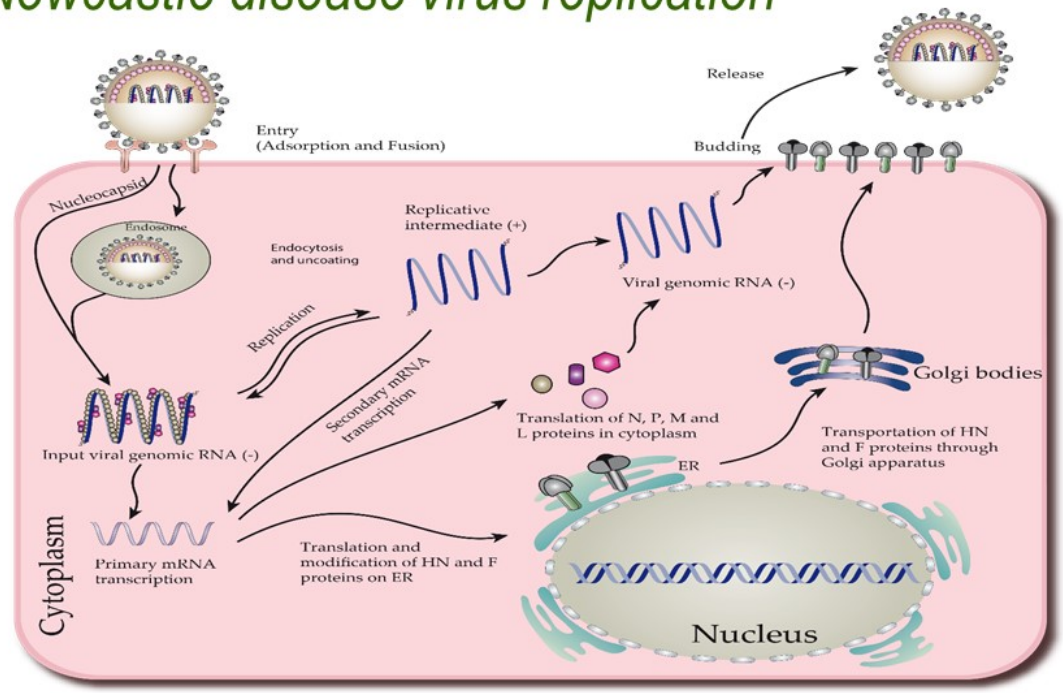
- NDV is a single-stranded,
- Negative-sense
- Non-segmented
- Enveloped RNA virus
- Genome length of 15.2 kb
- Six proteins and two non-structural proteins
- One serotype and 2 classes class I and class II
- Class II viruses are primarily responsible for the outbreaks





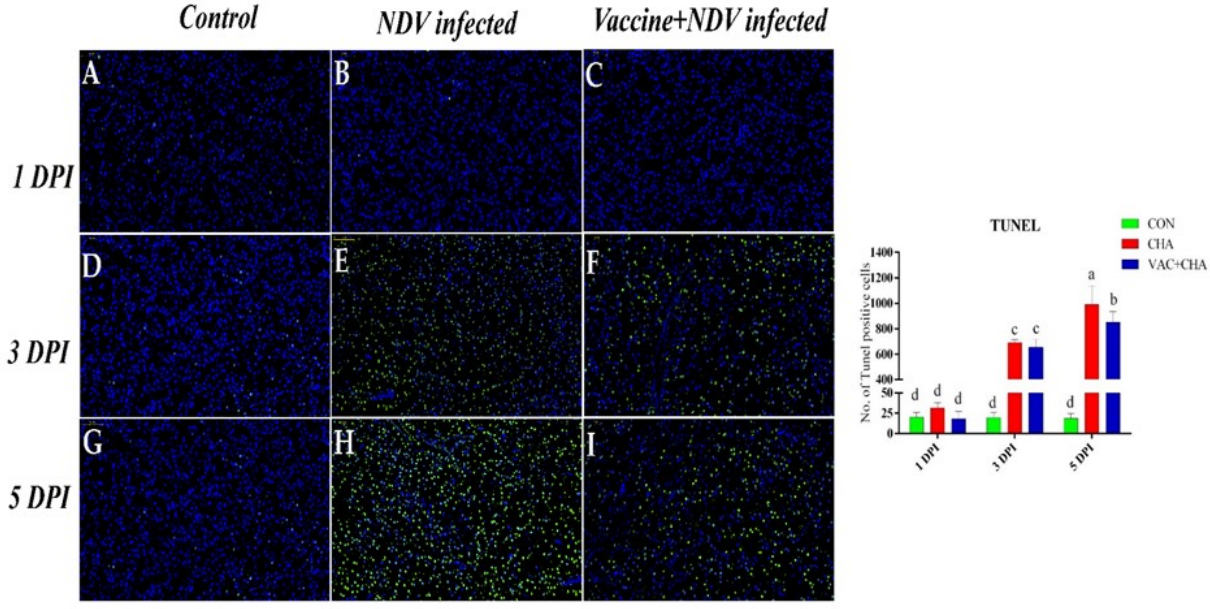
(Rehman et al., 2018, Veterinary Research, 49. 94; Mast and Demeestere, 2009, Diagnostic Pathology, 4. 5)

Newcastle disease virus replication



Rehman et al., 2018, Veterinary Research, 49. 94

Fluorescent micrographs of chicken pancreas infected with NDV and stained by TUNEL assay technique

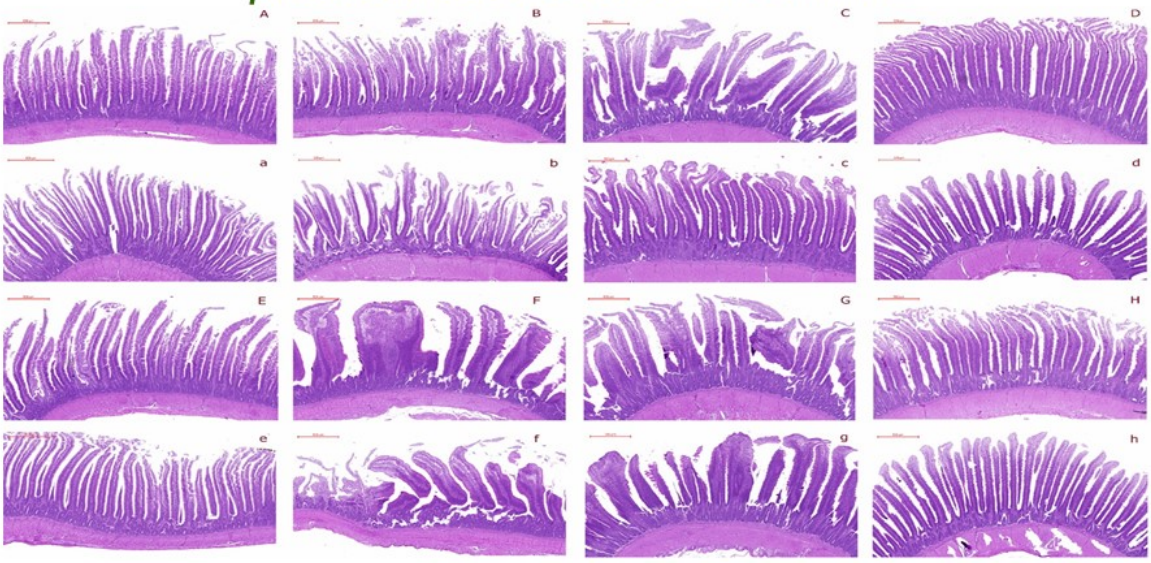


Effect of different treatments on intestinal histomorphology

	Days post infection	Parameters	CON	NS+CHA	VE50+CHA	VE100+CHA	SEM	P Value
Duodenum	3	Villus Height	1071.8 ^{a}	858.6 ^{b}	946.6 ^{ab}	1009.7 ^{a}	23.62	0.01
	3	IEL	25.9 ^{d}	33.2 ^{a}	30.6 ^{b}	28.5 ^{c}	0.54	0.00
	5	Villus Height	1149.3 ^{b}	883.8 ^{a}	926.2 ^{b}	987.9 ^{b}	23.17	0.00
	5	IEL	25.9 ^{c}	32.8 ^{a}	29.7 ^{b}	27.3 ^{c}	0.53	0.00
Jejunum	3	Villus Height	950.25 ^{b}	872.7 ^{b}	948.6 ^{b}	1098.8 ^{a}	21.65	0.00
	3	IEL	23.6 ^{b}	26.2 ^{a}	24.3 ^{b}	24.3 ^{b}	0.28	0.00
	5	Villus Height	930 ^{ab}	871 ^{b}	981.9 ^{a}	902 ^{b}	14.24	0.04
	5	IEL	23.6	21.9	23.2	22.3	0.60	0.75

Values with different letters in the same rows are significantly different ($P < 0.05$)
CON= non-supplemented, non-challenged control
NS+CHA= non-supplemented + challenged
VE50+CHA= vitamin E50 IU/day/ Kg body weight + challenged
VE100+CHA= vitamin E100 IU/day/ Kg body weight + challenged
SEM=pooled standard error of mean

NDV induced histopathological changes and protective effects of vitamin E



Panel marked with upper case letters (A to D) and (E to F) are showing the histopathological changes of CON, NS+CHA, VE50+CHA and VE100+CHA groups in the duodenum of chicken, at 3 and 5 DPI respectively. Similarly, pictures marked with lower case letters (a to d) and (e to f) are showing the histopathological changes of CON, NS+CHA, VE50+CHA and VE100+CHA groups in the jejunum of chicken at 3 and 5 DPI respectively.

Amino acid transporter

- Decreased mRNA expression of
  - CAT-1
    solute carrier family 7 member 1 (SLC7A1)
  - CAT-2
    solute carrier family 7 member 2 (SLC7A2)
  - PepT-1
    solute carrier family 15 member 1
  - rBAT/
    solute carrier family 3 member 1
  - EAAT-3
    solute carrier family 1 member 1
  - y+LAT-2/
    solute carrier family 7 member 6

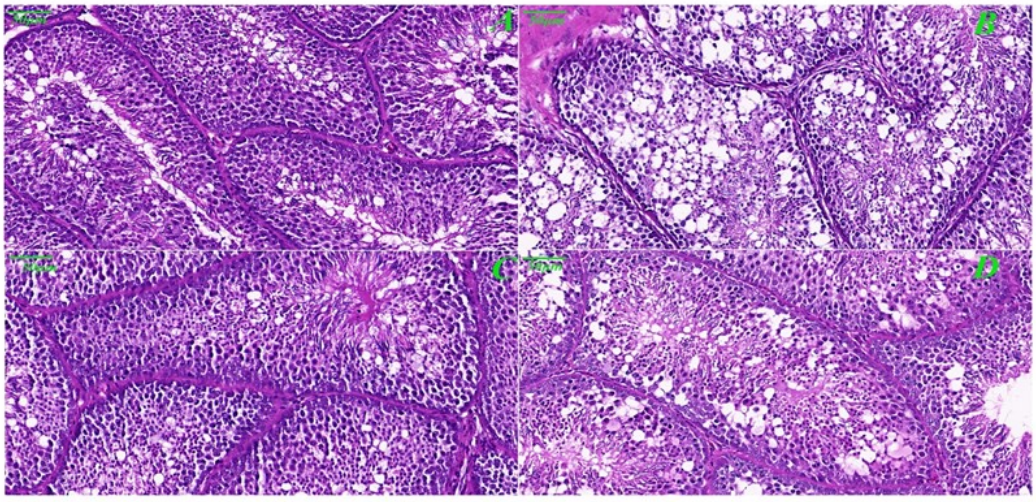
Mineral transporters

- Decreased mRNA expression of
  - ZnT1
    Gallus gallus solute carrier family 22 member 18
  - PiT-1
    solute carrier family 20 member 1
  - PiT-2
    solute carrier family 20 member 2
  - NaPi-IIb
    solute carrier family 34 member 2

