



Does LPAIV replicate in muscles of infected chickens?

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Haemagglutinin Protein of the Flu

Synthesized as an HA0
and
cleavage into HA1+HA2 subunit is crucial for infectivity

Amino acid sequence **at the cleavage site** determined
the enzymes responsible for the cleavage

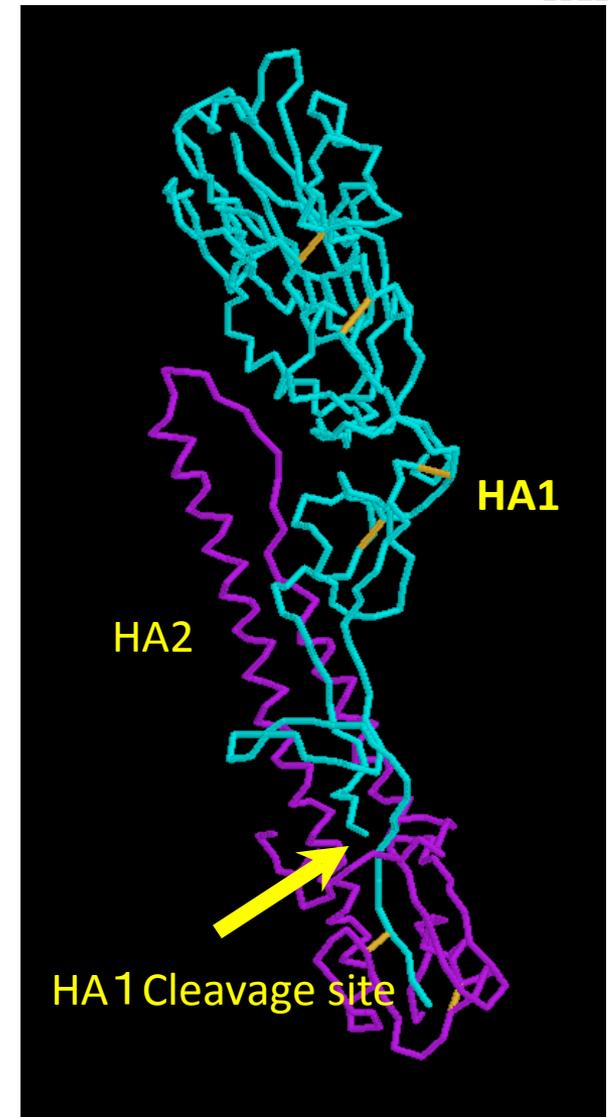
Tissue distribution of these enzymes differ and it
determined tissue tropism of the influenza virus

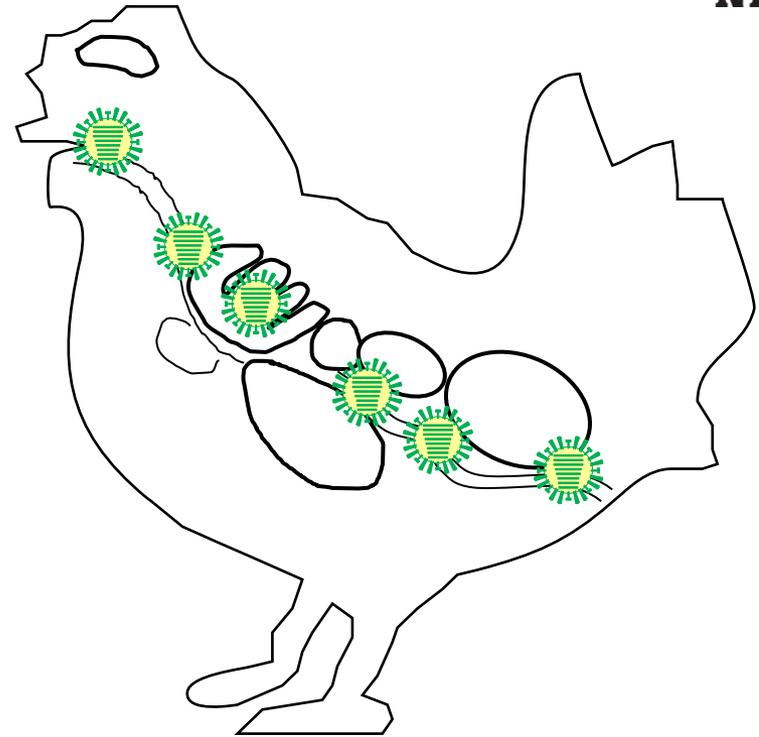
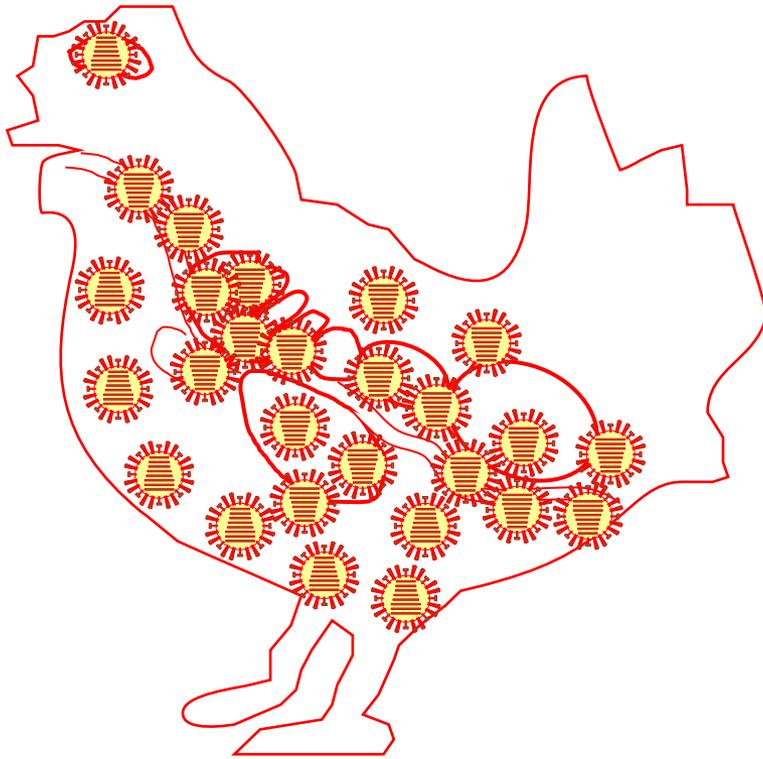
Highly pathogenic avian influenza virus

