



Challenges and Innovations in Avian Influenza Vaccines

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Presentation Outline

- **Challenges in sustaining the efficacy of avian influenza vaccines**
- **Approaches to enhance the potency and efficacy of avian influenza vaccines**
- **Approaches to improve multivalency and delivery methods for avian influenza vaccines**

Avian influenza viruses causing outbreaks in poultry

- **H5Nx (Low and High pathogenicity)**
>30 HA gene clades and sub-clades
- **H7Nx (Low and High pathogenicity)**
>25 HA gene clades and sub-clades
- **H9N2 (Low Pathogenicity)**
2 major HA lineages and >19 clades
- **H6Nx (Low Pathogenicity)**
Several HA lineages

H5N1, H5N2,
H5N3, H5N,
H5N5 H5N6,
H5N8, H5N9

H7N1,H7N2,
H7N3, H7N5
H7N6, H7N7,
H7N8, H7N9



Global epidemic of H5N1 viruses

Poultry outbreaks

>300 million birds culled or died in the last 3 years



A large number of Northern gannets and great skuas died due to HPAI H5N1 infection

Wild birds and mammalian species

>60 wild bird species (>200,000 bird birds)



Mammalian species

>5200 Mink in Spain in 2022

> 600 sea lions in Peru in 2023

> Several infection in Cattles in USA



Human Infections with Avian Influenza Viruses

H5N1 896 cases, (CFR of 52%) in 24 countries from 2003 to 19 July 2024)

H5N6: 93 cases (CFR 61%) (from 2014, last reported on 17 June 2024

H7N9: 1568 cases (CFR: 39%) mainly in China from 2013-2019.

H7N4 one reported case 14 Feb 2018

H9N2: 102 cases, two deaths Last reported case 15th June 2024.

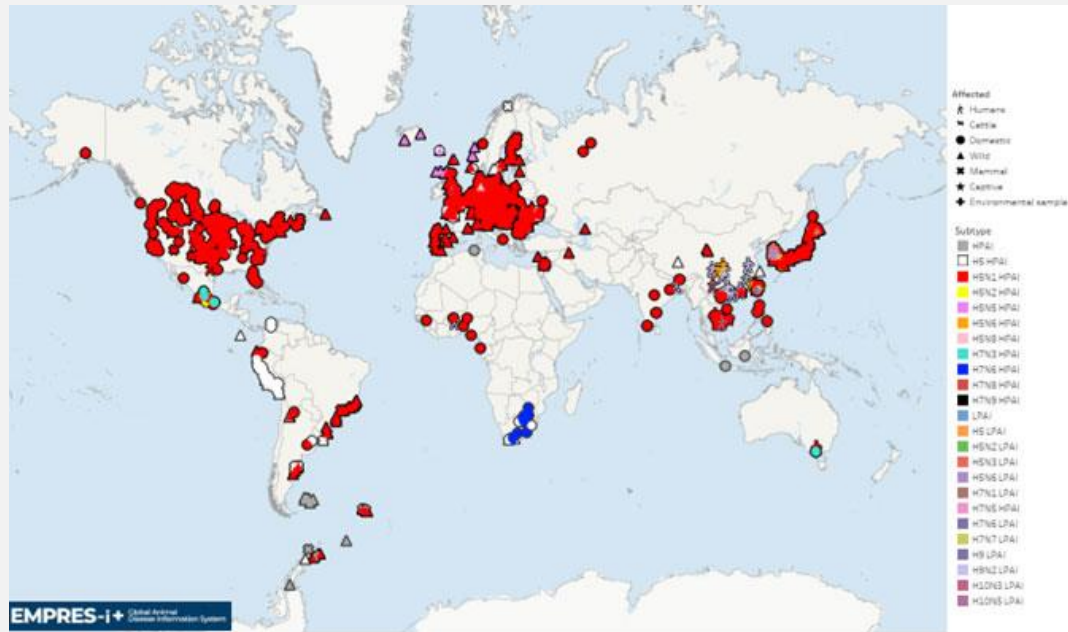
H3N8 three lab-confirmed cases Last 22 Feb 2023).

H10N3: three lab-confirmed cases. Last case 28 Feb 2024.

H10N5) one lab-confirmed case. 30 November 2023.

Recent reported HPAI Poultry outbreaks

Global distribution of AIV with zoonotic potential 1 October 2023 to 30 September 2024



>77 countries	H5N1
Australia	H7N9, H7N8, H7N3
China	H7N9
South Africa,	H7N6
Mexico	H7N3
Germany	H7N5
Mozambique	H7 (N untyped)

UK: 5 outbreaks in November/December 2025

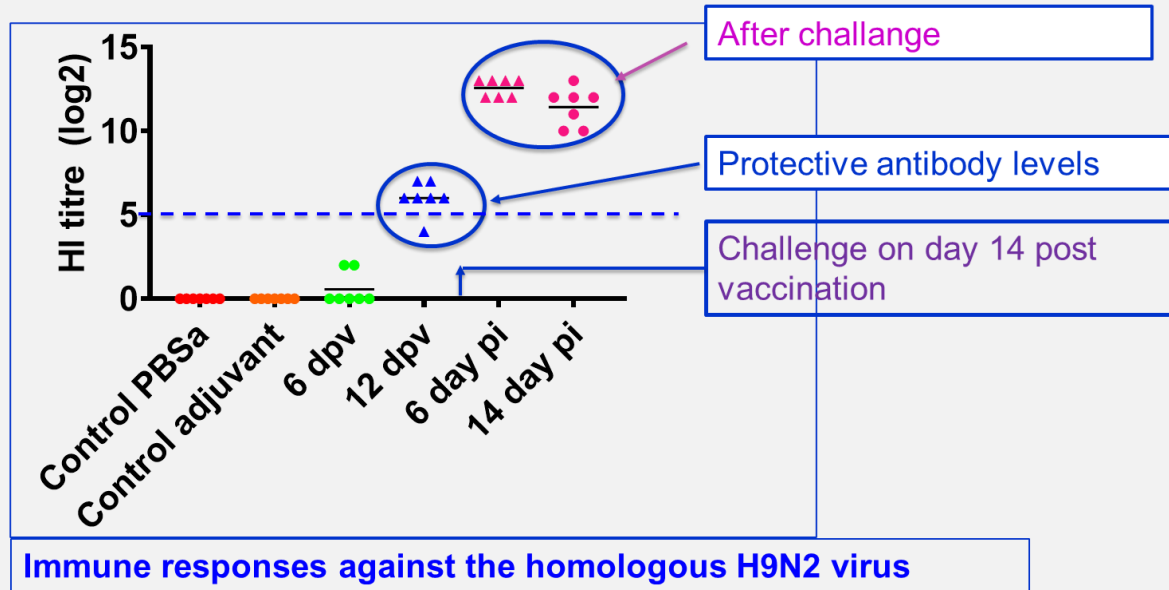
Control of AIV in poultry

- Stamping-out infected and at-risk flocks (sporadic infections)
- Vaccination

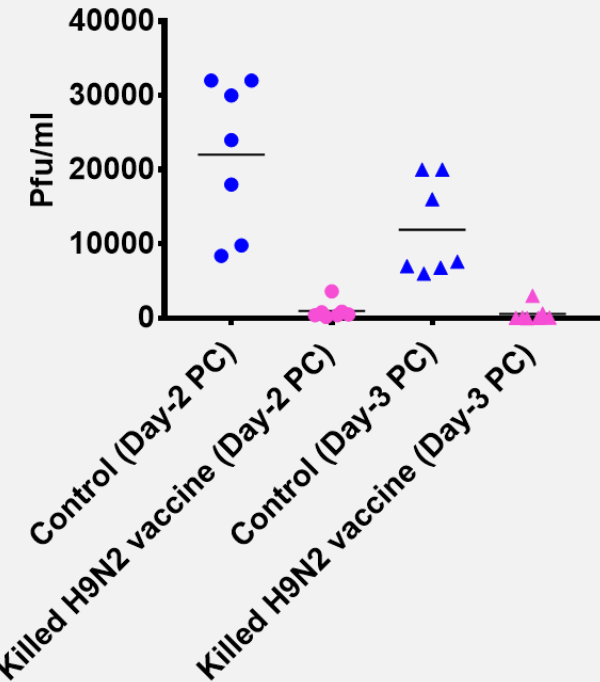


Do Avian Influenza Vaccines Provide Protection from Disease?

Vaccination of 7-day-old SPF chickens with inactivated H9N2 virus vaccine



- All vaccinated birds remained healthy and gained weight similar to control non- challenge birds
- All control birds showed clinical disease signs including diarrhoea and some respiratory symptoms and weight loss. **3 died**



Reduced virus shedding from vaccinated birds compared to control non-vaccinated birds

Do H5 vaccines provide protection from HPAI H5Nx viruses?

Over 300 million poultry died or were destroyed due to H5 virus infection during the last 3 years

Mainland China lost only 10,000 birds during this period because of vaccination.

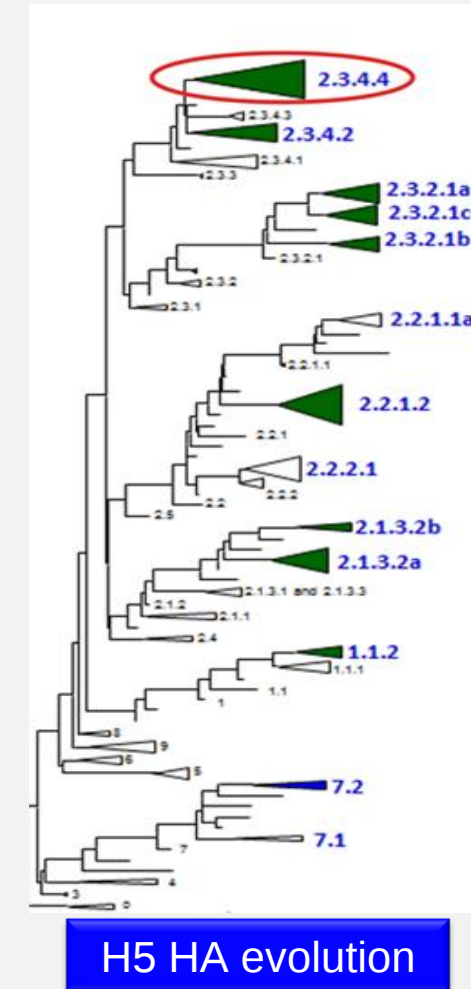
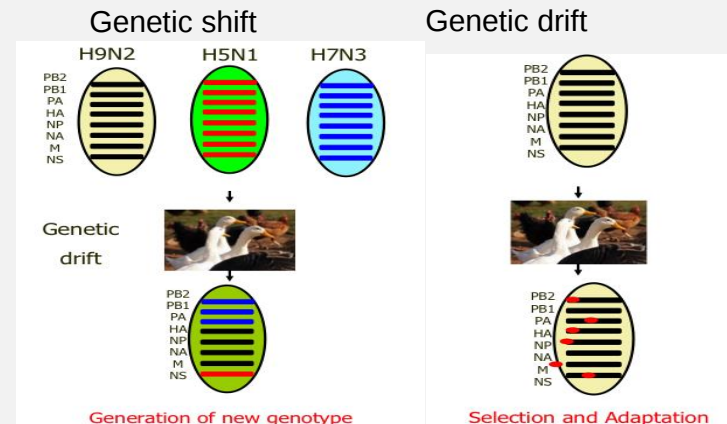
Last 20 years Hong Kong vaccinating their flocks with H5 vaccines; only one outbreaks reported at one farm in 2008 that used poorly antigenic matched vaccine.