Carbohydrate transporter

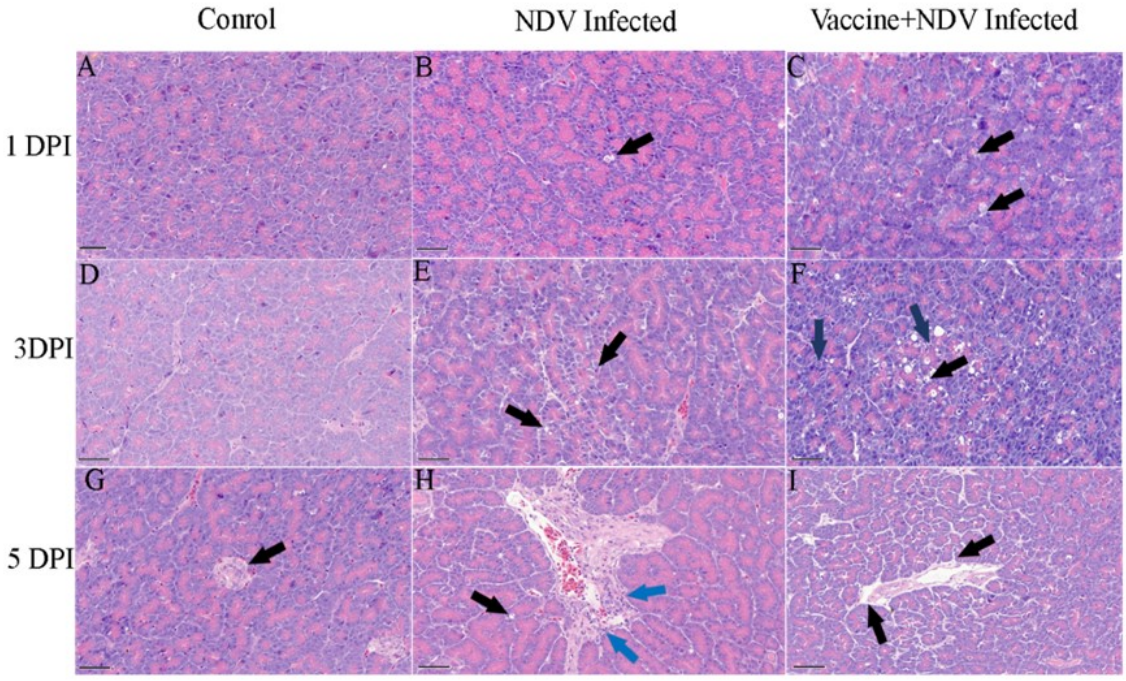
- Decreased mRNA expression of
  - SGLT-1/Solute carrier family 5 member 1 (SLC5A1)
  - GLUT-2/Solute carrier family 2 member 2
  - GLUT-5/solute carrier family 2 member 5 (SLC2A5)

Photomicrograph of NDV induced histopathological changes in the testis



- Normal progression of spermatogenesis (Panel A and C).
- Panel B indicate the decrease in number of Sertoli cells, individualized, shrunken spermatogonia with pyknotic nuclei
- Panel D denote multifocal, and segmented, spermatogenic columns, necrotic cells, lipid vacuoles and proteinaceous homogenous material

NDV induced Histological changes in the pancreas

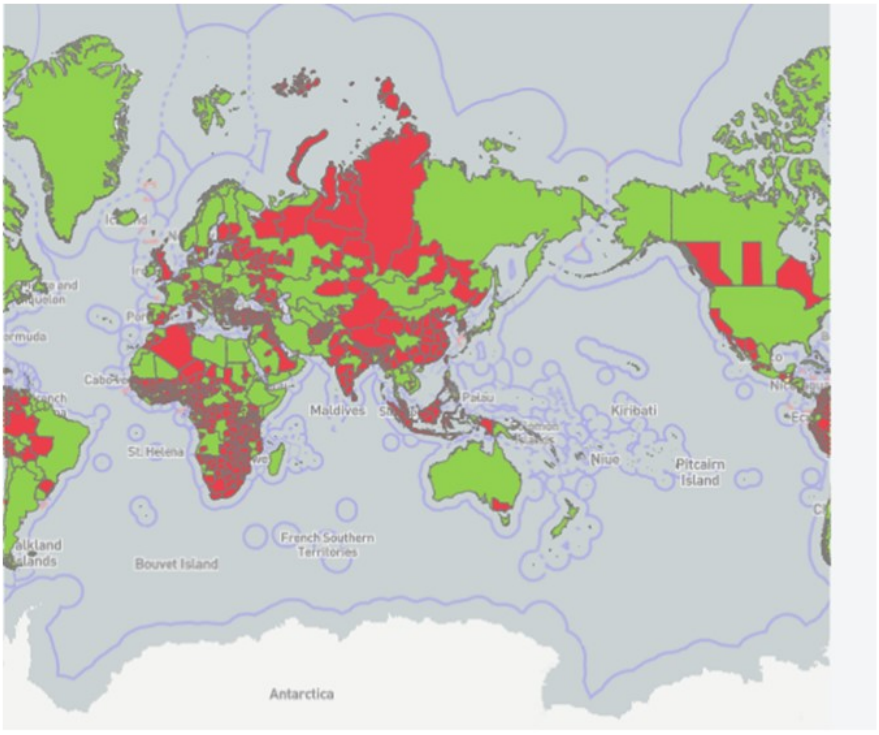


Current Scenario

Current Scenario

- Disease has been currently controlled in Canada, the United States and some western European countries
- It continues in parts of Africa, Asia and South America
- However, since wild birds can sometimes carry the virus without becoming ill, outbreaks can occur anywhere that poultry is raised

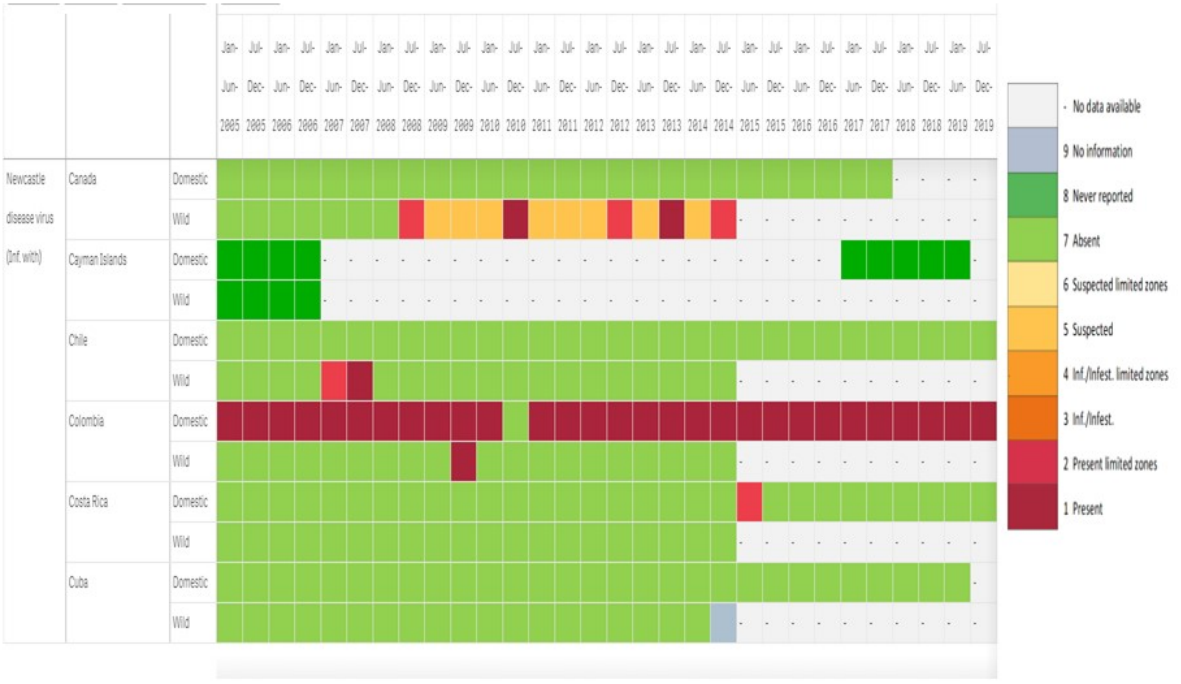
2005-2019 (World)



America

Disease status : To view this map, please select only one disease from the filter section above

America



NDV and Innate Immune Response

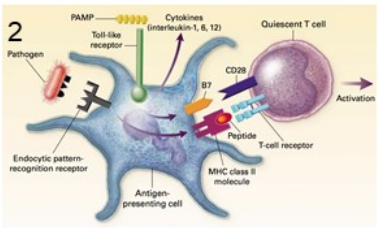
Innate immune system

- One of the two main immunity strategies found in vertebrates
- An older evolutionary defense strategy

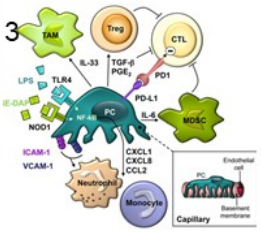
Main functions of Innate immune system

- Identification and removal of foreign substances
- Activation of the adaptive immune system
- Recruiting immune cells to sites of infection
- Acting as a physical and chemical barrier