Series of basic amino acids R/K, such as **RKKR**, at the cleavage sequence
⇒cleaved by the ubiquitously distributed enzyme, like Furin, in chicken
and
Resulted in systemic infections

Low pathogenic avian influenza virus

Single Arginine (R) at the cleavage site
⇒cleaved by localized enzyme in the respiratory and intestinal tracts in chicken
and
Resulted in local infections





Highly pathogenic strains
(portion of H5 and H7 subtypes)
RKKR etc.
⇒ **Systemic infection**
High mortality, Neurological signs

Most of type A influenza virus
LPAIV
RETR etc.
⇒ **Localized infection**
Asymptomatic ~ mild symptom
(respiratory symptom, egg drop etc.)

In theory,
meat from LPAIV infected chickens could not be contaminated
with the virus

In literature,
a few example of virus detection from muscles of LPAIV infected
chickens and turkey

Co-infection of *Staphylococcus aureus* or *Haemophilus paragallinarum*
exacerbates H9N2 influenza A virus infection in chickens. Kishida et al

→A/chicken/aq-Y-55/01, A/chicken/aq-Y- 135/01, isolated from from meat of
imported carcasses, were recovered from muscle samples of the
experimentally infected chickens.



How often we could detect LPAIV in muscle by experimental infection
and
if co-infection of *Mycoplasma* species enhance virus replication in chickens



LPAI strains	subtype	abbreviations
A/Turkey/Virginia/505477-17/2007	H5N1	Turkey/VA/07
A/Wild Bird/South Dakota/2307-3/2007	H5N2	W.Bird/SD/07
A/Muscovy Duck/New Jersey/912-1/2008	H5N2	M. duck/NJ/08
A/Duck/New York/2670-2/2009	H5N2	Duck/NY/09
A/Duck/Pennsylvania/34322-1/2009	H5N2	Duck/PA/09
A/Chicken/Ibaraki/1/2005	H5N2	Ck/Ibaraki/05
A/Pheasant/Idaho/2590-63/2008	H5N8	Pheasant/ID/08
A/Chicken/Arkansas/918-10/2008	H7N3	Ck/AR/08
A/Turkey/Nebraska/505577-3/2007	H7N9	Turkey/NE/07
A/Turkey/Minnesota/14135-4/2009	H7N9	Turkey/MN/09

Intranasal (IN) or intratracheal (IT) inoculation at 4 weeks of age of SPF White leghorn chickens
 10^6 EID₅₀ /head
 with or without pre-inoculation of Mycoplasma 7 day prior to Flu inoculation