Good quality antigenic matched vaccine to the field viruses do protect birds from clinical disease and its hidden impacts (weight gain or egg production

Avian influenza virus evolution and adaptation in poultry

Impact of Evolution on Biological Properties

- Antigenicity
- Vaccines & Diagnostic effectiveness
- Epidemiology
- Virulence
- Transmission
- Host-range



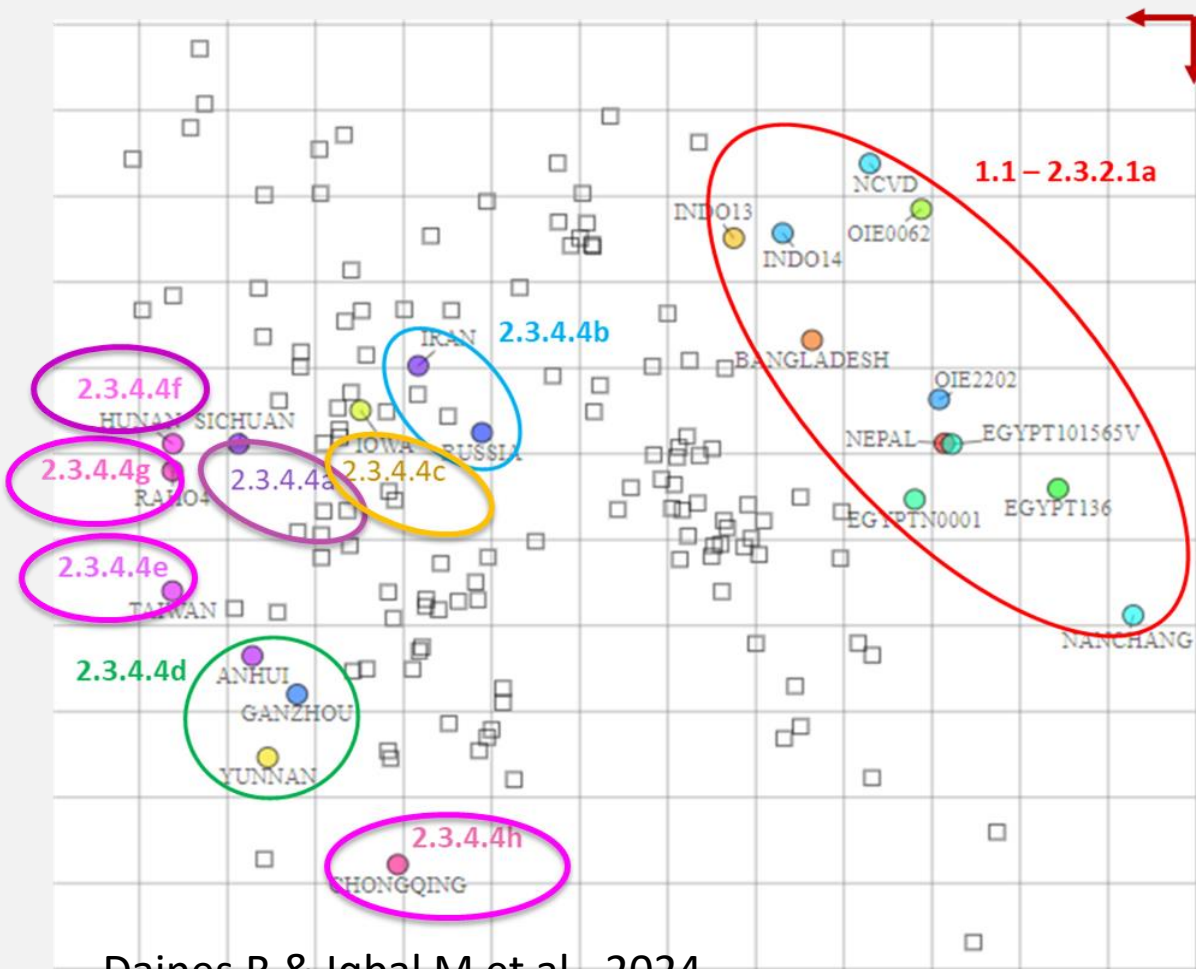
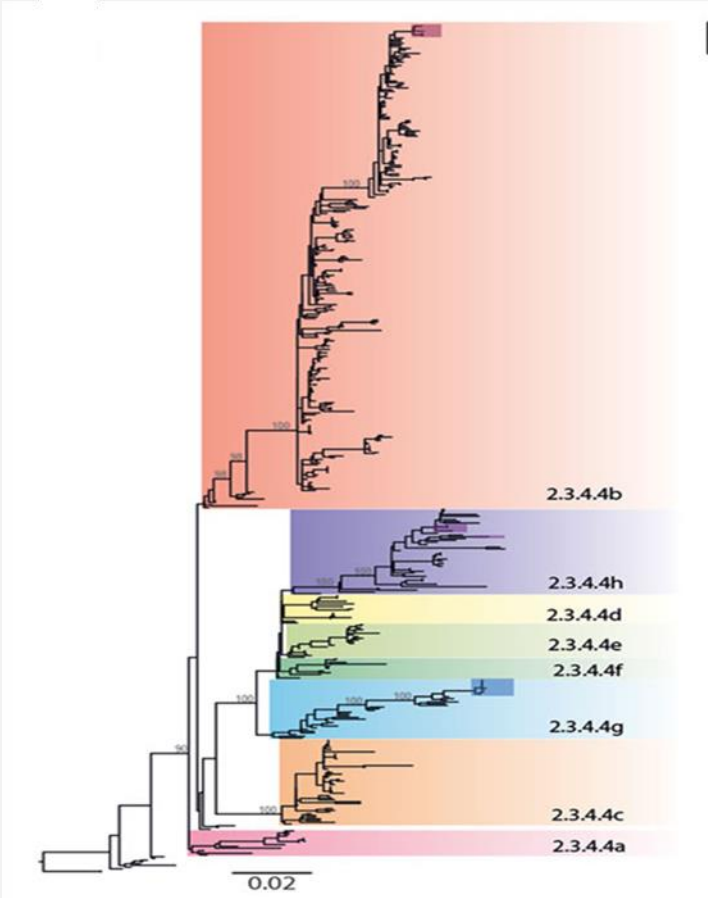
Evolution and antigenic diversity among H5 viruses

	Clade	Subtype	Strain Name	Antisera																					
				Nepal	Bangladesh	Indo 13	Yunnan	Iowa	OIE0062	Egypt 137	Egypt N0001	Egypt 101565v	Nanchang	Indo 14	NCVD	OIE2202	Ganzhou	Cheboksary	Aghakhan	Sichuan	Anhui	Taiwan	Hunan	Raho	Chongqing
Virus	2.3.2.1a	H5N1	A/Chicken/Nepal/T-359/2014	8.8	6.8	7.1	3.2	3.9	8.0	8.2	6.5	7.8	8.6	8.4	3.9	9.5	6.0	3.2	5.0	4.7	2.8	2.3	3.8	4.8	4.0
	2.2.2.1	H5N1	A/Chicken/Bangladesh/31289-1/2011	5.3	9.7	6.2	2.6	5.6	9.4	8.8	7.8	9.2	7.8	8.0	7.6	7.8	7.4	5.8	7.6	7.3	4.4	4.3	5.3	5.8	5.5
	2.1.3.2a	H5N1	A/Tree_Sparrow/Indonesia/D10013/2010	5.6	6.0	10.9	5.2	4.9	7.2	6.3	5.4	6.9	6.8	12.0	6.7	8.6	6.6	6.3	8.5	6.3	4.2	4.2	5.5	6.7	5.2
	2.3.4.4(d)	H5N6	A/Yunnan/0127/2015	2.9	4.1	7.7	10.1	5.0	3.5	3.8	3.6	4.7	4.5	5.8	1.0	2.5	11.8	4.7	6.7	6.8	9.4	8.0	5.0	5.8	8.0
	2.3.4.4c	H5N2	A/Chicken/Iowa/14589-1/2015	5.7	7.4	7.5	7.1	11.4	7.6	8.5	5.7	6.0	5.7	8.2	10.4	7.0	11.8	11.0	11.6	11.9	8.6	9.3	11.0	11.7	5.2
	1.1.1	H5N1	A/Duck/Vietnam/OIE/0062/2012	5.0	6.4	6.2	1.1	3.9	10.9	6.8	5.0	5.8	6.1	8.7	9.3	7.0	6.0	5.5	5.2	4.2	3.2	1.4	3.3	4.0	2.7
	2.2.1.2	H5N1	A/Turkey/Egypt/137/2013	5.4	5.3	4.4	1.6	1.5	5.6	8.9	7.8	8.7	8.8	6.0	4.1	8.5	4.3	1.7	2.6	3.3	1.8	1.5	3.0	3.3	4.2
	2.2.1	H5N1	A/Egypt/N0001/2015	7.0	7.2	5.4	3.2	3.5	6.9	9.9	9.5	10.5	8.9	7.7	5.6	8.7	6.0	2.9	3.8	4.8	4.2	1.5	3.3	4.9	5.8
	2.2.1	H5N1	A/Duck/Egypt/101565v/2010	4.9	6.6	5.3	2.4	4.2	7.0	8.9	8.3	10.4	9.7	7.7	5.7	8.6	6.8	4.6	3.7	4.7	3.8	2.0	3.0	4.3	5.2
	2.3.2.1b	H5N1	A/Duck/Nanchang/9789/2013	7.3	3.8	4.9	1.2	1.4	4.5	6.3	3.4	9.4	11.7	5.3	2.8	8.4	2.7	0.9	1.9	2.2	0.6	0.3	2.8	4.0	2.0
	1.1	H5N1	A/Chicken/Vietnam/NCVD/1192-2012	4.7	4.4	5.5	1.5	4.4	9.7	5.4	4.4	5.6	6.1	7.9	8.5	5.0	6.2	7.5	7.7	5.8	4.5	2.5	4.3	5.1	3.8
	2.1.3.2b	H5N1	A/Chicken/Indonesia/D100014/2010	5.8	5.9	11.1	4.9	5.7	7.3	7.3	5.8	6.2	6.9	12.0	6.2	9.2	5.3	3.5	9.2	6.2	4.1	3.9	5.8	6.4	3.9
	2.3.2.1c	H5N1	A/Duck/Vietnam/OIE/2202/2012	8.8	6.0	5.7	1.0	1.6	7.7	8.5	5.8	8.0	8.7	8.7	10.0	11.6	6.8	5.0	6.0	4.7	2.6	1.6	3.9	5.2	3.8
	2.3.4.4d	H5N6	A/Chicken/Ganzhou/GZ21/2015	4.6	4.9	7.2	8.5	4.7	4.6	4.0	3.6	5.7	4.7	6.4	4.6	5.1	10.6	6.8	6.9	7.8	8.6	7.3	6.5	8.0	8.7
	2.3.4.4b	H5N8	A/Chicken/Cheboksary/854/2018	6.6	8.8	10.3	9.8	12.0	9.4	8.7	8.0	10.2	7.8	10.8	8.6	7.1	10.6	9.7	11.2	11.0	9.4	9.1	11.0	10.7	7.5
	2.3.4.4b	H5N8	A/Crow/Aghakhan/2017	4.8	5.8	7.6	6.7	8.8	6.5	6.7	5.3	7.8	6.2	9.2	6.8	5.5	8.0	8.5	10.8	9.0	7.2	7.3	9.5	10.2	6.5
	2.3.4.4a	H5N6	A/Chicken/Sichuan/J1/2014	3.8	6.0	6.3	4.8	7.7	5.0	5.8	4.0	6.0	4.8	6.8	3.8	5.0	7.6	6.9	9.0	11.8	7.6	6.8	9.5	9.8	6.5
	2.3.4.4d	H5N6	A/Anhui/33162/2016	3.5	4.4	6.2	8.0	5.5	5.0	4.0	4.3	6.2	3.8	6.7	3.5	4.8	9.5	5.6	6.8	8.7	9.6	7.7	6.8	7.7	6.5
	2.3.4.4e	H5N6	A/Duck/Taiwan/1702004/2017	2.7	4.2	5.2	6.0	6.7	3.7	4.8	3.0	4.8	3.4	5.8	3.3	4.5	7.4	5.1	7.0	10.2	8.6	9.0	8.0	7.5	7.2
	2.3.4.4f	H5N6	A/Whooper swan/Hunan/4/2016	3.0	4.8	5.3	4.7	7.0	4.2	5.2	3.0	5.0	4.4	7.2	3.3	5.3	6.7	6.8	9.5	9.7	6.8	7.2	11.3	8.8	5.7
	2.3.4.4g	H5N6	A/Chicken/Vietnam/Raho4-Cd-20-421/2020	3.0	4.2	5.5	4.7	6.2	4.2	4.2	3.2	5.5	4.0	6.2	3.5	4.5	7.4	7.0	9.5	11.7	7.0	6.3	8.5	10.7	6.2
	2.3.4.4h	H5N6	A/Chongqing/00013/2021	3.2	4.2	5.0	6.0	2.0	4.7	4.7	3.5	5.8	4.0	5.5	3.3	4.5	7.9	4.0	5.5	6.8	6.8	4.7	5.3	6.3	10.7