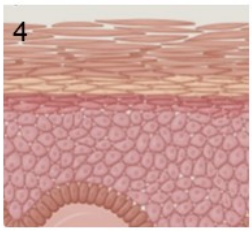
ProteinLounge



Ruslan Medzhitov, 2000



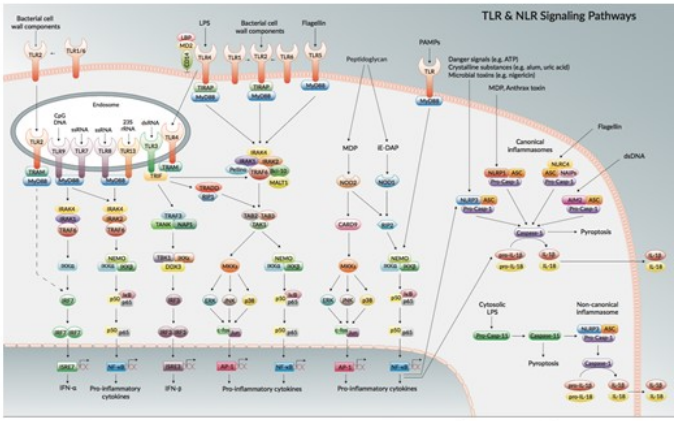
Rocío Navarro, 2016



A.D.A.M

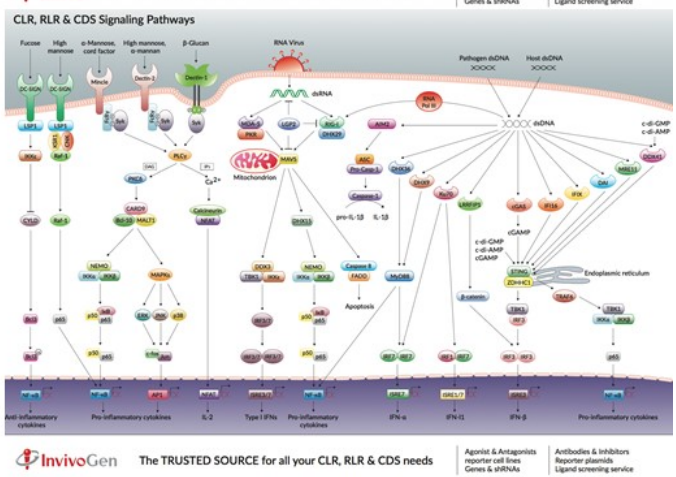
Bacterial/Virus Bacterial

TLR & NLR PATHWAY



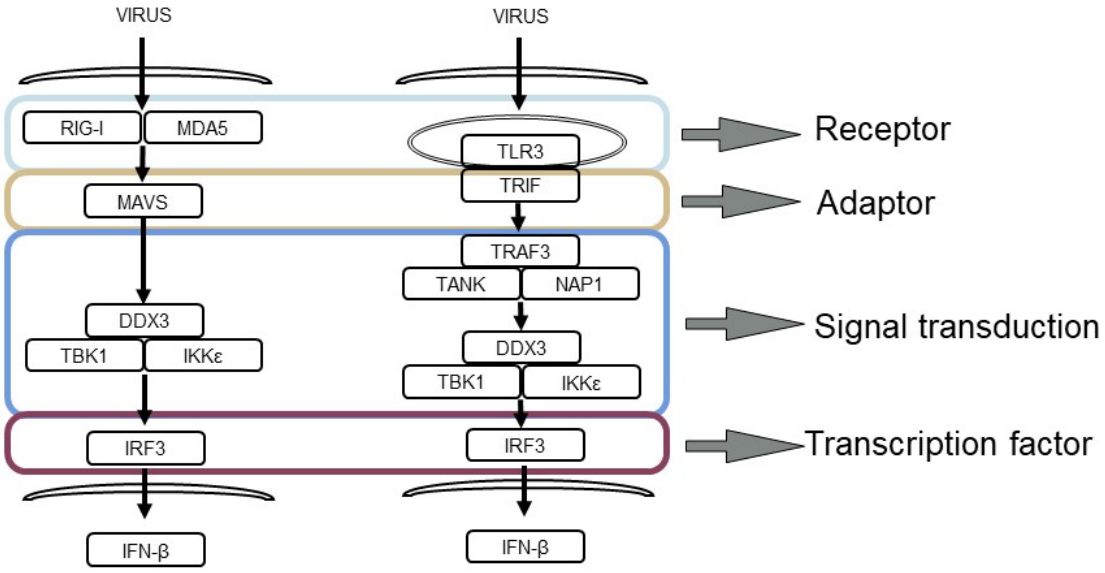
Fungus RNA DNA

CLR, RLR & CDS PathWAY



C-type lectin receptor
RIG-I-like receptors
Cytosolic DNA Sensors

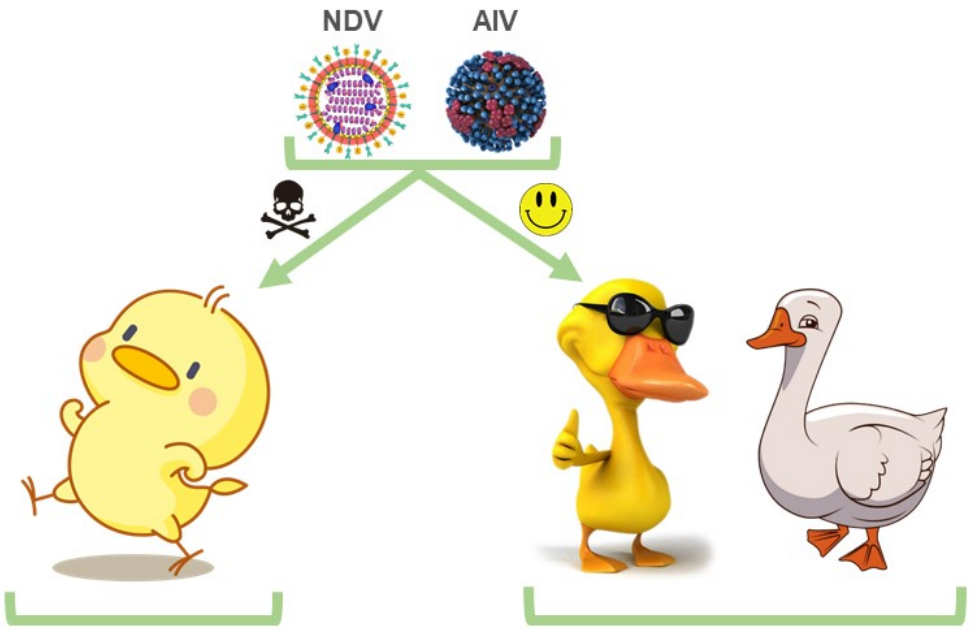
Virus Recognition



Subtle Differences

TLR9	RIG-I	IRF-3 IRF-7

TLR21	N/A	IRF-7
Keestra, 2010	Barber, 2010	Cormican, 2009

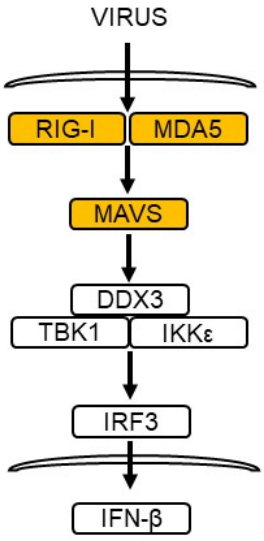


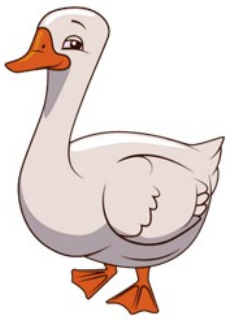
N/A	⇔	**RIG-I**	Barber, 2010
N/A	⇔	**β-defensins**	Huang, 2013
N/A	⇔	**BTNL** butyrophilin-like family	Huang, 2013

Question

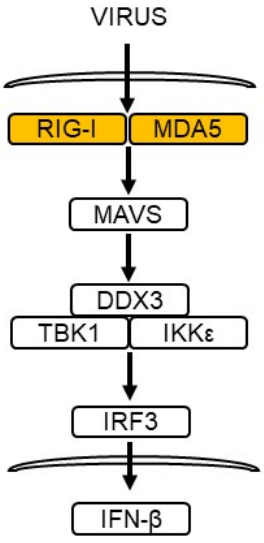
- 1 Role of RLR pathway in waterfowls?
- 2 How RLR pathway works in chickens?

Goose RIG-I/MDA5/MAVS play important roles in virus infection



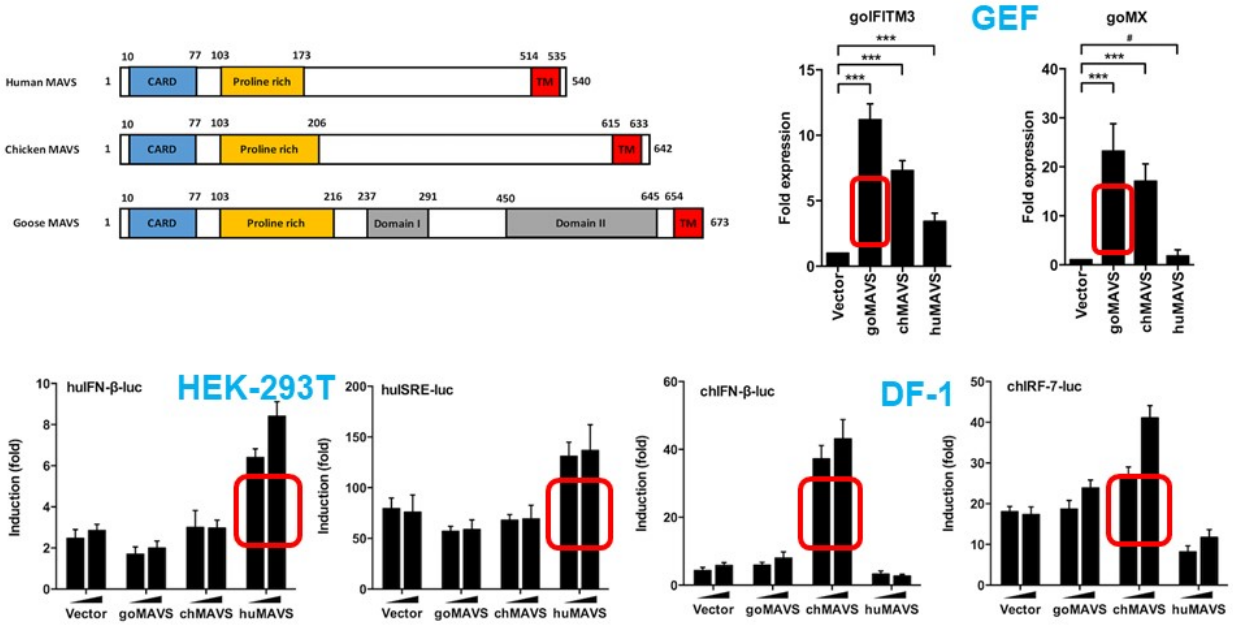


RIG-I/MDA5 Amino acid identity

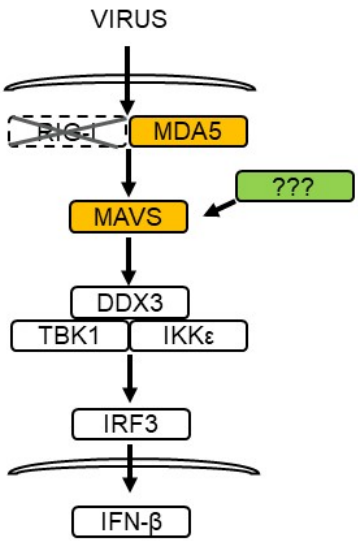


	Chicken	Duck	Human
Goose RIG-I	N/A	93.8%	50.8%
Goose MDA5	87.2%	94.5	63.9%

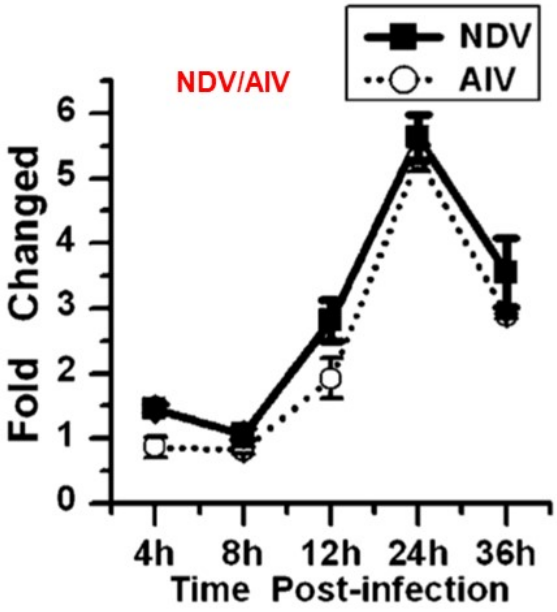
GoMAVS activates IFN-β in **a species-specific manner**.



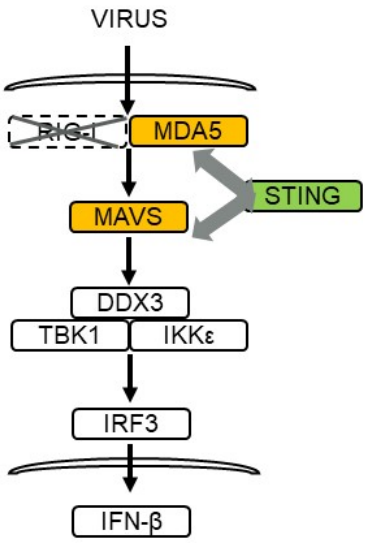
How RLR pathway works in chickens ?