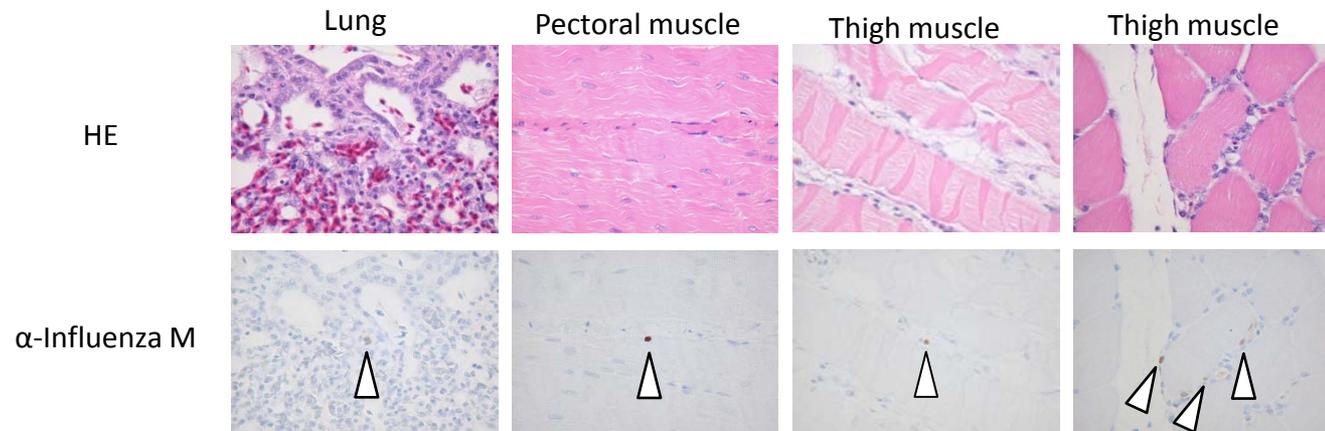
Bacteria

strain	species
R	<i>Mycoplasma gallisepticum</i>
1-3-SN	<i>Mycoplasma synoviae</i>

I.N inoculation study

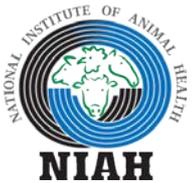
Strain	pi	Lung		Blood	Rectum	Tracheal
		Isolaton* ^a	qRT-PCR* ^b	Isolaton	Isolaton	Isolaton
Turkey/VA/07	2	2/3 (3.2, 3.0)	N/A* ^c	0/3	1/3 (1.9)	0/3
	4	0/3	N/A	0/3	0/3	0/3
	6	0/3	N/A	0/3	0/3	0/3

Strain	pi	Pectoral muscle		Thigh muscle	
		Isolaton	qRT-PCR	Isolaton	qRT-PCR
Turkey/VA/07	2	1/12 (1.9)	2/12 (2.9, 2.6)	1/12 (2.2)	2/12 (3.2, 3.1)
	4	0/3	0/3	0/3	0/3
	6	0/3	0/3	0/3	0/3





I.N inoculation study



Strain	pi	Lung		Tracheal	Cloacal
		Isolaton* ^a	qRT-PCR* ^b	Isolaton	Isolaton
W.Bird/SD/07	2	0/3	N/A	0/3	N/A
	4	0/3	N/A	0/3	N/A
	6	1/3 (1.9)	N/A	0/3	N/A
Pheasant/ID/08	2	0/3	N/A	1/3 (1.9)	N/A
	4	0/3	N/A	0/3	N/A
	6	0/3	N/A	0/3	N/A
Ck/AR/08	2	0/3	0/3	0/3	0/3
	4	0/3	0/3	1/3 (0.9)	0/3
	6	0/3	0/3	0/3	0/3

M. duck/NJ/08, Duck/NY/09, Duck/Penn/09, Turkey/NE/07 and Turkey/MN/09 were not recovered from organs or swabs examined

Summary of the results

Group	Incubation route	Pectoral muscle			Thigh muscle		
		Isolaton	qRT-PCR	IHC	Isolaton	qRT-PCR	IHC
Turkey/VA/07	i.n	1/54	2/54	1/2	1/54	4/54	3/4
Turkey/VA/07	i.t	0/27	0/27	N/A	0/27	1/27	0/1
Ck/Ibaraki/05	i.n	0/18	0/18	N/A	0/18	0/18	N/A
Ck/AR/08	i.n	0/27	0/27	N/A	0/27	0/27	N/A
Ck/AR/08	i.t	0/36	0/36	N/A	0/36	0/36	N/A
Pheasant/ID/08	i.n	0/9	N/A	N/A	0/9	N/A	N/A
W.Bird/SD/07	i.n	0/9	N/A	N/A	0/9	N/A	N/A
M. duck/NJ/08	i.n	0/9	N/A	N/A	0/9	N/A	N/A
Duck/NY/09	i.n	0/9	N/A	N/A	0/9	N/A	N/A
Duck/PA/09	i.n	0/9	N/A	N/A	0/9	N/A	N/A
Turkey/NE/07	i.n	0/9	N/A	N/A	0/9	N/A	N/A
Turkey/MN/09	i.n	0/9	N/A	N/A	0/9	N/A	N/A

Conclusion

- Isolation of the infectious virus from muscle is a rare event in case of LPAIV infection, considering the amount of the virus infected in this study (10^6EID_{50}).
- Risk of contamination of chicken meat by LPAIV is considerably low comparing to that by HPAIV