Homologous HI
Titre **11.7**
log

Heterologous HI
Titre **0.9 log₂**

Significant antigenic differences among H5 viruses infecting poultry in different regions



1 square = 1 antigenic unit = 2log HI titre

Circles – viruses
Squares – sera

Daines R & Iqbal M et al., 2024

Why antigenic match between vaccine and field viruses is critical for protective efficacy

Virus	HA Clade	HI antibody titre of antiserum	
		H5-Re11	H5-Re12
H5-Re11	2.3.4.4h	512	32
H5Re12	2.3.4.4f	16	512
WS/SX/4-1/2020	2.3.4.4b	16	8
BS/BJ/1/2021	2.3.4.4b	16	8
GS/HuN/S11288/2021	2.3.4.4b	16	8
WS/SD/SC195/2021	2.3.4.4b	16	8

Multiple variants of H5Nx clades 2.3.4.4b are prevalent in China

Cui et al., SCLS, 2022

China tries to update its vaccine seed to follow virus evolution

Vaccine	H5 Clade	HA donor virus	Time period
H5-Re1	0	GD/GD/1/1996(H5N1)	03/2004–03/2008
H5-Re4	7.2	CK/SX/2/2006(H5N1)	07/2006–04/2014
H5-Re5	2.3.4	DK/AH/1/2006(H5N1)	03/2008–06/2012
H5-Re6	2.3.2	DK/GD/S1322/2010(H5N1)	06/2012–09/2017
H5-Re7	7.2	CK/LN/S4092/2011(H5N1)	04/2014–09/2017
H5-Re8	2.3.4.4g	CK/GZ/4/2013(H5N1)	12/2015–12/2018
H5-Re11	2.3.4.4h	DK/GZ/S4184/2017(H5N6)	12/2018–12/2021
H5-Re12	2.3.2.1f	CK/LN/SD007/2017(H5N1)	12/2018–12/2021
H5-Re13	2.3.4.4h	DK/FJ/S1424/2020(H5N6)	01/2022– current
H5-Re14	2.3.4.4b	WH/SX/4-1/2020(H5N8)	01/2022–current

What about the co-circulation of multiple antigenic variants? (Vaccines against the dominant strains)

Antigenic diversity among H9N2 viruses infecting poultry in different regions

Virus	Antisera															
	UDL1/08	UDL2/08	SA/D363/06	BD/0994	Iran/B10/02	Leb/1080	Em/R66	UAE/302	Pak/2/99	HK/G1	HK/33982	HK/1073	mal/Eng	Knot/Eng	HK/91/76	Wis/66
UDL1/08	8192	512	4096	2048	4096	2048	512	2048	512	128	64	1024	128	32	512	<64
UDL2/08	4096	1024	2048	2048	1024	1024	256	1024	512	64	32	1024	256	64	512	128
Env/BD	512	64	64	512	256	256	16	64	128	32	32	256	16	<8	64	<64
BD/0994	2048	256	512	512	1024	512	128	256	256	64	32	512	64	32	256	<64
UAE/D1556	128	64	64	128	32	16	16	8	<32	64	16	256	32	<8	64	<64
egy/D7100	8192	256	512	128	1024	2048	256	512	512	64	64	1024	256	64	512	128
Ind/WB	4096	512	2048	4096	2048	2048	256	1024	512	128	64	1024	128	32	512	128
Isr/239	8192	512	1024	512	2048	2048	256	1024	512	128	64	1024	128	64	512	<64
Leb/1080	4096	256	512	2048	2048	8192	1024	512	512	256	64	1024	128	64	512	<64
Em/R66	128	64	32	16	64	128	256	256	128	64	16	256	32	32	128	<64
HK/G1	256	32	16	2048	128	128	256	128	128	512	128	1024	16	32	256	<64
HK/33982	128	32	16	1024	128	64	32	128	64	64	1024	256	4	<8	128	128
VN/38	64	32	16	1024	64	64	32	64	64	32	512	256	8	<8	128	128
HK/G9	2048	128	256	128	1024	1024	128	256	256	64	32	1024	128	32	256	<64
WZ/606	128	256	64	2048	64	128	32	64	64	64	128	512	64	64	256	<64
HK/3239	4096	256	256	128	1024	1024	128	256	256	64	32	1024	64	32	256	<64
Wis/66	512	128	16	16	32	32	32	64	128	128	256	256	512	64	1024	512

An antigenic match between the vaccine and field viruses is required to induce optimal immunity against antigenic variants.

HA gene diversity of H9N2 viruses infecting poultry in Bangladesh during 2021-2022

3 different genetic sub-groups
of H9N2 viruses are co-
circulating in the same region

R48, I114, D198, I226,
D262, I359, I404, M451

A43, H48, T54, L115,
T198, I269, R276, N363

S22, Q48, T54, L69, K112,
V114, G149, Q150, Q162,
N198, V288, H315, K363,
K443, M444, R480, K492,
E496

Co circulation of H5HA clades:
2.3.2.1 (a-c) and 3.4.4.4 (b, h)

A similar situation in Egypt, China and
Indonesia

Summary-I

Challenges facing vaccine efficacy

- Virus evolution & antigenic diversity
- Emergence of new virus variants
- Co-circulation of multiple subtypes and variants (H5, H7, H9, H6)
- Poor quality Vaccines (antigenic mismatch to the field challenge)
- MDA interference
- Mixed infection of different viruses (Immunosuppressive pathogens)

Desirable formulation of vaccines

- **Potent:** Single dose induces faster, stronger, and durable immunity against co-circulating variants
- **Effective:** Protect from clinical disease, reduced shedding and transmission.
- **Affordable:** Cheap to produce and easy to deliver.
- **Safe:** No adverse impact on the host or environment.
- **Stable:** Retain efficacy for at least 1 year at the indicated temperature.
- **DIVA:** Allow differentiation between infected and vaccinated animals.

Desirable outcomes of vaccination

- **No effect on production and reduced related hidden impacts**
- **No trade embargoes**
- **Reduced or eradication of enzootic circulation**

AIV Vaccine Formulations

- **Whole Inactivated Virus Vaccines:**
 - Inactivated field or reverse genetic-based adjuvanted vaccines
- **Viral Vectored Vaccines:**
 - Herpesvirus of turkeys (HVT), Newcastle disease virus (NDV), Duck enteritis virus (DEV) Duck enteritis virus (DEV); Fowl pox virus (FPV); Infectious laryngotracheitis virus (ILTV)
- **Subunit Recombinant Protein Vaccines:**
 - Recombinant HA protein produced in cultured cells
- **DNA/ RNA Vaccines:**
 - Requiring more innovations to be cost-effective for poultry

Types of AIV vaccines

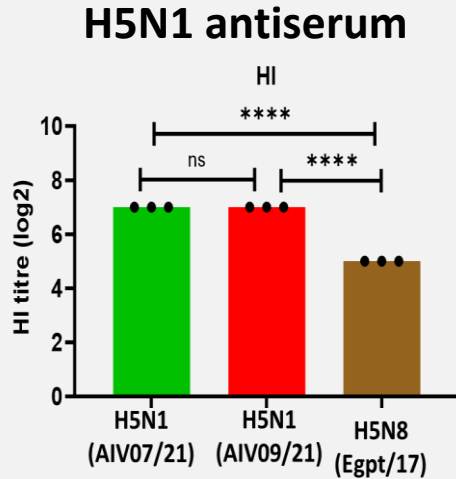
Vaccines	Challenges
Whole inactivated virus vaccines: (Isolation and inactivation of field viruses)	Field viruses may contain hidden pathogens or may not replicate in high titres, (repeated doses costs)
Viral vectored vaccines: (HVT, NDV, DEV) FPV, ILTV)	Reduced replication and expression of recombinant antigens in the presence of Maternal derived antibodies (MDA)/ pre-existing host immunity
Subunit vaccine: (Recombinant influenza haemagglutinin (HA) protein)	Effectiveness of single protein antigen, stability of antigen and production costs
mRNA/DNA vaccines	Requiring more innovations to be cost-effective for poultry

Genetic Homology and vaccine Seed Selection

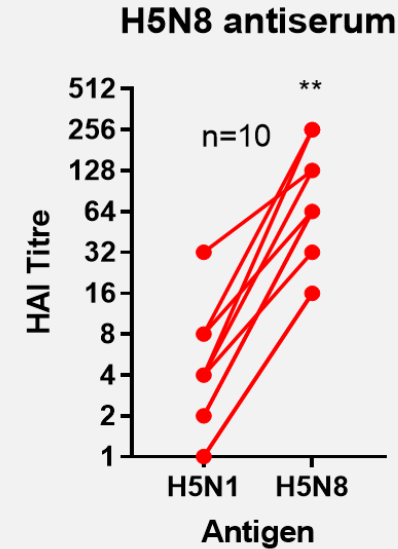
Cross-reactivity of monoclonal antibodies against closely related and divergent H9N2 viruses									
	Lineage	Amino acid homology (%)	H9N2 Monoclonal antibodies against UDL01/08						
			EC12	CG12	HA9	JF7	JF8	ID2	IB3
chicken/Pakistan/UDL-01/2008	G1	100	2560	5120	5120	5120	2560	2560	2560
chicken/Pakistan/UDL-02/2008	G1	96	2560	2560	5120	5120	40	160	2560
Environment/Bangladesh/10306/2011	G1	96	80	80	80	40	< ^c	<	20