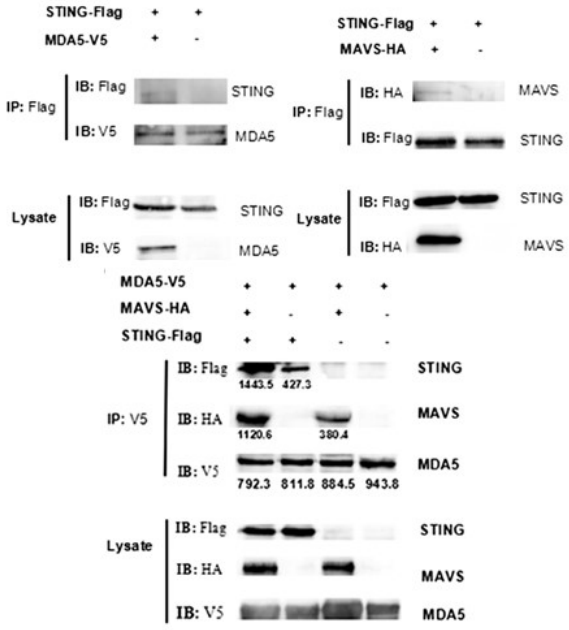


ChSTING mRNA level is up-regulated by RNA virus

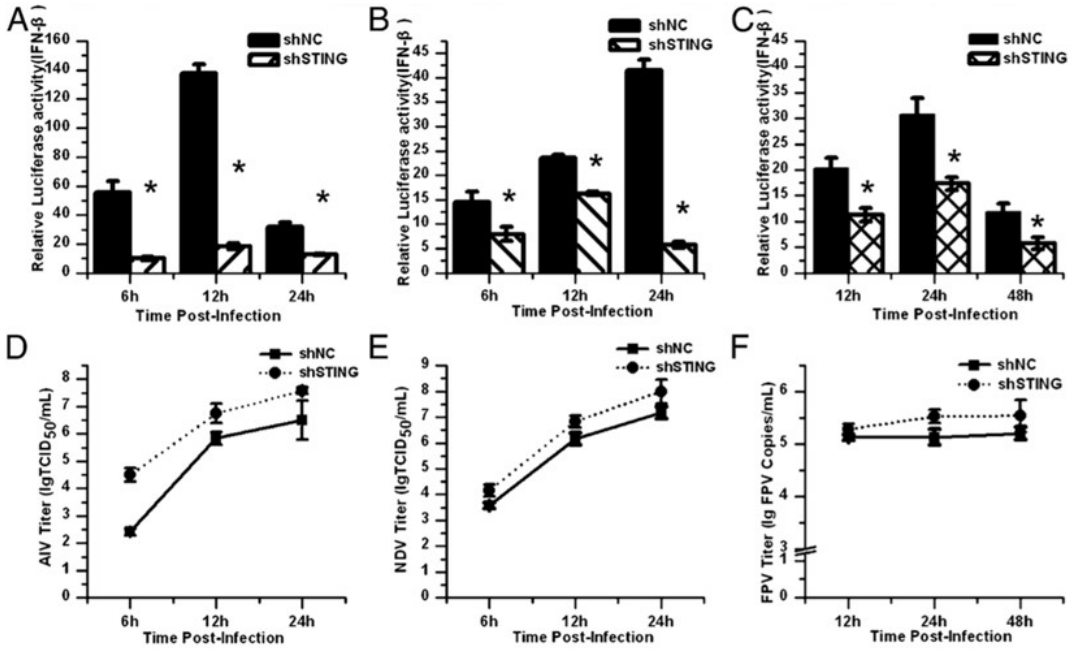


ChSTING/chMDA5/chMAVS form a complex

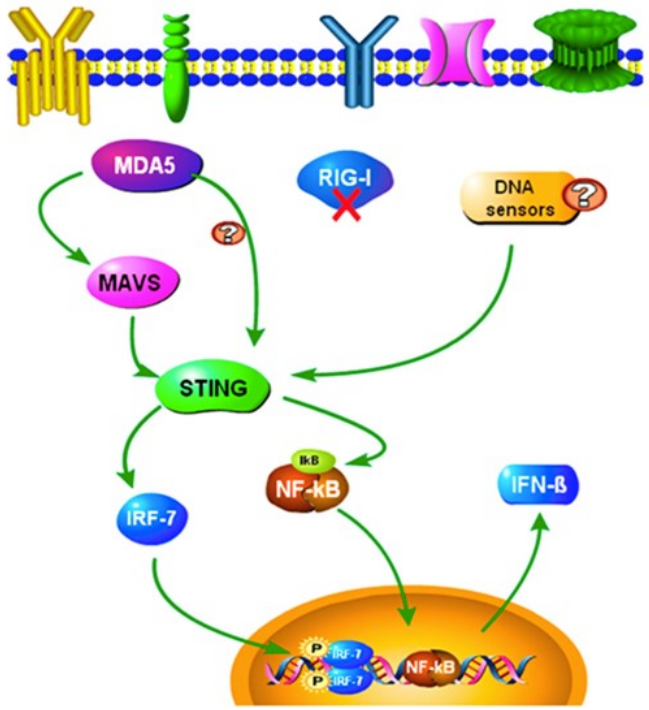




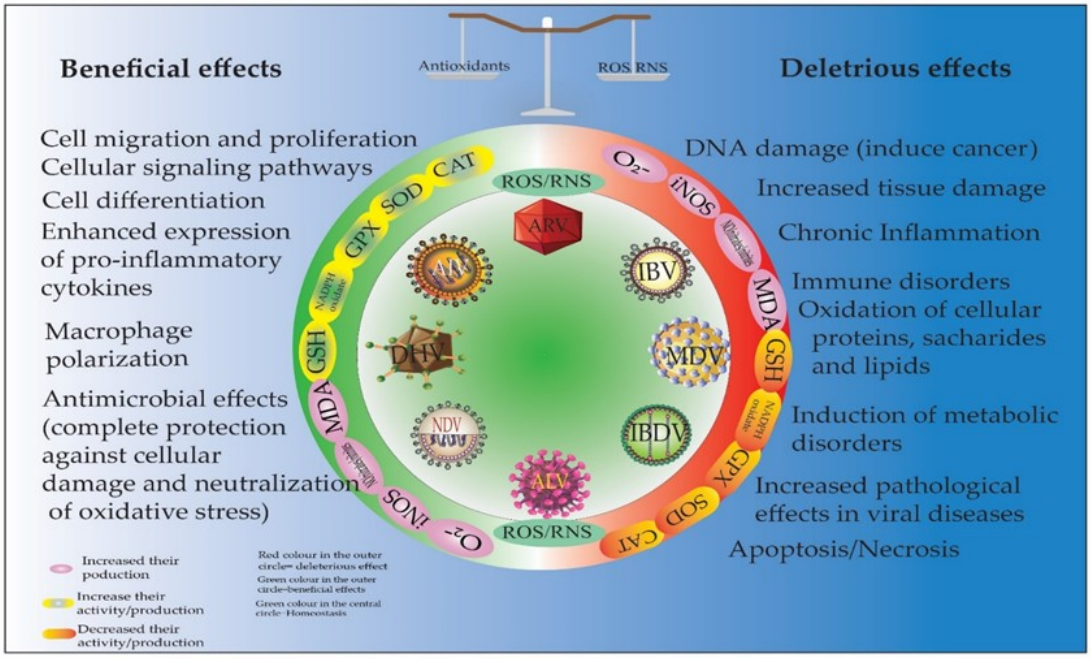
ChSTING inhibits virus infection



Working model

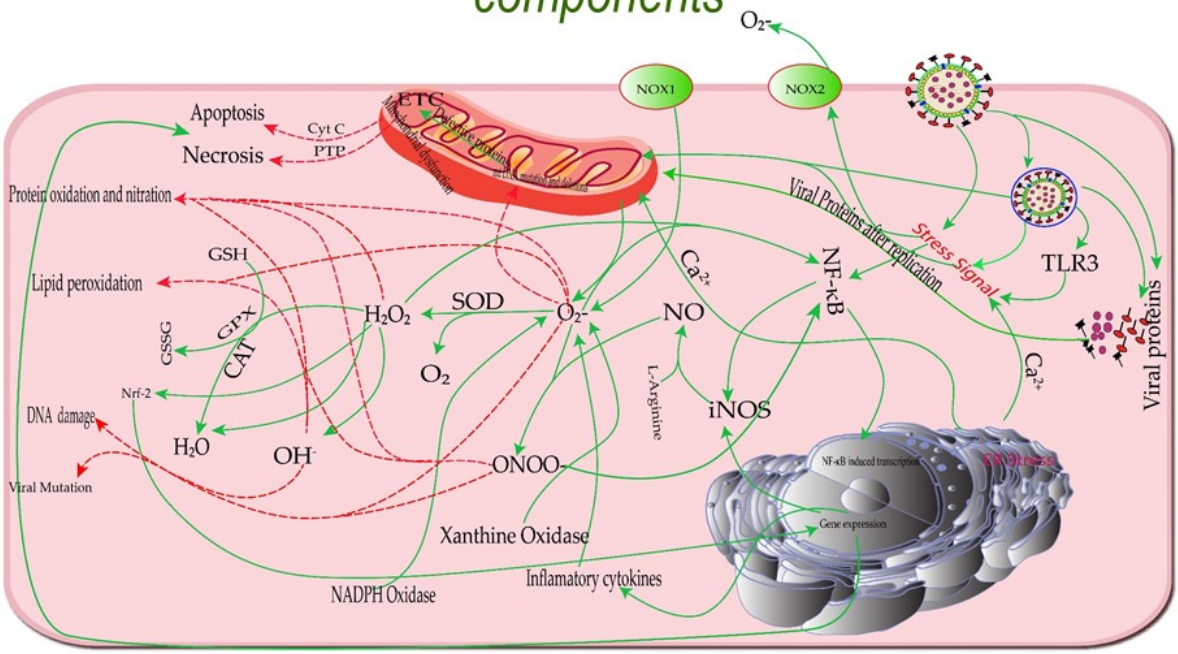


Are these reactive species always induce pathological effects?

