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HPAIV H5 evolution requires adaptation of the hemagglutinin by elevation of the fusion competence activation pH.

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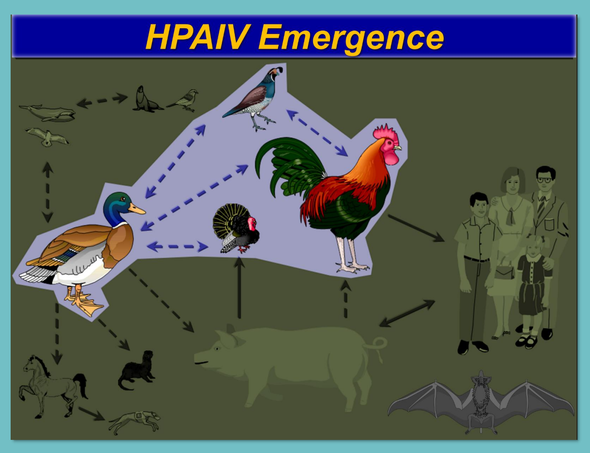
INTRODUCTION

Highly pathogenic avian influenza viruses (HPAIV) cause fatal disease in poultry leading to enormous losses world-wide. Moreover, repeated zoonotic infections have raised serious concerns of a novel pandemic.

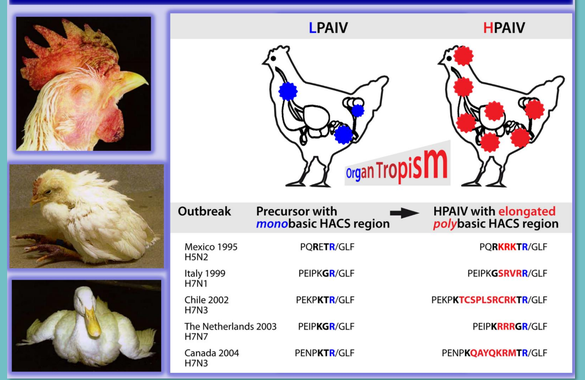
Previously, we found that, beside the essential polybasic hemagglutinin (HA) cleavage site (HACS), a lethal phenotype in chicken requires **additional virulence determinants** in the HA and other genes as NA, PB2, NP, and M. **Here, we focused our follow-up search on the HA.**

Using the reverse genetics systems from the clade 2.2.2. HPAIV A/Swan/Germany/R65/2006 (H5N1, **R65**) and the low-pathogenic strain A/Teal/Germany/WV632/2005 (H5N1, **TG05**), **we generated several HA reassortants, HA1/HA2 chimeras and point mutants to investigate the HA fusion competence activation pH and the virulence in chicken and mice.**

HPAIV Emergence



HPAIV Features



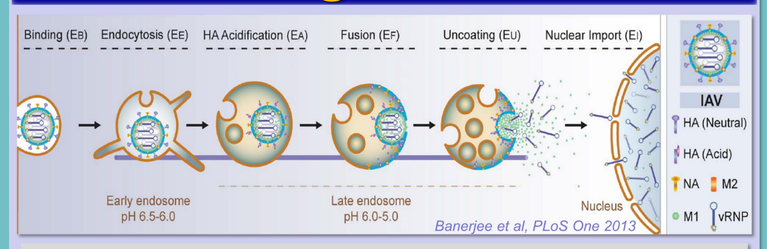
CONCLUSIONS

The two HA1 amino acids **SI @123/124 form an essential virulence determinant in H5 HPAIV**.

Whereas this motif is retained in contemporary H5 HPAIV including novel reassortants with different neuraminidase subtypes, **the first Asian HPAIV, the Goose/Guangdong strains from 1996/97 carried 123S only.**

Overall, HA adaptation of H5N1 HPAIV to elevate their HA fusion activation pH is required for early evolution.

HA cleavage is essential.