Genetic homology not always linked with antigenic homology

Antigenic differences between current H5N1 and previous H5N8 HPAIV viruses



H5N1 antisera showed significantly less cross-reactivity against H5N8 virus



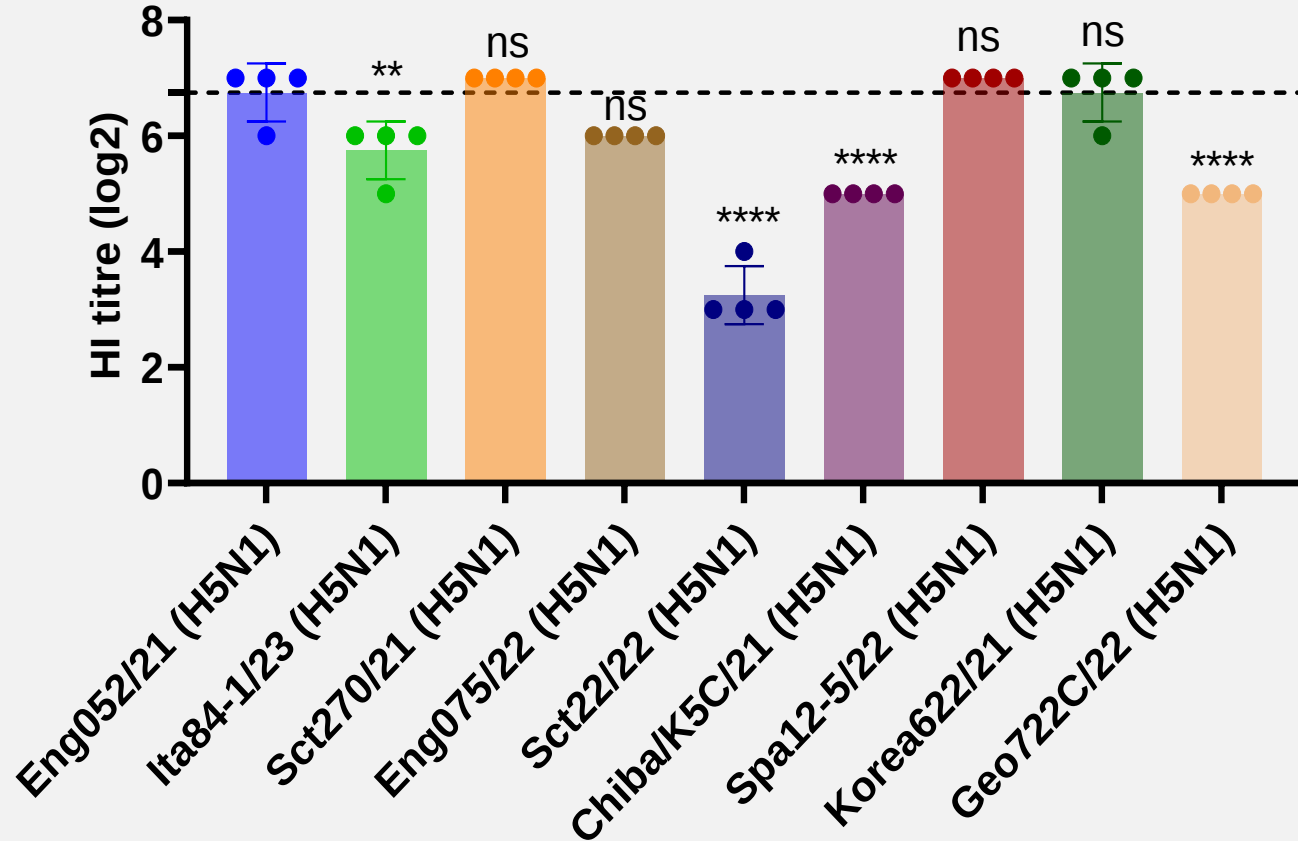
H5N8 antisera showed significantly less cross-reactivity against H5N1 virus

Single amino acid mutation (N236D) influences the antigenicity of current H5N1 and previous H5N8 viruses

H5Nx Clade 2.3.4.4b viruses	HA Amino acid position 236	HI titers of monoclonal antibody #19
H5N8 (A/Duck/Egypt/SS19/2017)	N	256
H5N1 (A/Ck/England/085598/2022	D	0
H5N1:(A/Ck/England/053052/2021	D	0
H5N1 (A/Ck/Scotland/054477/2021	D	0
H5N1 (A/Ck/England/085598/2022	D	0

Several antigenic variants of H5N1 clade 2.3.4.4b have been identified.

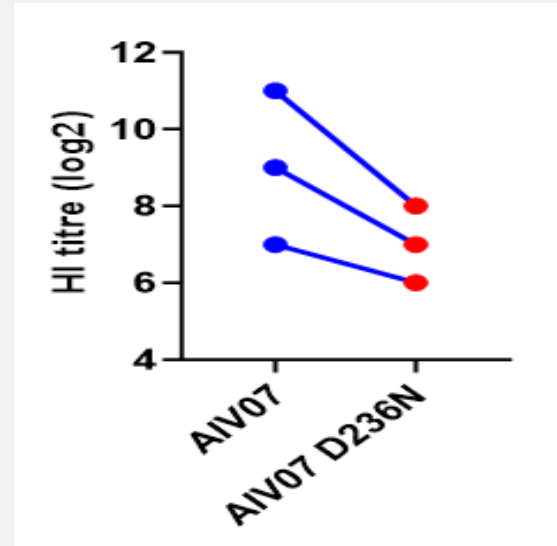
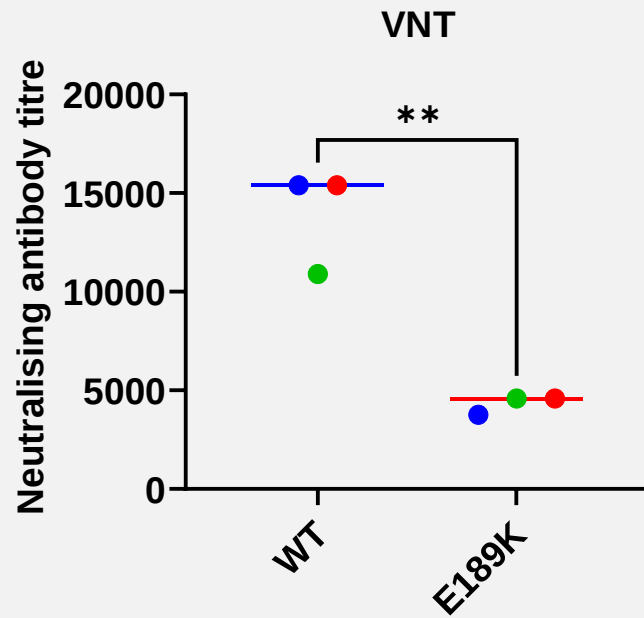
HI



Some emerging strains of H5N1 are displaying antigenic changes.

Mutations identified that modulates the antigenic change.

Recent H5N1 clade 2.3.4.4b viruses carry mutations that may compromise vaccine efficacy.



A single amino acid mutation can cause significant antigenic change

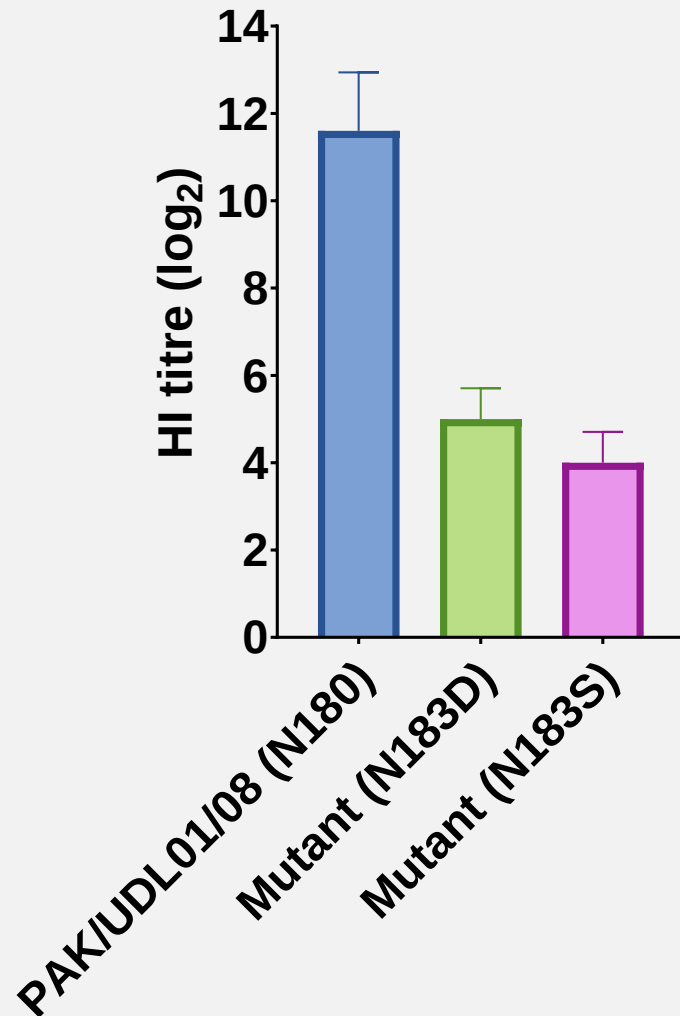
H9N2: with a single mutation, the virus can overcome vaccine effectiveness

Virus	Amino acid at position 180	HI titres (Polyclonal antisera)
H9N2	A	256 (8 log ₂)
H9N2	T	16 (4 log ₂)
H9N2	V	16 (4 log ₂)

Currently, variants with this mutation are prevalent in most countries in Asia and Middle East

(16x) 4-fold log₂ change in HI titres due to A180T/V substitution

Significant antigenic change with H9HA mutations N183D



Currently, H9N2 viruses containing HA with N/D/S183 are co-circulating in poultry in many countries in Asia and Middle East.

H9N2 immune escape variants via deletion of 4 amino acids in the HA that almost completely lose antigenic cross-reactivity

Virus	HI Titre (polyclonal antisera to wild type virus)	
Wild type	1024	(10 log ₂)
Deletion mutant	8	(3 log ₂)

4-7 amino acids
deletion at the 220-
loop region of HA
antigen



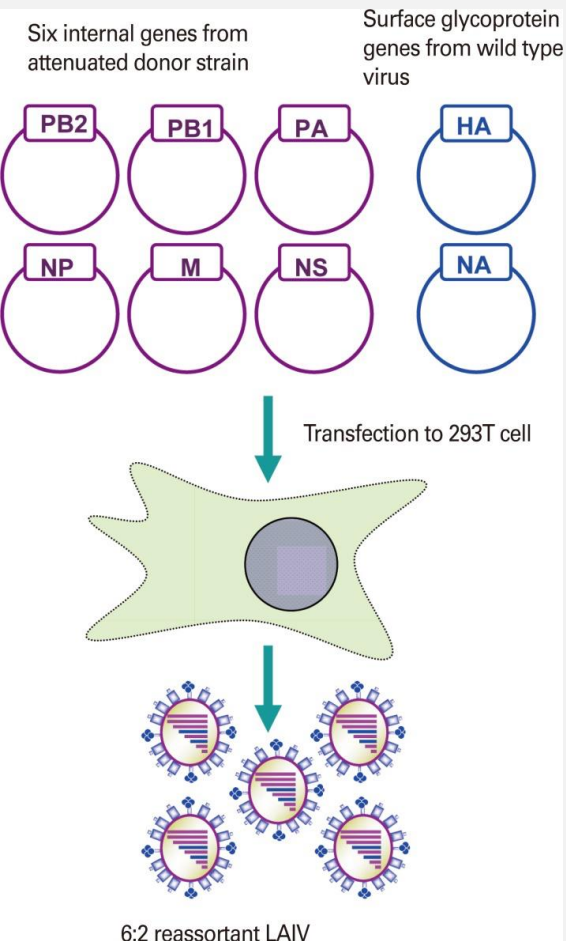
Summary-II

- **Antigenically distinct strains of H5 and H9 viruses are co-infecting poultry.**
- **Surveillance of variants and updating vaccines against variants is critical for reducing the ALVs impacts**

Innovations overcoming challenges in AIV vaccines

- **Bioinformatics:** Selecting more cross-reactive antigens
- **Reverse genetic:** Increasing safety and yield and reducing contaminating agents
- **Engineering antigenic epitopes:** Identifying and modifying epitopes inducing stronger and broader immunity
- **Manipulating viral vectors:** Expressing multiple antigens targeting variants
- **Passive immunisation:** Expressing multiple antibodies targeting variants

Recombinant Inactivated Influenza vaccines produced using reverse genetic (RG) viruses