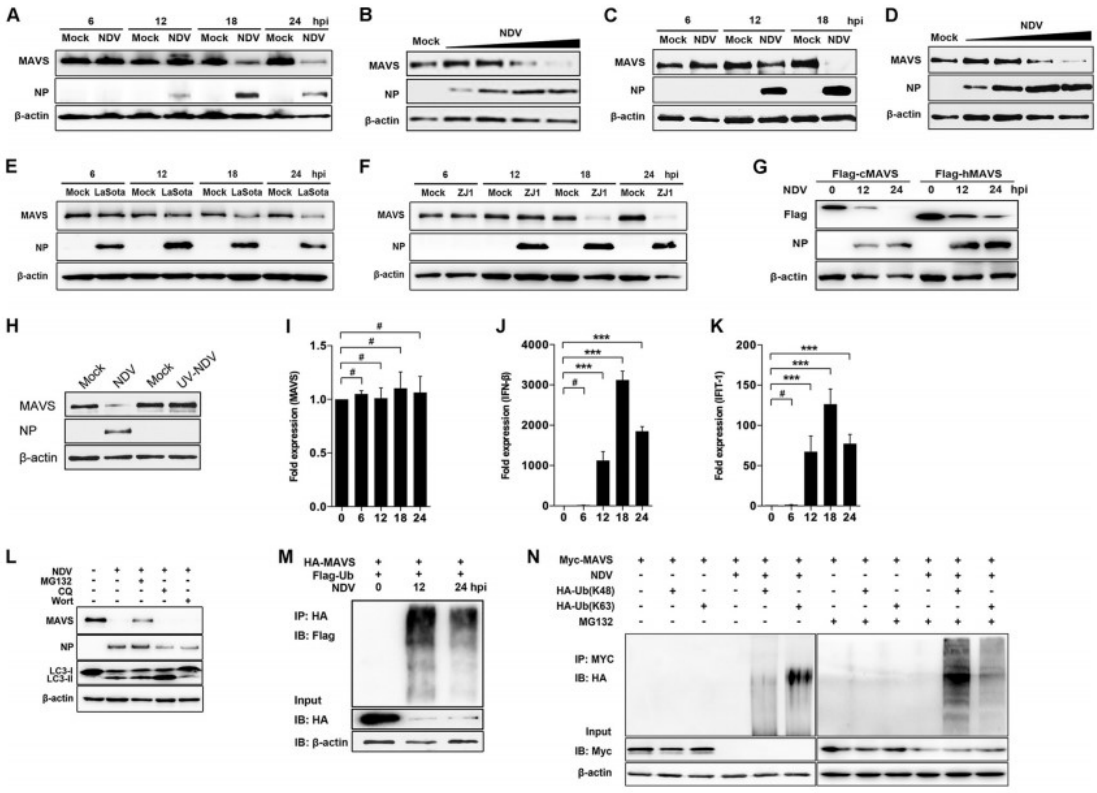


(Rehman et al., 2018, Oxidative Medicine and Cellular Longevity, 2018. 14)

Basic mechanisms of viral cross-talk with the cellular pathways to cause oxidative damage to cellular components

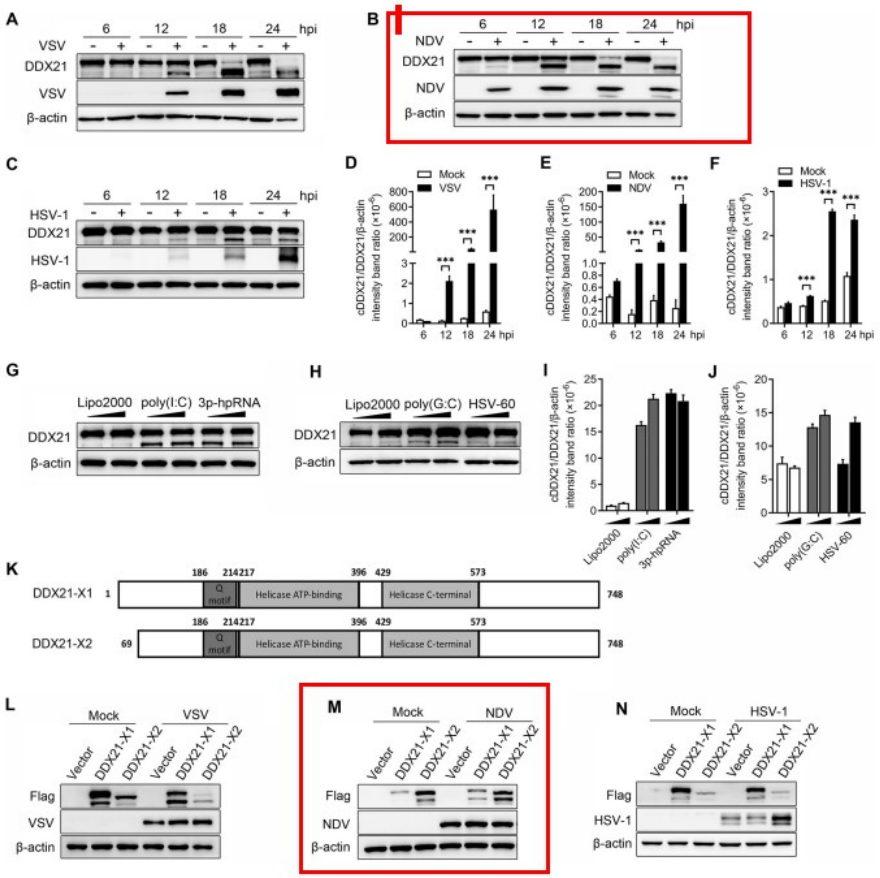


(Rehman et al., 2018, Oxidative Medicine and Cellular Longevity, 2018. 14)

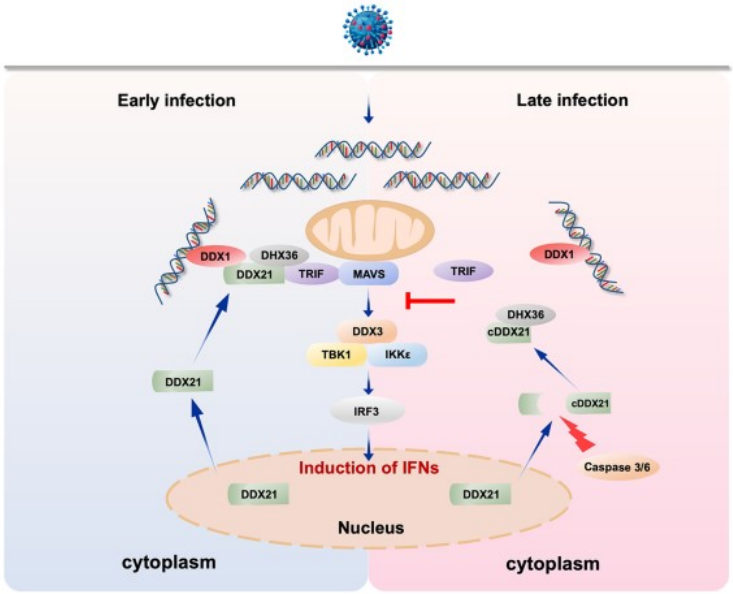


Caspase-Dependent Cleavage of DDX21 Suppresses Host Innate Immunity

- DEAD (Glu-Asp-Ala-Glu) box RNA helicases have been proven to contribute to antiviral innate immunity
- The DDX21 RNA helicase was identified as a nuclear protein involved in rRNA processing and RNA unwinding
- DDX21 was also proven to be the scaffold protein in the complex of DDX1-DDX21-DHX36, which senses double-strand RNA and initiates downstream innate immunity
- Here, we identified that DDX21 undergoes caspase-dependent cleavage after virus infection and treatment with RNA/DNA ligands, especially for RNA virus and ligands
- Caspase-3/6 cleaves DDX21 at D126 and promotes its translocation from the nucleus to the cytoplasm in response to virus infection
- The cytoplasmic cleaved DDX21 negatively regulates the interferon beta (IFN-b) signaling pathway by suppressing the formation of the DDX1-DDX21-DHX36 complex
- Thus, our data identify DDX21 as a regulator of immune balance and most importantly uncover a potential role of DDX21 cleavage in the innate immune response to virus



Proposed model



NDVs Differ in their Pathogenicity

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ORIGINAL ARTICLE

WILEY Transboundary and Emerging Diseases

Potential of genotype VII Newcastle disease viruses to cause differential infections in chickens and ducks

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Abstract

Newcastle disease (ND), caused by ND virus (NDV), is one of the most infectious and economically important diseases of the poultry industry worldwide. While infections are reported in a wide range of avian species, the pathogenicity of chicken-origin virulent NDV isolates in ducks remains elusive. In this study, two NDV strains were isolated and biologically and genetically characterized from an outbreak in chickens and apparently healthy ducks. Pathogenicity assessment indices, including the mean death time (MDT), intracerebral pathogenicity index (ICPI) and cleavage motifs in the fusion (F) protein, indicated that both isolates were velogenic in nature. While these isolates carried pathogenic characteristics, interestingly they showed differential pathogenicity in ducks. The chicken-origin isolate caused high (70%) mortality, whereas the duck-origin virus resulted in low (20%) mortality in 4-week-old ducks. Intriguingly, both isolates showed comparable disease pathologies in chickens. Full-genome sequence analysis showed that the virus genome contains 15 192 nucleotides and carried features that are characteristic of velogenic strains of NDV. A phylogenetic analysis revealed that both isolates clustered in class II and genotype VII. However, there were several mutations in the functionally important regions of the fusion (F) and haemagglutinin-neuraminidase (HN) proteins, which may be responsible for the differential pathogenicity of these viruses in ducks. In summary, these results suggest that NDV strains with the same genotype show dif-

Pathogenicity indexes for the NDV isolates

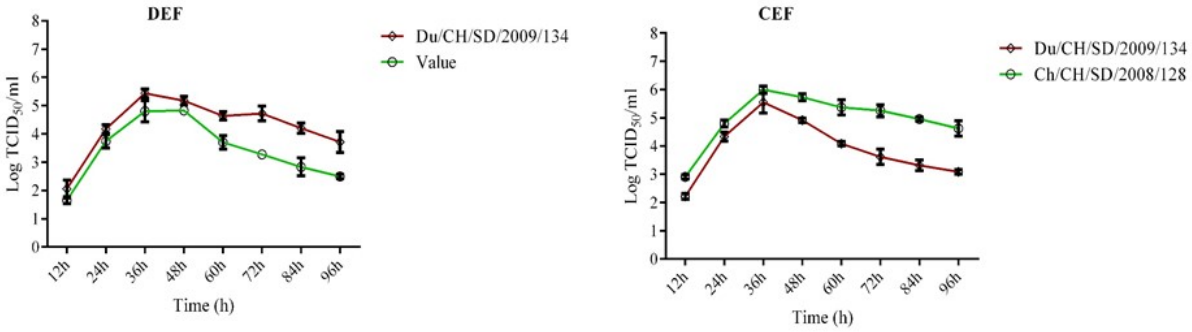
Isolate name	Origin	Host	[a]HA	[b]$TCID_{50}$	[c]ELD_{50}	[d]MDT (h)	[e]ICPI
Ch/CH/SD/2008/128	Shandong, China	Chicken	2^8	7.80	8.31	55h	1.90
Du/CH/SD/2009/134	Shandong, China	Duck	2^9	7.35	7.16	59h	1.81

1

[a]HA=Haemagglutination
[b]$TCID_{50}$=50% tissue culture infective dose
[c]ELD_{50}=50% embryo lethal dose
[d]MDT (h)=Mean death time in embryonated eggs (hours)
[e]ICPI=Intracerebral pathogenicity index in day-old chicks

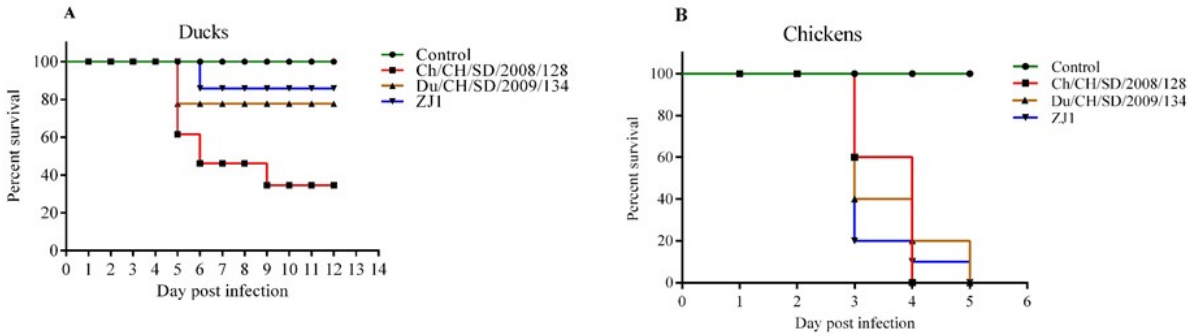
Virus growth kinetics

The growth kinetics of both viruses were determined under multiple cycle growth conditions in chicken embryo fibroblast