***HA cleavage** is absolutely required for an **acid pH-triggered conformation change** enabling **membrane fusion** resulting in **uncoating** of the vRNPs into the cytosol.*

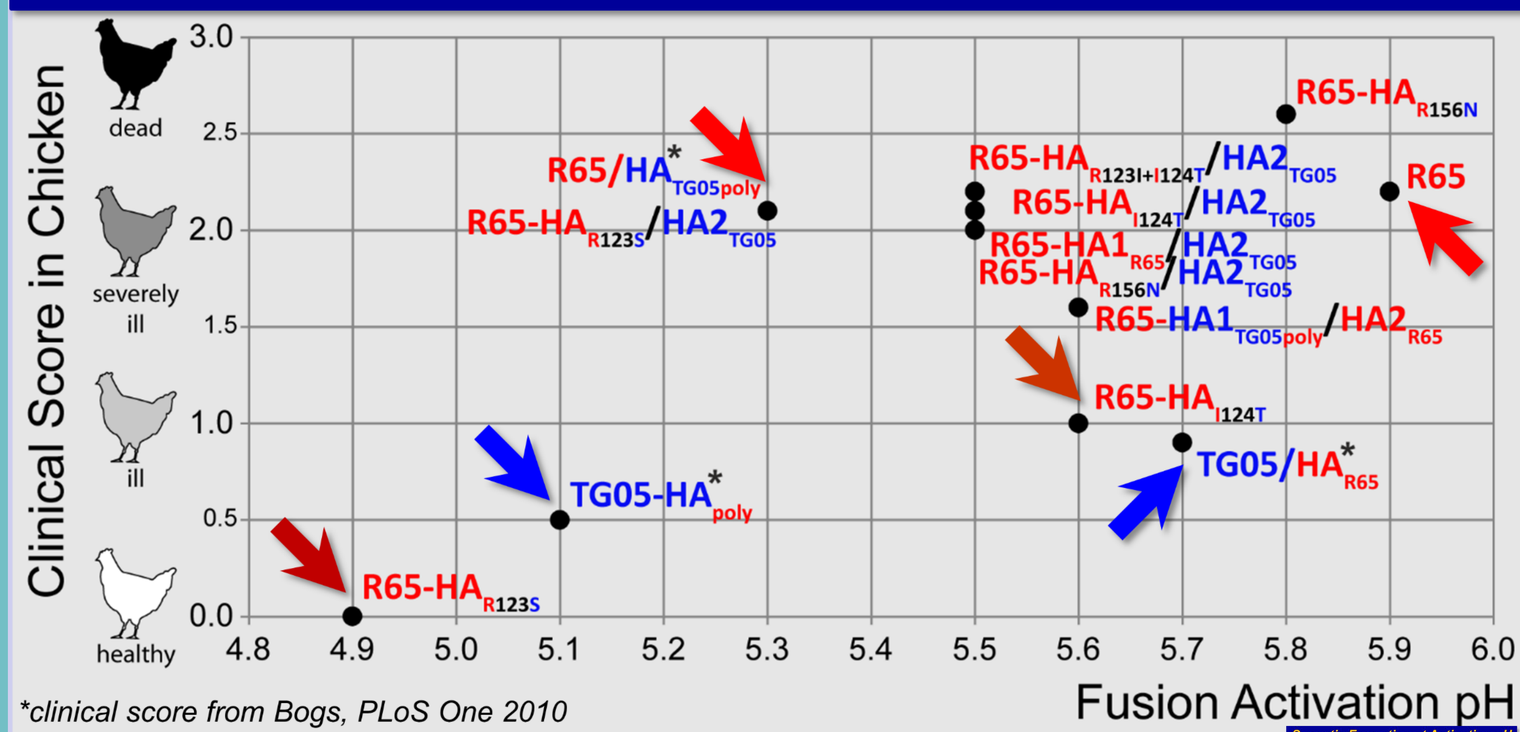
RESULTS

R65 HA variants with exchanged HA1 and/or the point mutations **S123R or I124T were found to display a notable decrease of activation pH up to one magnitude.**

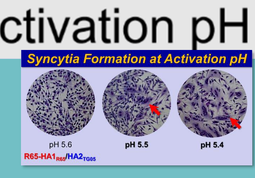
Furthermore, this pH decrease is **paralleled by extreme reduction of virulence down to 0% both in chicken and mice.**

Among 3385 full-length HA protein public sequences from H5 HPAIV, we found the single exchanges HA1 123**S** or 124**I** at a very low frequency of 0.5% each, but **in dual combination at the majority of 97.5%.**