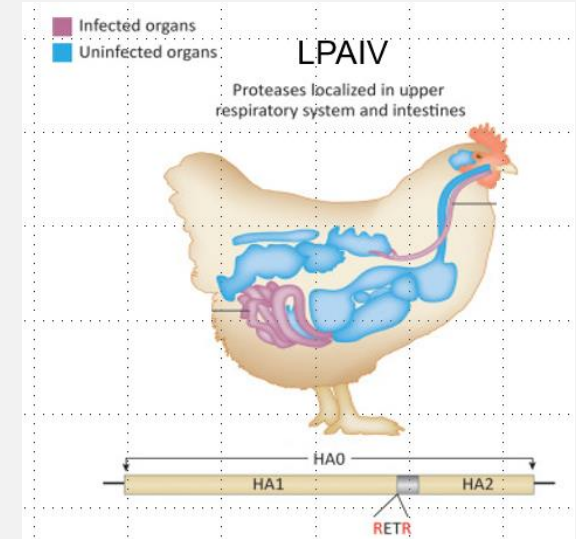
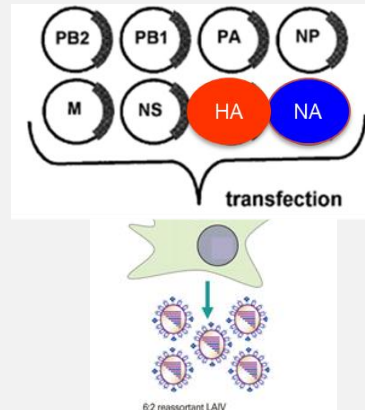
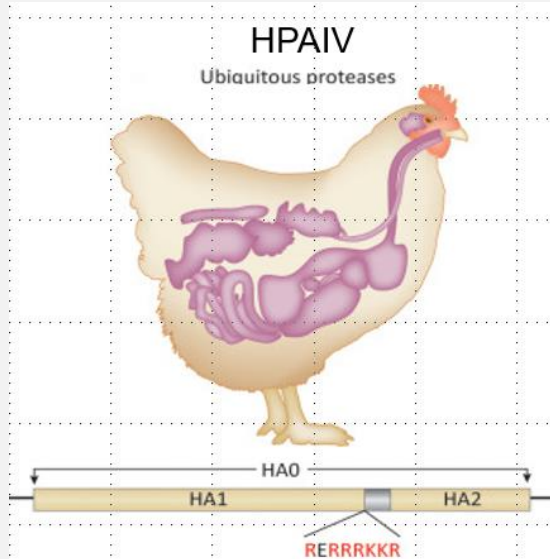


- **Replace/modify genes:**
- **To antigenically match to field viruses**
- **To increase the safety of vaccine production**
- **To increase the breadth of Immunity**
- **To increase the vaccine seed replication and yield**
- **To Produce vaccine seeds with no containing field pathogens**

Increased safety and yield of H5 Vaccines: HPAIV to LPAIV

'De-engineering'

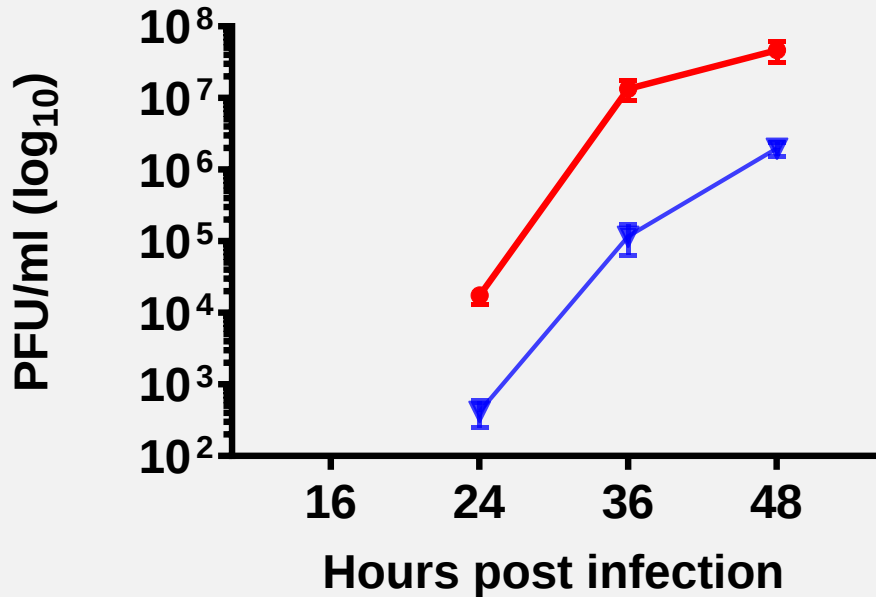
Alteration of HA cleavage site



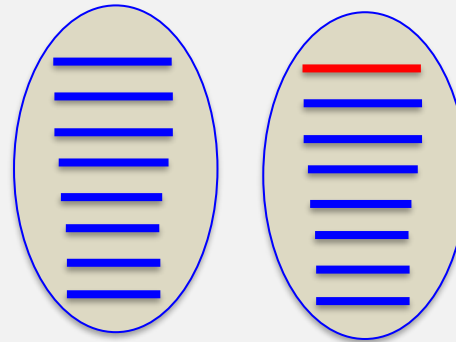
Deletion of HA cleavage site multi-basic motif completely removes the ability of the virus to kill embryos

Increase vaccine yield: optimise viral gene constellation

- Reassortant 7+1
(H9N2-UDL/Pakistan+PB2/Vietnam-H9N2)
- ▼ Wildtype (H9N2-UDL/Pakistan-H9N2)



H9N2 (A/ chicken/Pakistan/UDL/01/08) showed $>1.5\log_{10}$ increase in replication titers by replacement of **PB2 gene** from Vietnam (A/chicken/Vietnam/2015) H9N2 virus.



Wildtype (UDL/08_
Reassortant (UDL/08)

Vaccine does from 1 egg:

- Wild-type: 100 doses
- Reassortant: 1500 doses

Identify mutations that compromise the antigenicity and efficacy of Vaccines

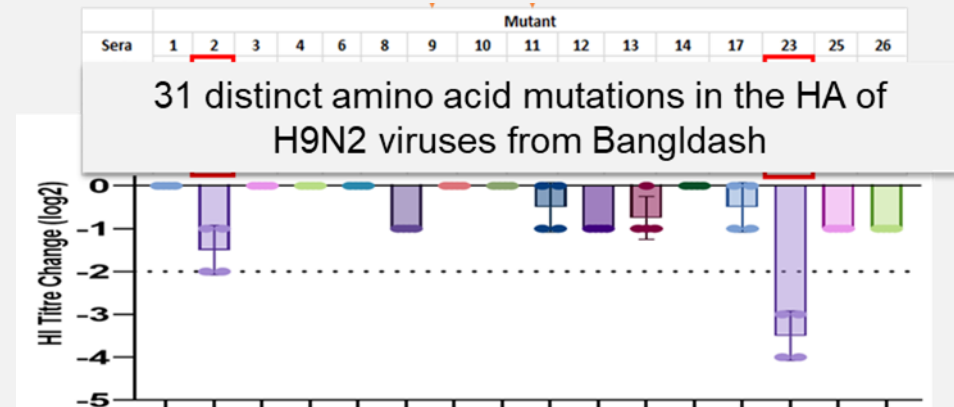


**R48, I114, D198, I226,
D262, I359, I404, M451**

**A43, H48, T54, L115,
T198, I269, R276, N363**

**S22, Q48, T54, L69, K112,
V114, G149, Q150, Q162,
N198, V288, H315, K363,
K443, M444, R480, K492,
E496**

Mutation		
Amino acid	Nucleotide (Codon)	Codon End NT#
T22S	ACT --> AGC	66
A43S	GCA --> TCA	129
H48R	CAT --> CGT	144
H48Q	CAT --> CAA	144
T54K	ACA --> AAA	162
L69S	CTT --> TCA	207
Q112K	CAA --> AAA	336
I114V	ATC --> GTC	342
Q115L	CAA --> CTA	345
G149S	GGT --> AGT	447
F150Q	TTT --> CAA	450
F150L	TTT --> CTT	450
R162Q	CGG --> CAG	486
N198D	AAT --> GAC	594
N198T	AAT --> ACT	594
V226I	GTA --> ATA	678
N262D	AAT --> GAT	786
N262S	AAT --> AGC	786
V269I	GTA --> ATA	807
V288I	GTC --> ATC	864
P315H	CCT --> CAT	945
V359I	TTT --> TCT	1077
Q363K	CAG --> AAG	1089
Q363N	CAG --> AAT	1089
I404L	ATT --> CTT	1212
K443R	AAA --> AGA	1329
R444M	AGA --> ATG	1332
L451M	CTG --> ATG	1353
R480K	AGG --> AAA	1440
K492R	AAA --> AGA	1476
I496V	ATA --> GTA	1488



Use Bioinformatics and reverse genetic approaches to select broadly cross-reactive vaccine seeds

Vaccine seed Selection

BD/H9-79948

R48, I114, D198, I226,
D262, I359, I404, M451

BD/H9/79924

Sera ID (Bird)	HI Titre (log2 HAU)		
	79948	79924	79943
145	>12	>12	>12
146	>12	>12	>12
147	>12	>12	>12
148	11	10.5	10.5
149	>12	>12	>12
150	11	11	11.5

A43, H48, T54, L115,
T198, I269, R276, N363

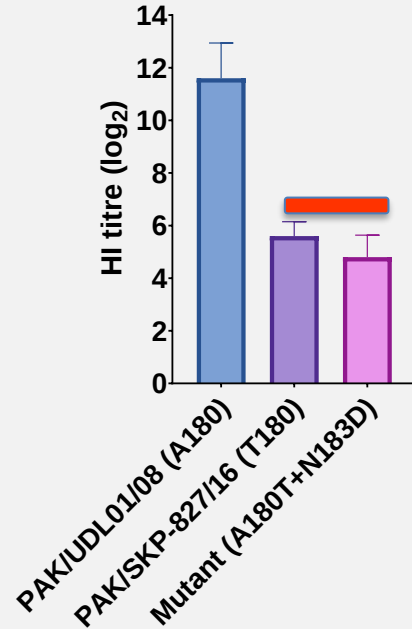
BD/H9/079943

S22, Q48, T54, L69, K112,
V114, G149, Q150, Q162,
N198, V288, H315, K363,
K443, M444, R480, K492,
E496

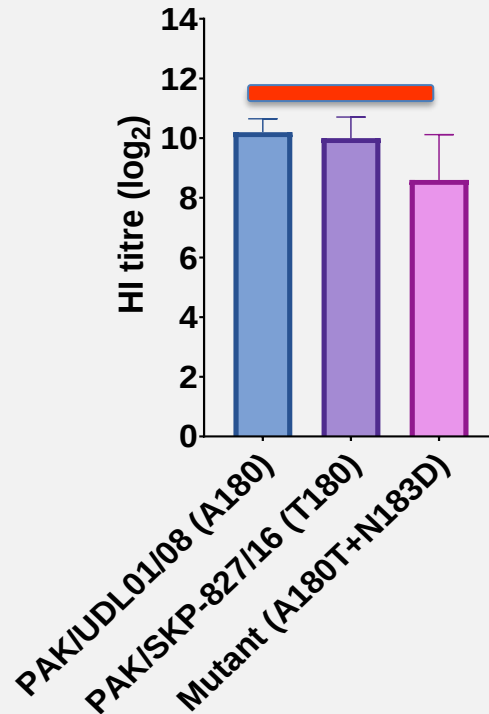
BD/H9-079948

Engineered antigenic epitopes increased the antigenic cross-reactivity against variants

Chicken antisera against H9N2 virus with (A180)



Chicken antisera against H9N2 with (T180)



A180T mutation increased the binding affinity of HA to cell receptors

H9N2 Vaccine with HA (T180) showed increased cross-reactivity against variants.

Modification of antigenic epitopes enhances cross-reactivity against variants.