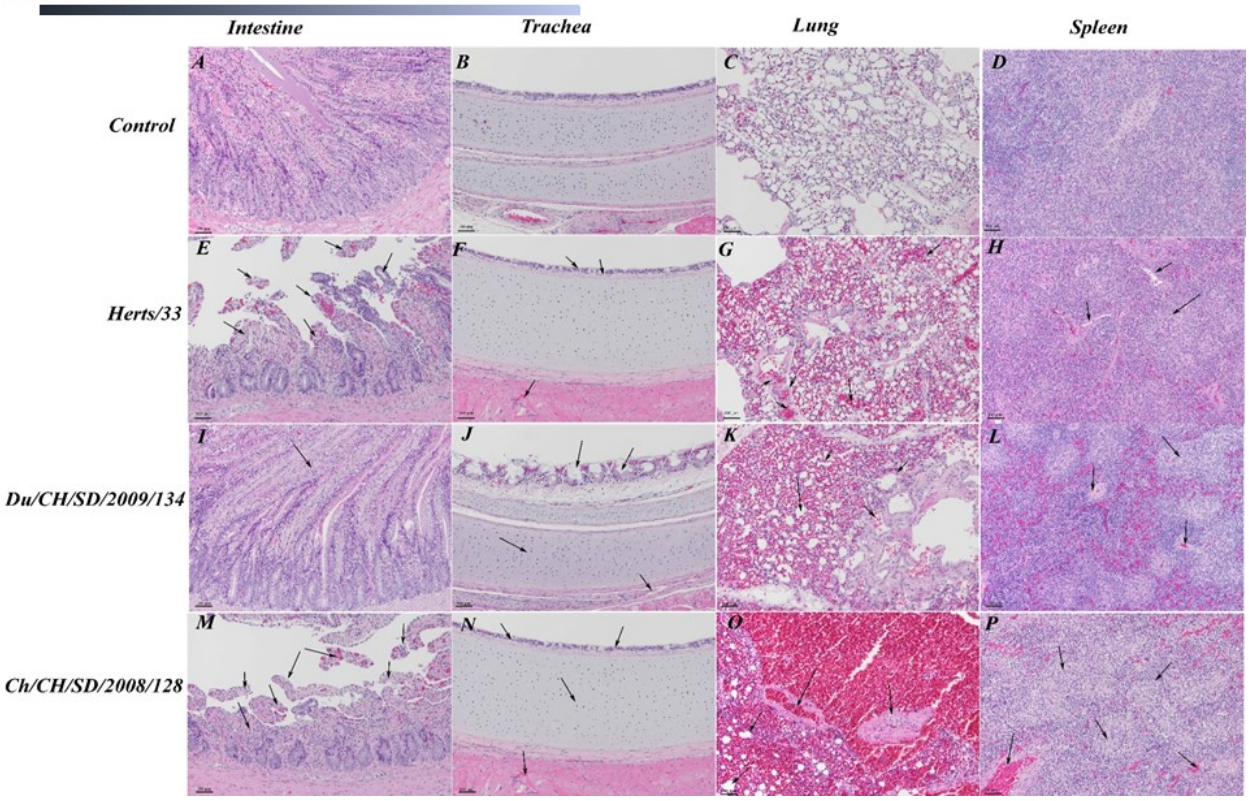


Survival rate

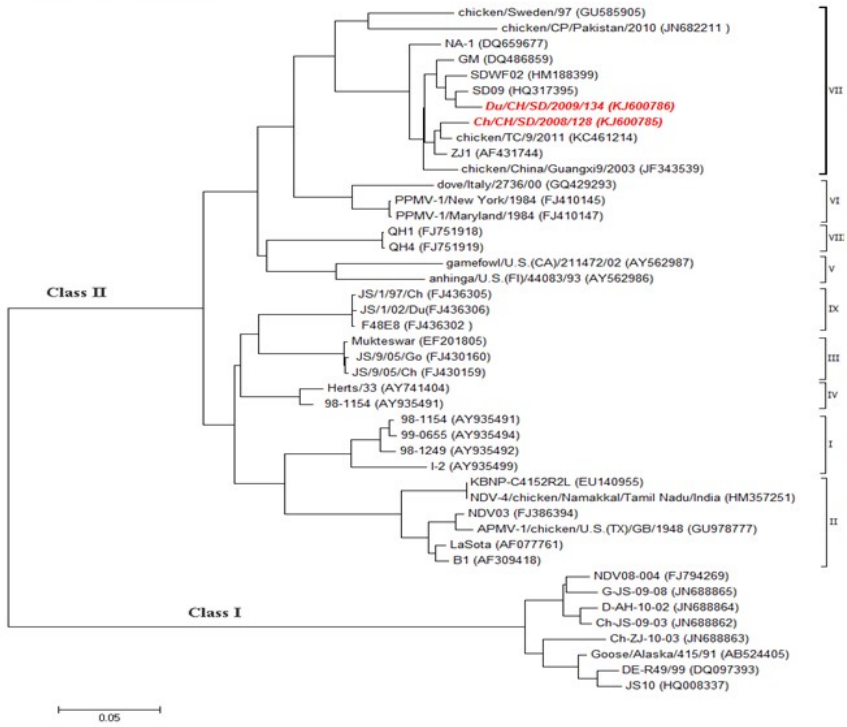
- Chickens and ducks were infected with
  - Ch/CH/SD/2008/128 (70% mortality in ducks)
  - Du/CH/SD/2009/134 (20% mortality in ducks)
  - ZJ1 (10% mortality in ducks)
  - PBS



Histopathological changes induced by the different isolates



Phylogenetic analysis



Genetic analysis of NDV isolates

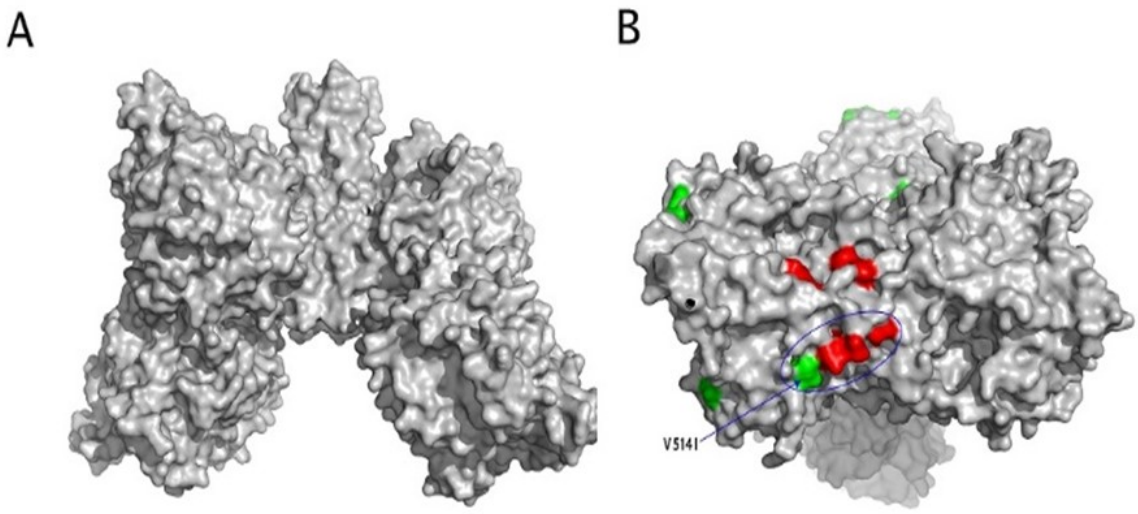
Genomic features of the NDV isolates Du/CH/SD/2009/134 and Ch/CH/SD/2008/128

Gene	Gene start		Gene end		Intergenic sequences
	Sequences	Start	Sequences	End	
NP	ACGGGTAGAA	56	CCCAAGGTAT	1798	TAGAAAAAAAT
P	ACGGGTAGAA	1810	CATT(C)aAGAAAT	3250	TAAGAAAAAAT
M	ACGGGTAGAA	3262	TCTAGCAAAT	4493	TAGAAAAAAC
F	ACGGGTAGAA	4504	GTAGAAGACT	6285	TAAGAAAAAACTACTGGGAACAAGCAAC CAAAGAGCAATAC
HN	ACGGGTAGAA	6327	ATCACTTTAT	8318	TAAGAAAAAATACAGAAAGCATTGAGATG TAAGGGAAAACAACCAACAAGAGGGAAC
L	ACGGGTAGGA	8376	TAGAAAAAAG	15079	

Amino acid changes in the HN proteins of genotype VII NDV strains

Virus strain	Amino acid residues											
	9	102	138	141	216	309	323	331	355	477	479	514
BPO1	V	I	K	I	T	D	N	E	A	I	H	V
ZJ1	-	-	-	-	-	-	-	-	-	-	-	-
SDWF02	-	-	-	-	-	-	-	-	-	-	-	-
NA-1	-	-	-	-	-	-	-	-	-	-	-	-
China/Guangxi9/2003	-	-	-	-	-	-	-	-	-	-	-	-
Du/CH/SD/2009/134	-	-	E	L	I	-	-	-	V	V	-	-
Ch/CH/SD/2008/128	M	T	-	-	-	N	D	K	-	-	Y	I

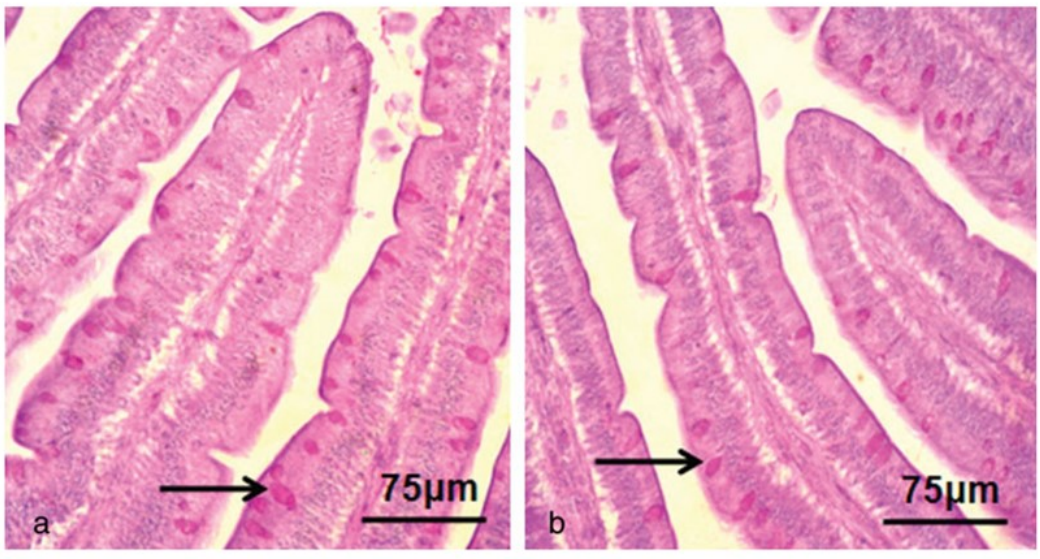
Crystal structure of the NDV HN protein



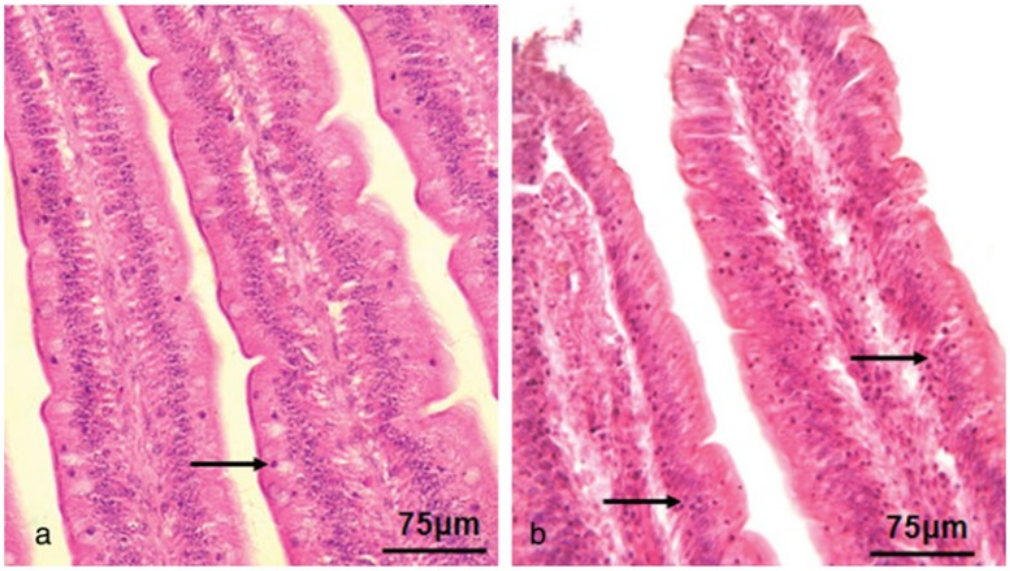
Adoptive immune responses

- Age
- Type (broiler, layer, breeder)
- Genetics
- Vaccine
- Health Status
- Biosecurity

Intestinal Goblet cells



Intestinal intraepithelial lymphocytes



Avian Pathology (December 2008) **37(6)**, 579–585

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ORIGINAL ARTICLES

Increased mast cell density during the infection with velogenic Newcastle disease virus in chickens

Quan Sun†, Decheng Wang†, Ruiping She*, Wengui Li, Shuai Liu, Deping Han, Yinghua Wang and Ye Ding

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In addition to their well-characterized role in allergic inflammation, recent data confirm that mast cells play a more extensive role in a variety of viral infections. The contribution of mast cells to Newcastle disease pathogenesis has not been investigated. We evaluated mast cell activity after Newcastle disease virus (NDV) infection in specific pathogen free chickens using cytochemical and immunocytochemical analyses. The results were as follows. Severe tissue damage was observed in the proventriculus, duodenum, jejunum and caecal tonsil, and NDV antigens were detected and presented extensively in these tissues. Second, in the NDV-infected group, the mast cell population was increased markedly in the proventriculus, duodenum, jejunum and caecal tonsil at 24, 48, 72 and 96 h after infection ($P < 0.01$). However, very few mast cells were

Mast cells and innate immunity: master troupes of the avian immune system

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Mast cells (MCs) are granulated cells of haematopoietic lineage and constitute a major sensory arm of the immune system. MCs dually guard hosts and regulate immune responses against invading pathogens. This property of the MCs is attributed to their adaptability to detect stress signals and pathogens, and the production of signal specific mediators to engage immune cells for clearance of infectious agents. Pathogen-specific signals establish basis for the initiation of adoptive immune responses. These immune regulatory roles of MCs have opened avenues to engage different MCs activators which culminate in effective passive immunisation. The molecular mechanisms and dynamics of functionalities of MCs

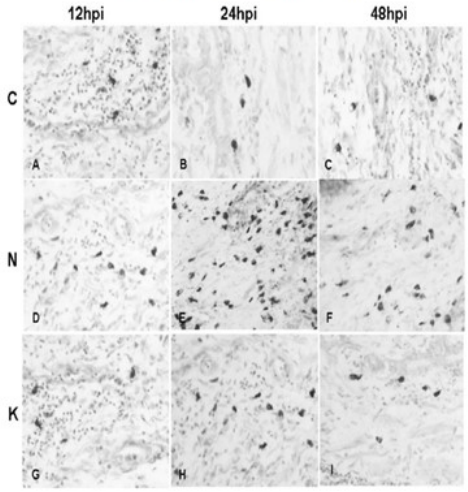

Figure 2. The distribution of mast cells in proventriculus. Mast cells in the proventriculus of the birds are distributed throughout lamina propria of mucosa from 12 to 48 h postinfection (hpi). C = control group (A, B, and C); N = Newcastle disease virus-infected group (D, E, and F); K = ketotifen-pretreated group (G, H, and I). Improved toluidine blue stain. Original magnification ×400.

Evidence for a role of mast cells in the mucosal injury induced by Newcastle disease virus

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ABSTRACT We have previously demonstrated that mast cells were significantly increased during Newcastle disease virus (NDV) infection, but their precise role in the process is unknown. In this study, we investigated the role of mast cells in this process by using ketotifen, a mast cell membrane stabilizer. A total of 60 specific-pathogen-free chickens were randomly divided into 3 groups of 20 birds each (NDV-infected group, ketotifen-pretreated group, and the control group). The ketotifen-pretreated group was administered orally with ketotifen before NDV infection. Six 12, 24, and 48 h postinfection, 5 chickens from each treatment were killed. Tissues of proventriculus were collected to quantify mast cells, the content of tryptase and histamine by cytochemistry, immunohistochemistry, and fluorescence analysis, respectively. The results showed that the population of mast cells and the content of tryptase and histamine were increased significantly in the proventriculus ($P < 0.01$) of infected birds compared with the control group. An acute mucosal injury was observed in the infected chickens. In contrast, among chickens pretreated with ketotifen, followed by NDV infection, the mast cells number and the content of tryptase and histamine were decreased significantly ($P < 0.01$). Likely as a result, the mucosal injury was remitted remarkably. The overall results of this experiment suggest that mast cells are implicated in NDV-induced mucosal injury. Inhibition of mast cell mediator release may represent a novel strategy to modulate this process.

Key words: mast cell, tryptase, histamine, Newcastle disease virus

2009 Poultry Science 88:554–561
doi:10.3382/ps.2008-00468

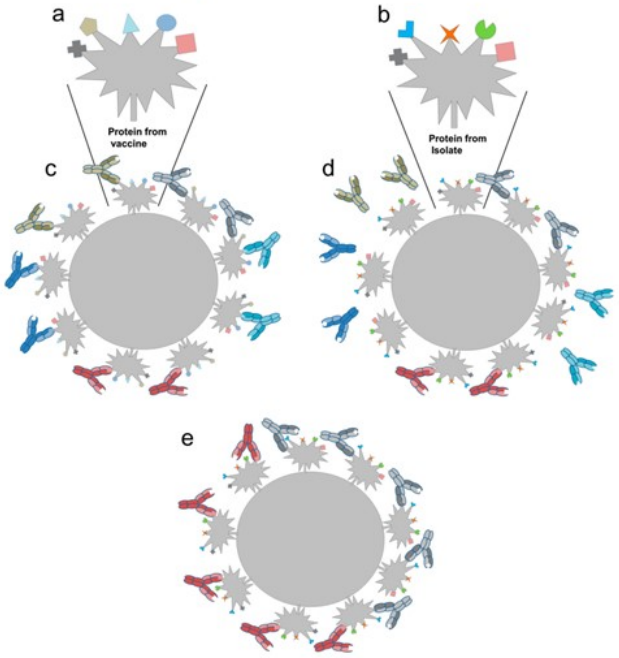
Control Strategies

Newcastle Disease Control

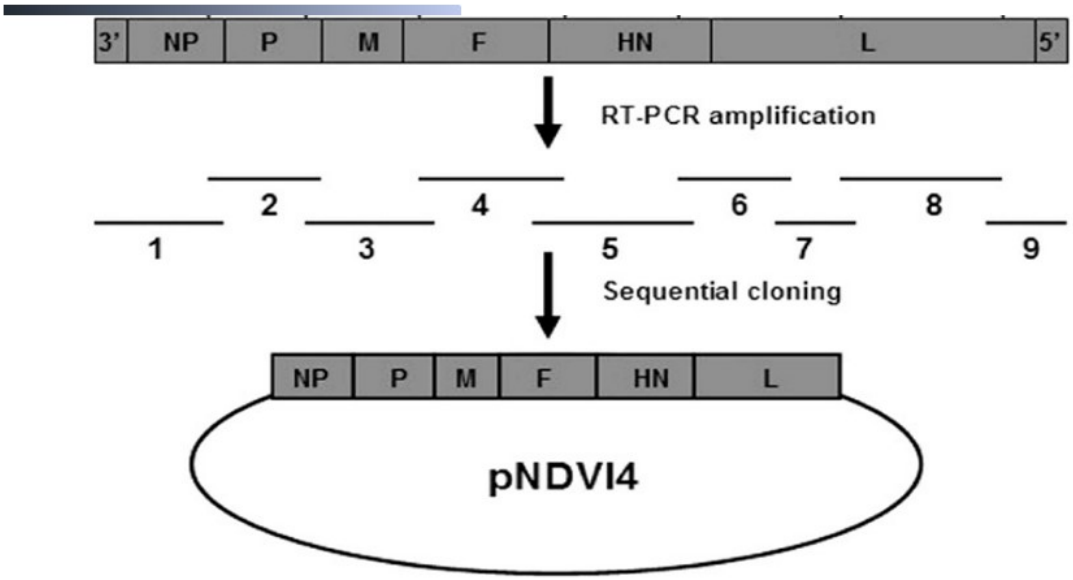
- Production of genetically resistant strains
- Vaccination
  - Commonly used live vaccine (LaSOta)
  - Killed Vaccines
  - Genotype matched vaccines

Model for titre-dependent escape

- (a) Viral protein (e.g. HN) of a vaccine strain; each shape represents a neutralizing epitope. (b) Viral protein with three mutated neutralizing epitopes. (c) The vaccine virus is neutralized by low antibody titres. Antibodies with different specificities spread among protein copies, enabling coverage of all copies. (d) The mutated protein displays only two epitopes in common with the vaccine epitopes, leaving some of the viral protein copies intact and thus able to escape neutralization by the vaccine-induced antibodies. (e) High antibody titre may compensate for loss of neutralizing epitopes by covering all protein molecules and preventing escape.



Genotype matched vaccine



Schematic representation of cloning strategy of the complete JS5/05 virus genome. Nine overlapped cDNA fragments spanning the entire genome were generated and cloned into the transcription vector TVT7R (0, 0).

Biologic properties and HI antibody titers in the vaccinated chickens

Biological properties

Virus	Pathogenicity: Mean death time in eggs	Pathogenicity: Intracerebral pathogenicity index	Pathogenicity: Intravenous pathogenicity index	Virus titer: EID_{50}/ml	Virus titer: HA
rNDV/I4	48 hr	1.96	2.55	$10^{9.5}$	8 $\log_2$
NDV/AI4	>120 hr	0.13	0	$10^{9.8}$	10 $\log_2$

Antibody titers

	Vaccine: Oil-AI4			Vaccine: Oil-Las		
HI antigen	7 days pi	14 days pi	21 days pi	7 days pi	14 days pi	21 days pi
NDV/AI4	**8**[A]	**79**	**294**	2	16	108
LaSota	5	17	137	**3**	**23**	**128**

Comparison of viral shedding

Chicken

	Samples (positive/total): Day 3 pc		Day 5 pc		Day 7 pc	
Group	OS	CS	OS	CS	OS	CS
PBS-C	10/10	10/10	NS	NS	NS	NS
Oil-AI4-C	4/10*	0/10	1/10	1/10	0/10	0/10
Oil-Las-C	9/10	0/10	2/10	1/10	0/10	0/10

Geese

	Samples (positive/total): Day 2 pc		Day 4 pc		Day 6 pc	
Group[A]	OS	CS	OS	CS	OS	CS
PBS-C	10/10	10/10	10/10	10/10	10/10	10/10
Oil-AI4-C	1/10	0/10	0/10	0/10	0/10	0/10
Oil-Las-C	5/10	1/10	1/10	1/10	0/10	0/10

C= challenge with 10^5 EID50 JS3/05; OS =oropharyngeal swabs; CS 5 cloacal swabs; NS 5 no survivors

What will be the effect of genotype matched vaccines on the virus evolution?