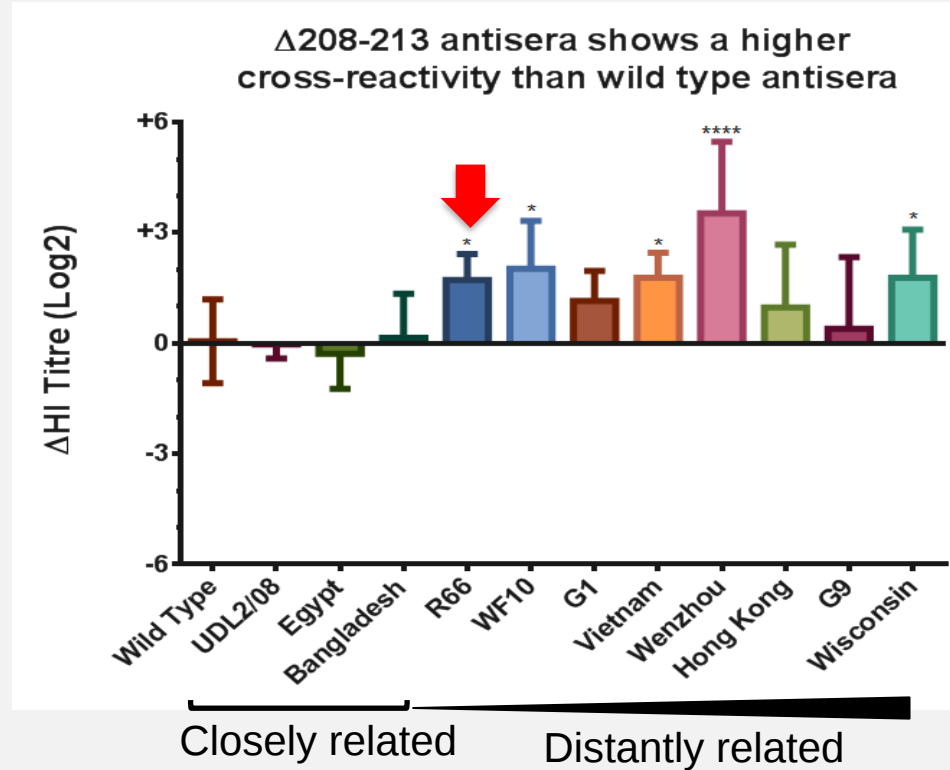
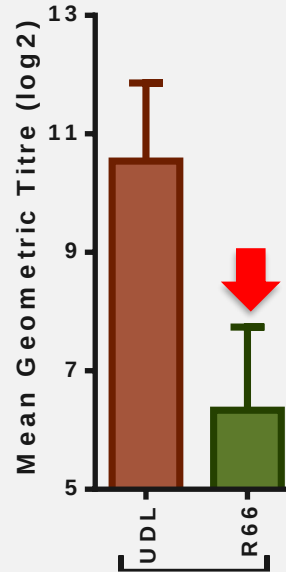
Changing glycosylation sites increased antigenic cross-reactivity

Mutant viruses (Geometric mean HI titre)

Antisera	T180+N134
H9N2 (A180)	167
H9N2 (T180)	2366

Compared to wild type H9N2 virus with A180, the mutant with **T180A+N134 with glycosylation at residue 134** induces greater and broader antigenic cross-reactivity

Deleting dominant antigenic epitopes enhances breadth of immunity against variants



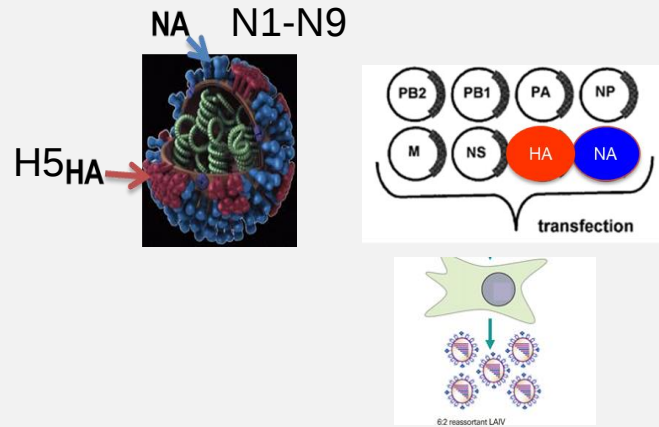
220 loop region
(aa 207-215)



Antisera raised against 120 loop deletion mutant showed broader cross-reactivity

Do changes in NA antigens subtype influence the antigenicity of H5 vaccines?

Generated RG vaccine viruses representing same H5HA and different N-N9 NA subtypes



Antisera raised in chickens against each H5Nx vaccine virus

H5N1

H5N2

H5N3

H5N4

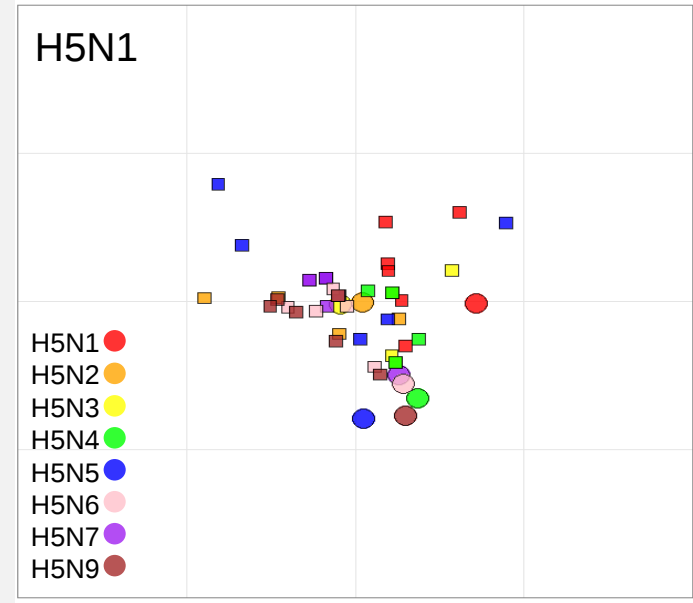
H5N5

H5N6

H5N7

H5N8

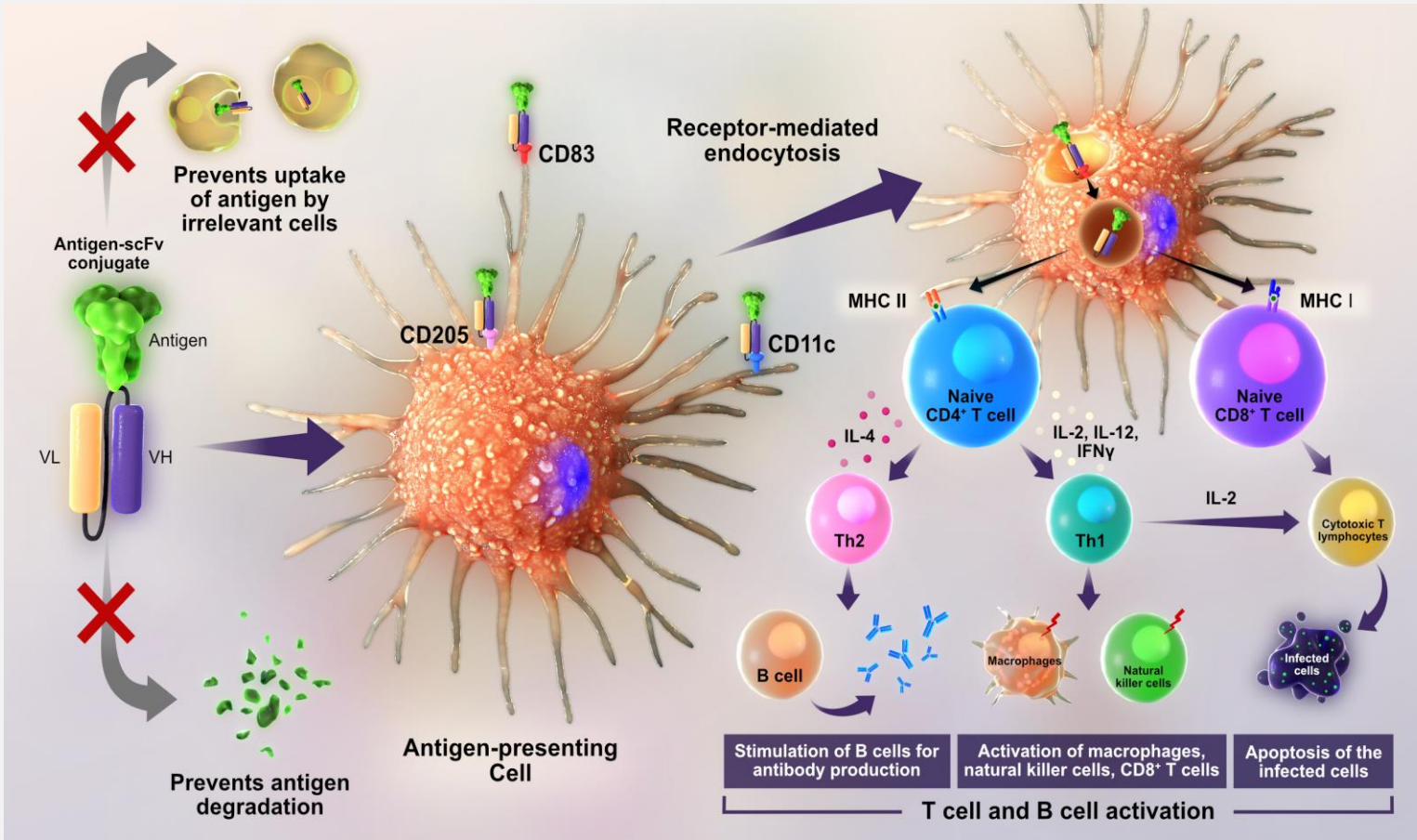
H5N9



The spatial distribution of H5Nx viruses shows little antigenic influence on HI assays.

NA-antigen-specific antibodies play a crucial role in virus neutralization.

Enhancing Breadth and Cross-Reactivity by Modifying Antigens



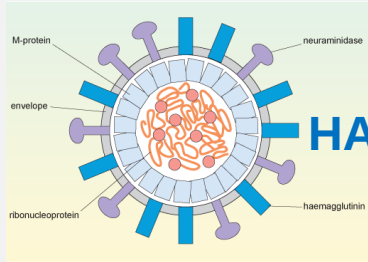
Targeted Delivery of Vaccine Antigens to Chicken Immune Cells (Antigen Presenting Cells)

Targeted Antigen Delivery Vaccines (TADV)

- Shrestha, & Iqbal et al., (2022) *Nature npj Vaccines* 7 (1), 33.
- Shrestha, & Iqbal et al., (2021) *Nature npj Vaccines* 6, 90
- Shrestha, & Iqbal et al., (2021) *Vaccines* 9 (7), 784

Production of Target Antigen Delivery Vaccines

H9HA antigen fused with chicken APCs- receptors (CD83, CD11c, Dec205) specific antibodies



HA antigen



Trimerization signal

Easy
Cell Culture

S2 Cells

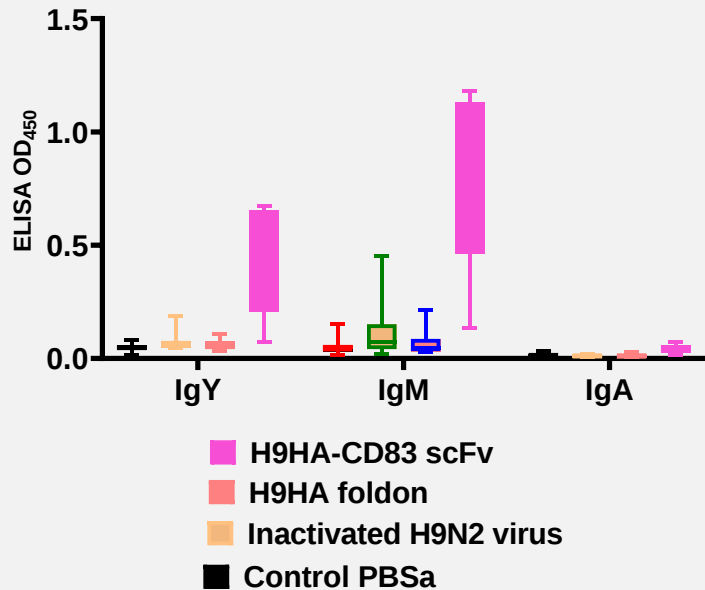
- Grow at room temperature
- Do not require CO₂ incubator
- Grow to high density in serum-free medium

Expression levels: ~ 100 mg/L in *Drosophila*
insect cell culture supernatants
(20ug/dose ~5000 vaccine doses per litre)

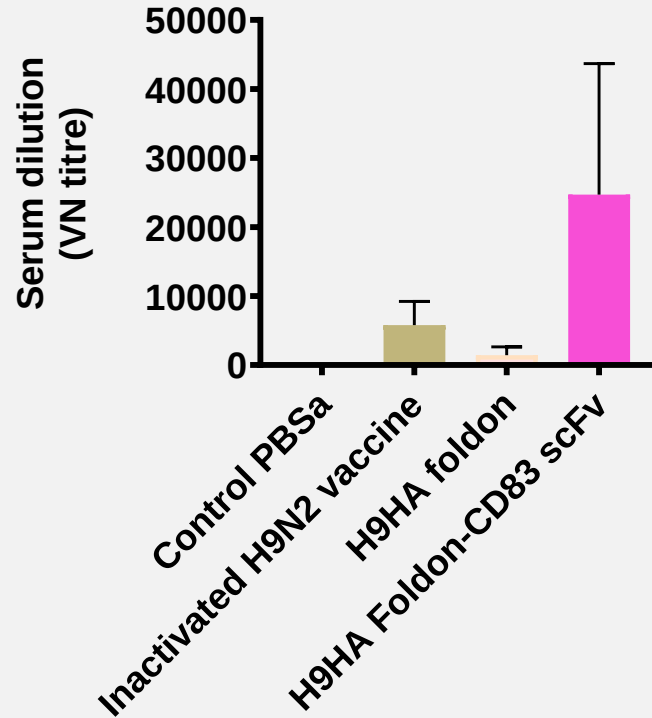
- H9HA-CD83 scFv
- H9HA (control)

TADV induces faster and stronger immune responses and protects chickens from morbidity and mortality

Day 6 Post vaccination

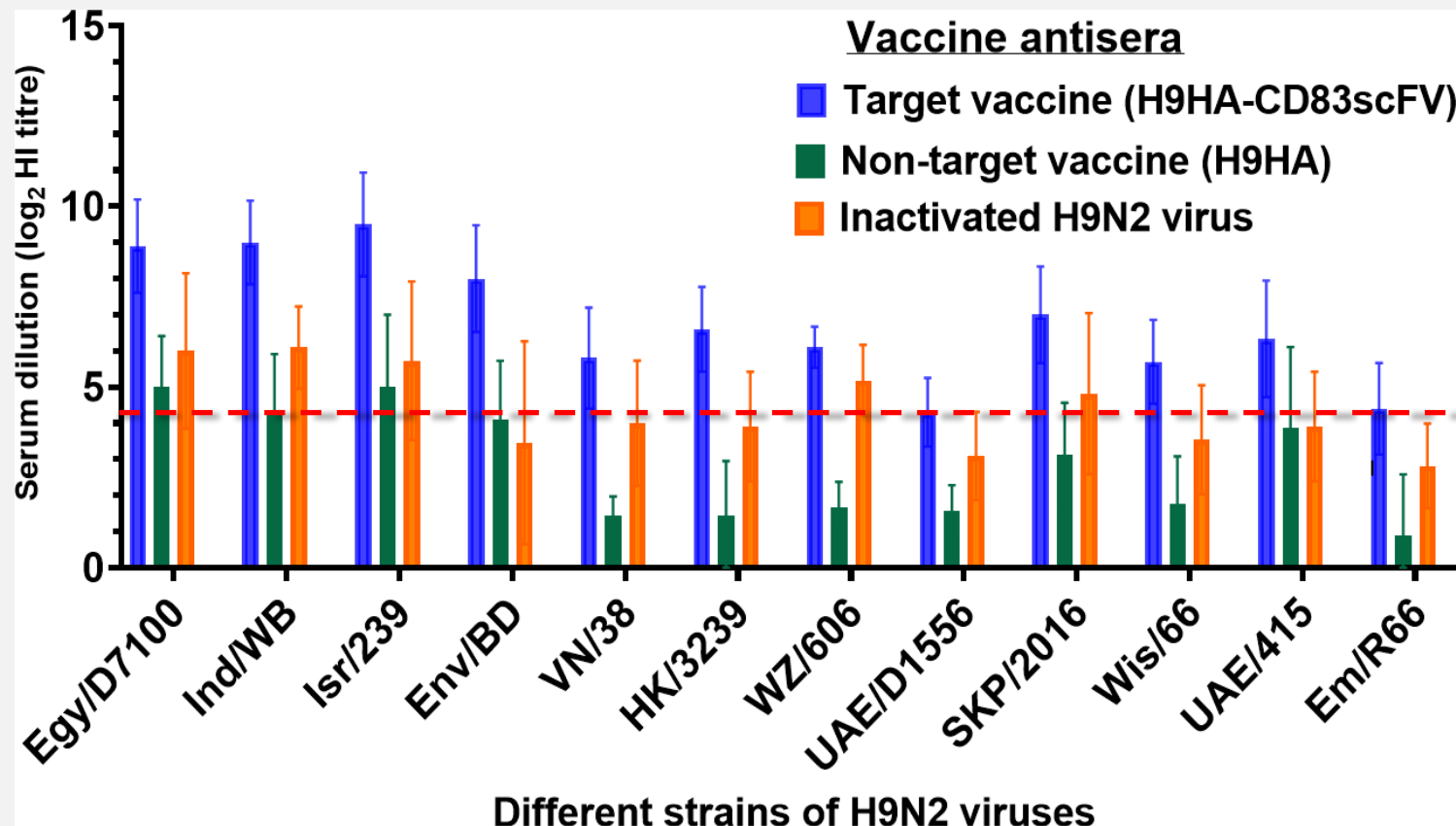


Day 28 post-vaccination



- **Vaccinated: 100% survival, no clinical disease and a significant reduction in shedding of challenge virus**
- **Control: 57% survival, moderate to severe clinical disease, weight loss on day 3-4 post-infection.**

Target vaccine (H9HA-CD83scFv) showed broader cross-reactivity against variant H9N2 avian influenza viruses



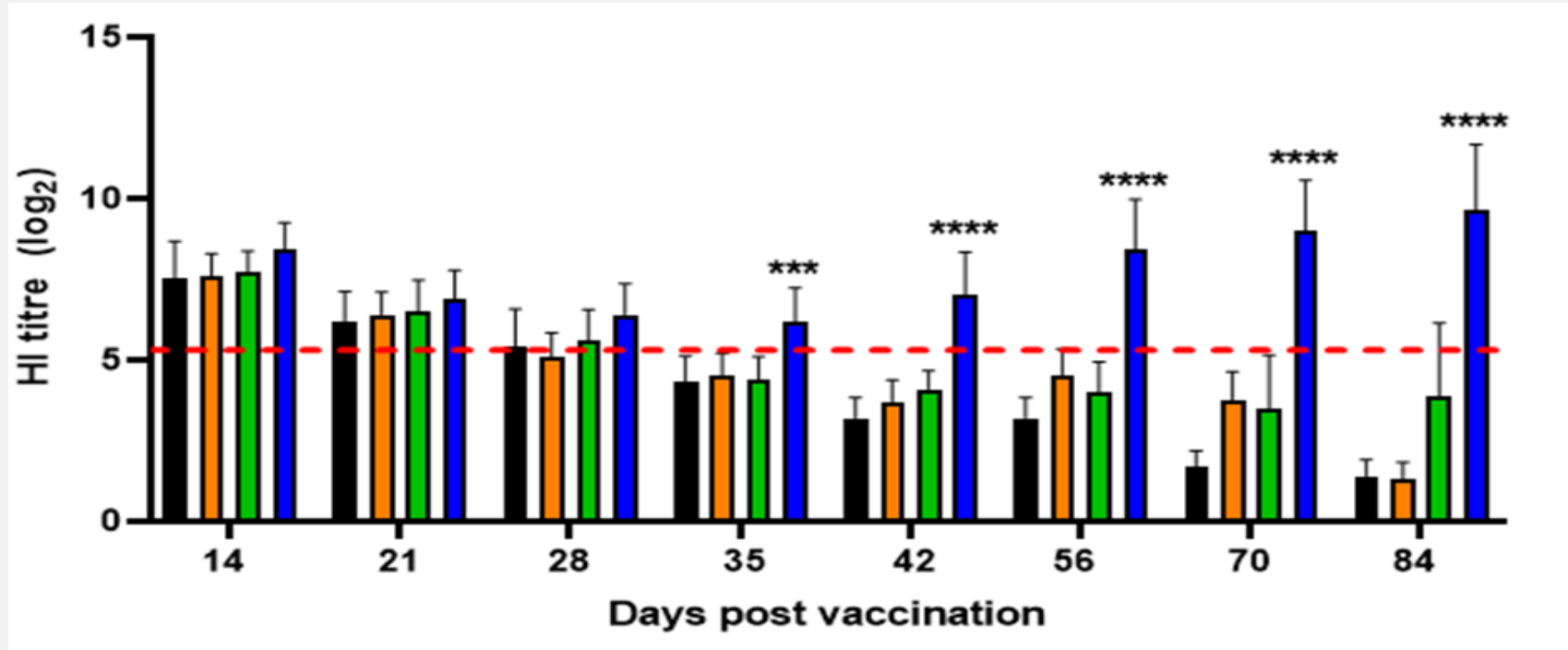
	H9N2 Strain	Alias
1	A/chicken/Egypt/D7100/2013	Egy/D7100
2	A/chicken/India/WB-NIV1057169/2010	Ind/WB
3	A/chicken/Israel/239/2013	Isr/239
4	A/environment/Bangladesh/10306/2011	Env/BD
5	A/Chinese hwamei/Vietnam/38/2006	VN/38
6	A/Hong Kong/3239/2008	HK/3239
7	A/chicken/Emirates/R66/2002	Em/R66
8	A/chicken/Wenzhou/606/2013	WZ/606
9	A/quail/United Arab Emirates/D1556/2011	UAE/D1556
10	A/chicken/Pakistan/SKP/827/2016	SKP/2016
11	A/turkey/Wisconsin/1/1966	Wis/66

Cross-reactivity of target vaccine (H9HA-CD83scFv) antisera against antigenically divergent H9N2 virus strains

Target vaccine (H9HA-CD83scFv) overcome maternal derived antibody Interference



(MDA ++ HI 9.2 log₂)



Predictive protective antibody titres (HI ≥ 5.3 log₂).

Vaccination to one-day-old chicks with very high levels of maternal derived antibody

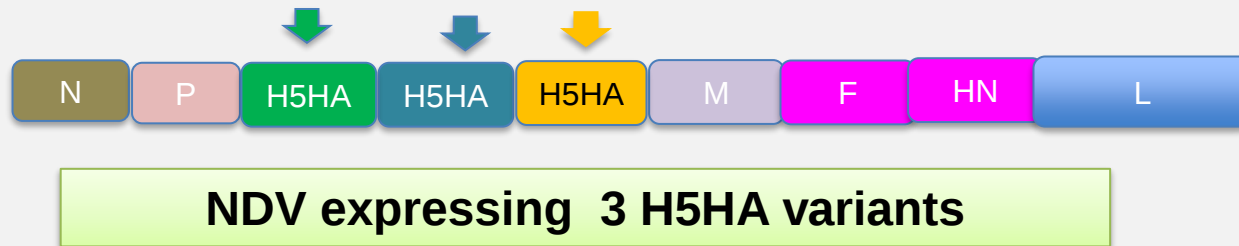
Summary-III

The breadth of vaccines against variants can be increased by selecting cross-reactive antigens and delivering them via targeted antigen delivery approaches.