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0022-538X/04/$08.00+0 DOI: 10.1128/JVI.78.15.8372–8381.2004

Effect of Vaccine Use in the Evolution of Mexican Lineage H5N2 Avian Influenza Virus

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Received 3 January 2004/Accepted 16 March 2004

An outbreak of avian influenza (AI) caused by a low-pathogenic H5N2 type A influenza virus began in Mexico in 1993 and several highly pathogenic strains of the virus emerged in 1994-1995. The highly pathogenic virus has not been reported since 1996, but the low-pathogenic virus remains endemic in Mexico and has spread to two adjacent countries, Guatemala and El Salvador. Measures implemented to control the outbreak and eradicate the virus in Mexico have included a widespread vaccination program in effect since 1995. Because this is the first case of long-term use of AI vaccines in poultry, the Mexican lineage virus presented us with a unique opportunity to examine the evolution of type A influenza virus circulating in poultry populations where there was elevated herd immunity due to maternal and active immunity. We analyzed the coding sequence of the HA1 subunit and the NS gene of 52 Mexican lineage viruses that were isolated between 1993 and 2002. Phylogenetic analysis indicated the presence of multiple sublineages of Mexican lineage isolates at the time vaccine was introduced. Further, most of the viruses isolated after the introduction of vaccine belonged to sublineages separate from the vaccine's sublineage. Serologic analysis using hemagglutination inhibition and virus neutralization tests showed major antigenic differences among isolates belonging to the different sublineages. Vaccine protection studies further confirmed the in vitro serologic results indicating that commercial vaccine was not able to prevent virus shedding when chickens were challenged with antigenically different isolates. These findings indicate that multilineage antigenic drift, which has not been observed in AI virus, is occurring in the Mexican lineage AI viruses and the persistence of the virus in the field is likely aided by its large antigenic difference from the vaccine strain.

Comparison of the protective antigen variabilities of prevalent Newcastle disease viruses in response to homologous/heterologous genotype vaccines

Yonghua Li,[*,1] Zaib Ur Rehman,[*,†,1] Mengjiao Li,[*] Zahid Manzoor,[†] Wei Liu,[*] Xusheng Qiu,[*] Yingjie Sun,[*] Ying Liao,[*] Lei Tan,[*] Cuiping Song,[*] Weiwei Liu,[*] Shengqing Yu,[*] Chan Ding,[*,‡] and Chunchun Meng[*,2]

[*]*Shanghai Veterinary Research Institute (SHVRI), Chinese Academy of Agricultural Sciences (CAAS), Shanghai 200241, China;* [†]*Faculty of Veterinary and Animal Sciences, PMAS Arid Agriculture University, Rawalpindi 46300 Pakistan; and* [‡]*Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou, 225009, PR China*

ABSTRACT The genotype VII Newcastle disease virus (**NDV**) vaccine has begun to replace the traditional genotype II NDV vaccine and is widely used in the commercial poultry of China. However, the effect of homologous and heterogeneous anti-NDV serum on the evolution of prevalent NDV is unknown. To understand the effect of genotype II and VII anti-NDV serum on the evolution of genotype VII NDV strains, ZJ1 (waterfowl origin) and CH/SD/2008/128 (ND128; chicken origin) were used for serial passage of 30 generations in DF-1 cells without anti-NDV serum or with genotype II and VII anti-NDV serum independently. The F and HN genes of the 2 viruses were amplified for the 10th, 20th, and 30th generations of each serial passage group and compared with their respective original viruses. We found that there was only one mutation at position 248 in the F gene of ZJ1 due to the serum pressure of genotype VII anti-NDV. Similarly, mutations at residue 527 of the F gene, and position 9 and 319 of the HN gene of ND128 were noted in both anti-NDV serum groups. The results show that the nonsynonymous (**NS**)-to-synonymous (**S**) ratio of the F gene of ZJ1 virus was 1.6, and for the HN gene, it was 2.5 in the anti-II serum group. In the anti-VII serum group, the NS/S ratio for the F gene was 2.1, and for the HN gene, it was 2.5. The NS/S ratio of the F gene of the ND128 virus was 0.8, and for the HN gene, it was 3 in the anti-II serum group. Furthermore, the NS/S ratio of the F gene was 0.8, and the HN gene was 2.3 in the anti-VII group. Taken together, our findings highlight that there was no significant difference in the variation of protective antigens in genotype VII NDV under the selection pressure of homologous and heterogeneous genotype NDV inactivated vaccines.

Table 5. Comparison of NS/S ratios of the F gene during continuous passage with or without anti-NDV serum.

	ZJ1 F gene												ND128 F gene											
	Without anti-serum				Anti-II serum				Anti-VII serum				Without anti-serum				Anti-II serum				Anti-VII serum			
	1	2	3	All	1	2	3	All	1	2	3	All	1	2	3	All	1	2	3	All	1	2	3	All
NS[1]	2	2	2	6	3	4	3	10	5	5	5	15	1	1	1	3	2	2	2	6	3	2	2	7
S[2]	1	2	2	5	2	2	2	6	3	2	2	7	2	2	2	6	2	2	3	7	3	3	2	8
NS/S	2	1	1	1.2	1.5	1	1.5	1.6	1.6	2.5	2.5	2.1	0.5	0.5	0.5	0.5	1	1	0.6	0.8	1	0.6	1	0.8

[1]NS represents nonsynonymous mutation.

[2]S represents synonymous mutation.

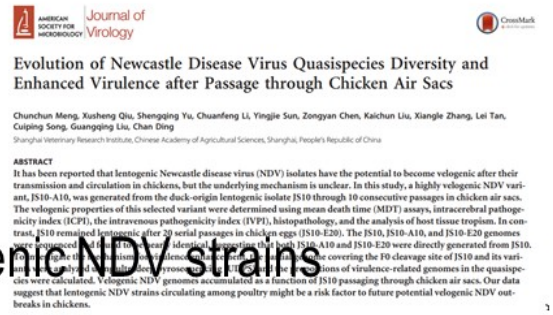
Table 6. Comparison of NS/S ratios of the HN gene during continuous passage with or without anti-NDV serum.

	ZJ1 HN gene												ND128 HN gene											
	Without anti-serum				Anti-II serum				Anti-VII serum				Without anti-serum				Anti-II serum				Anti-VII serum			
	1	2	3	All	1	2	3	All	1	2	3	All	1	2	3	All	1	2	3	All	1	2	3	All
NS[1]	3	7	8	18	2	6	2	10	4	7	7	18	2	2	1	5	5	7	6	18	5	6	3	14
S[2]	1	3	2	6	2	1	1	4	3	1	3	7	2	2	1	5	2	2	3	6	2	2	2	6
NS/S	3	2.3	4	3	1	6	2	2.5	1.3	7	2.3	2.5	1	1	1	1	2.5	3.5	3	3	2.5	3	1.5	2.3

[1]NS represents nonsynonymous mutation.
[2]S represents synonymous mutation.

Future threats

- Class 1 NDVs
- Waterfowl can harbor lentogenic NDV strains
- Extensive transmission from waterfowls to chickens
- LBMs play an important role in the spread of class I NDV
- Rural Poultry



Journal of Virology

Evolution of Newcastle Disease Virus Quasispecies Diversity and Enhanced Virulence after Passage through Chicken Air Sacs

Chunchun Meng, Xusheng Qiu, Shengqing Yu, Chuanfeng Li, Yingjie Sun, Zongyan Chen, Kaichun Liu, Xiangle Zhang, Lei Tan, Cuiping Song, Guangqing Liu, Chan Ding

Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Shanghai, People's Republic of China

ABSTRACT

It has been reported that lentogenic Newcastle disease virus (NDV) isolates have the potential to become velogenic after their transmission and circulation in chickens, but the underlying mechanism is unclear. In this study, a highly velogenic NDV variant, JS10-A10, was generated from the duck-origin lentogenic isolate JS10 through 10 consecutive passages in chicken air sacs. The velogenic properties of this selected variant were determined using mean death time (MDT) assays, intracerebral pathogenicity index (ICPI), the intravenous pathogenicity index (IVPI), histopathology, and the analysis of host tissue tropism. In contrast, JS10 remained lentogenic after 20 serial passages in chicken eggs (JS10-E20). The JS10, JS10-A10, and JS10-E20 genomes were sequenced and found to be nearly identical, suggesting that both JS10-A10 and JS10-E20 were directly generated from JS10. [illegible] the nucleotide variations [illegible] covering the F0 cleavage site of JS10 and its variants [illegible] and deep sequencing [illegible] the compositions of virulence-related genomes in the quasispecies were calculated. Velogenic NDV genomes accumulated as a function of JS10 passaging through chicken air sacs. Our data suggest that lentogenic NDV strains circulating among poultry might be a risk factor to future potential velogenic NDV outbreaks in chickens.

Virologica Sinica (2021) 36:818–822

LETTER

Surveillance of Class I Newcastle Disease Virus at Live Bird Markets and Commercial Poultry Farms in Eastern China Reveals the Epidemic Characteristics

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Dear Editor,

Newcastle disease (ND), caused by virulent Newcastle disease virus (NDV), is a highly contagious and economically devastating viral disease of birds (Habib *et al.* 2018). NDV, also termed as avian paramyxovirus type 1 (APMV-1), belongs to the genus *Orthoavulavirus* in the family *Paramyxoviridae* according to the International Committee on Taxonomy of Viruses (ICTV) (Amarasinghe *et al.* 2019). According to the latest phylogenetic classification system, NDVs can be further divided into two groups: class I and class II. There are three sub-genotypes in a single class I genotype 1, including sub-genotype 1.1.1, 1.1.2, and 1.2 (Dimitrov *et al.* 2019). Class I NDV, with the genome size of 15,198 nucleotides, is distributed globally and isolated frequently from wild birds and domestic poultry (Miller *et al.* 2010). Waterfowl can harbor lentogenic NDV strains and act as a natural reservoir for NDV (Kim *et al.* 2007; Wang *et al.* 2016), and class I NDVs have been reported to transfer from waterfowls to chickens and circulated in chicken flocks extensively in China (Zhu *et al.* 2014; Chen *et al.* 2020). Although class I NDVs are generally avirulent, they are likely to increase their virulence. For instance, class I NDVs can enhance virulence through several passages in chicken due to the mutations at the F cleavage site (Yu *et al.* 2002), and they can also evolve into a virulent virus through only a few point mutations (Collins *et al.* 1998). Furthermore, the 1990 Ireland ND outbreak was caused by a virulent class I NDV (Alexander *et al.* 1992). However, owing to its avirulence, class I NDVs are often underreported or neglected within other surveillance efforts. To better evaluate the prevalence of class I NDVs, we performed the continuous surveillance of class I NDVs and revealed the epidemic characteristics of class I NDVs at live bird markets (LBMs) and commercial poultry farms in eastern China.

During the active surveillance program of NDV from January 2017 to September 2018, we randomly collected samples from LBMs and commercial poultry farms from different provinces in eastern China, including Jiangsu, Shandong, Hebei and Liaoning. Cloacal and oropharyngeal swabs were collected monthly from each flock. Herein, a total of 1608 swab samples were collected from poultry at settled LBMs, including chicken samples (630/1608, 39.7%), duck samples (380/1608, 23.6%), goose samples (582/1608, 36.2%), and a few pigeon samples (7/1608, 0.4%). Furthermore, we also obtained samples from commercial poultry farms regularly, including commercial chickens (n = 400) and ducks (n = 800). Briefly, com-