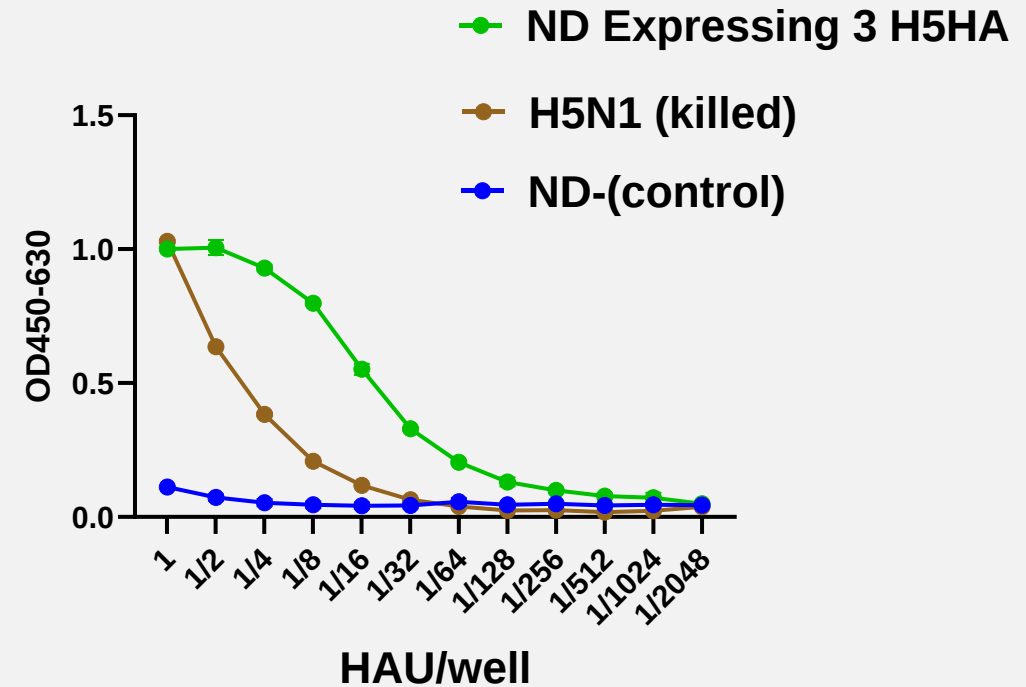
Vaccines production, formulation and delivery approaches

- **Whole inactivated virus vaccines:**
 - Vaccine seed replicate well producing high titres ($> \times 10^9$) in culture media (cultured cells or eggs). .
- **Viral vectored vaccines:**
 - Herpesvirus of turkeys (HVT);
 - Newcastle disease virus (NDV);
- **Subunit vaccine:**
 - Recombinant influenza haemagglutinin (HA) protein produced in cell culture systems.

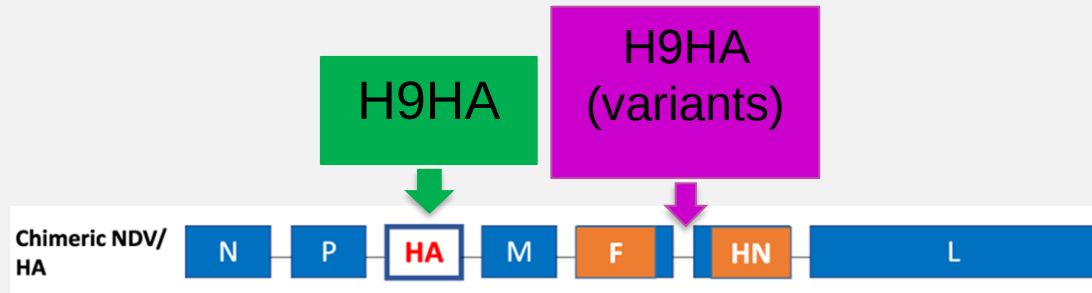
Increase multivalency, strength, and breadth of AIV vaccines by expressing 3 H5HA variants in NDV



A single dose of adjuvant killed recombinant NDV to day-old chicks induced strong immunity (>8 HI titres at 21-days post vaccination) against both AIV and NDV.



Recombinant NDV (genotype VII) expressing Two H9 HA variants



H9HA HI Titres (log₂)

Bird ID	D21
276	8
277	7
279	8
280	6
281	7
282	6
283	7
284	7
Mean	7

NDV- HI titres (log₂)

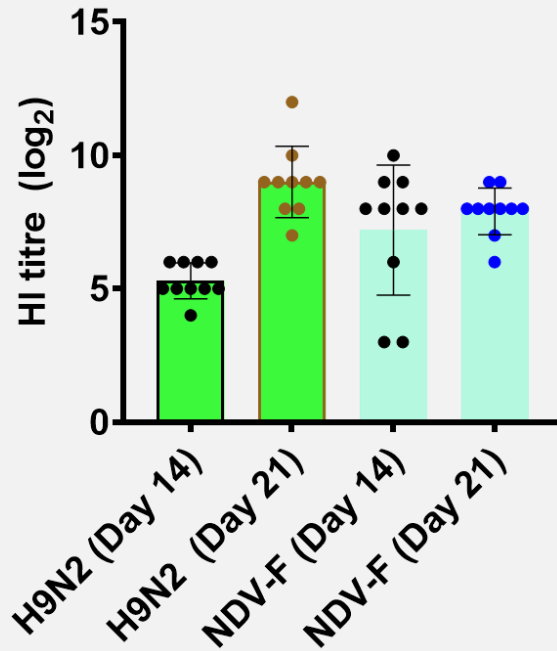
Bird ID	D21
276	9
277	8
279	9
280	8
281	9
282	8
283	9
284	8
Mean	8.5

Up to 3 different antigens into rHVT vectored vaccines

Singe dose of rHVT vaccine to day-old chicks induced immunity against H9-AIV, NDV, and MDV.

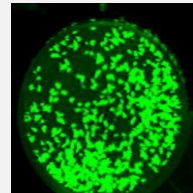
one-day old chicks

(Vaccine dose: 1×10^3 pfu /bird).

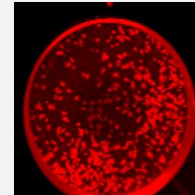


CEF cells infected with rHVT-H9HA – NDV-F stained with H9N2 and NV virus-specific anti-serum

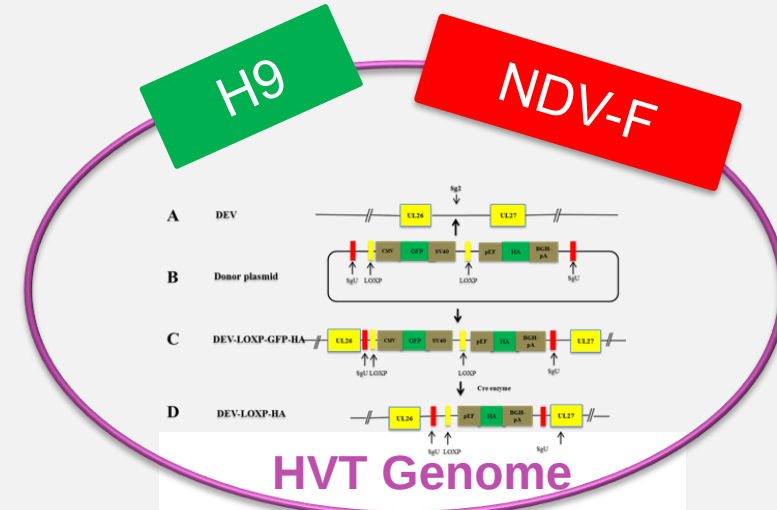
rHVT-H9HA



rHVT-NDV-F



rHVT-H9HA & NDV-F (gVII)



rHVT vaccine expressing both H9HA and NDV-F

H9N2 challenge

- Vaccinated group: all 10/10 showed protection from clinical diseases and death.
- Control group: all 10/10 showed disease signs and 3 /10 were died.

Passive Immunisation against avian influenza viruses



vaccines



[Vaccines \(Basel\)](#). 2020 Mar; 8(1): 118.

PMCID: PMC7157677

Published online 2020 Mar 3. doi: [10.3390/vaccines8010118](https://doi.org/10.3390/vaccines8010118)

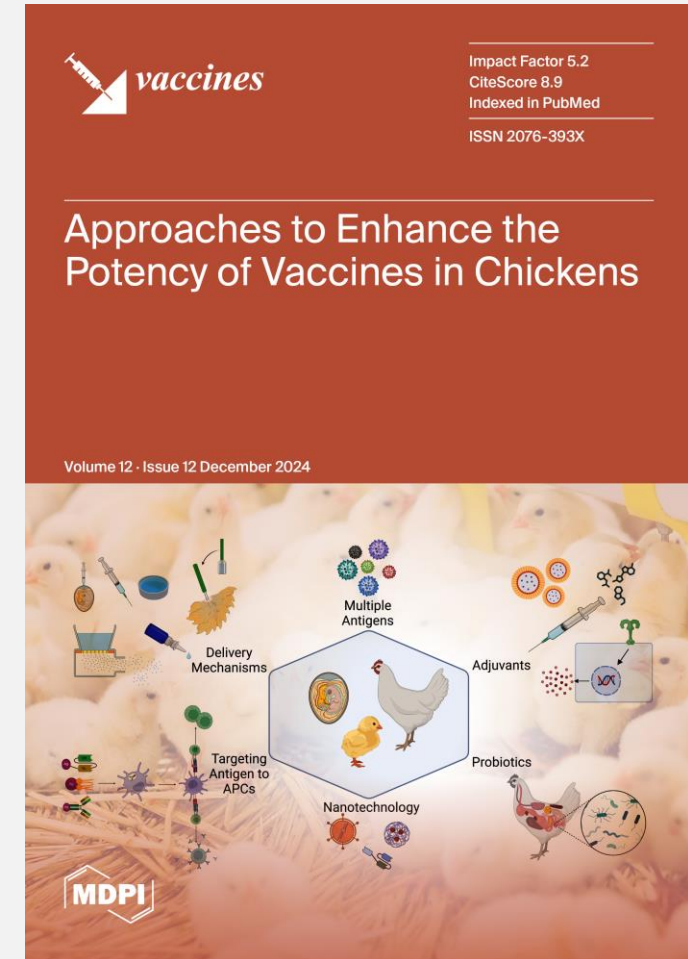
PMID: [32138253](https://pubmed.ncbi.nlm.nih.gov/32138253/)

Engineered Recombinant Single Chain Variable Fragment of Monoclonal Antibody Provides Protection to Chickens Infected with H9N2 Avian Influenza

[Deimante Lukosaityte](#),¹ [Jean-Remy Sadeyen](#),¹ [Angita Shrestha](#),¹ [Joshua E. Sealy](#),¹ [Sushant Bhat](#),¹ [Pengxiang Chang](#),¹ [Paul Digard](#),² and [Munir Iqbal](#)^{1,*}

Summary

- Improved approaches for rapid generation of recombinant killed virus, subunit and vectored vaccines.
- Implementing innovative approaches can enhance the efficacy, duration, and breadth of poultry vaccine



<https://www.mdpi.com/2076-393X/12/12/1337>

Impacts

- **Cost-effectiveness (reduced number of doses and delivery costs)**
- **Reduction in losses and improved productivity**
- **Reduced prevalence of viruses**
- **Animal welfare**
- **Public health: reduced virus prevalence leads to reduced zoonotic transmission.**

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AIV group (current team)

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- **Oenone Bodman-Harris**
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