Activation pH vs Clinical Score



*clinical score from Bogs, PLoS One 2010

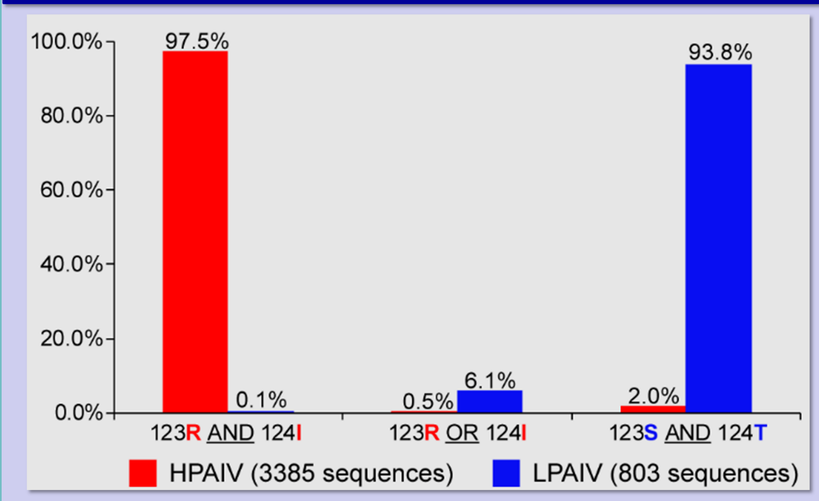


HA Mutants and Virulence

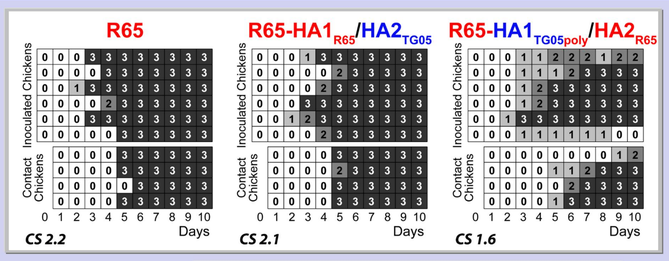
Virus	Dead/Total	Score
TG05-Ha$_{poly}$*	0/10	0.0
R65	6/6	2.2
TG05/HA$_{R65}$*	3/10	0.9
R65/HA$_{TG05poly}$*	8/10	2.1
R65-HA1$_{TG05}$/HA2$_{R56}$	4/6	1.6
R65-HA1$_{R65}$/HA2$_{TG05}$	6/6	2.1
R65-HA$_{R156N}$	3/3	2.6
R65-HA1$_{R156N}$/HA2$_{TG05}$	3/3	2.0
R65-HA$_{R123S}$	0/6	0.0
R65-HA1$_{R123S}$/HA2$_{TG05}$	3/3	2.1
R65-HA$_{I124T}$	3/6	1.0
R65-HA1$_{I124T}$/HA2$_{TG05}$	3/3	2.1
R65-HA1$_{R123S+T124T}$/HA2$_{TG05}$	3/3	2.2

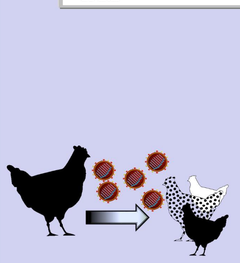
*virus and data from Bogs, PLoS One 2010

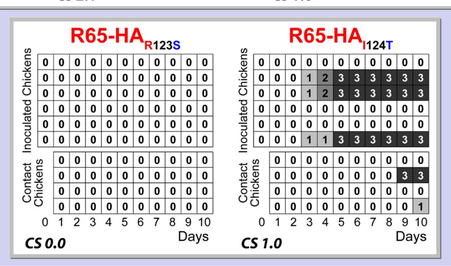
Amino Acid Frequencies @ HA 123/124



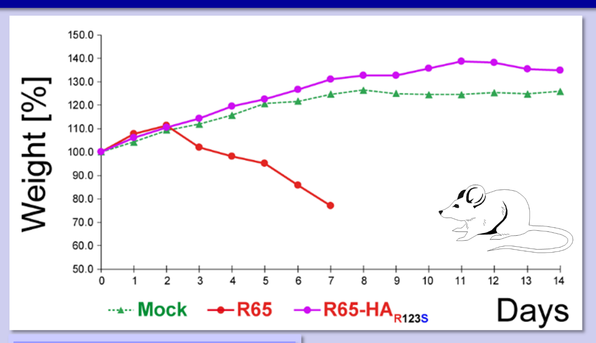
Contact Transmission







Virulence in Mice



Virus	Dead/Total
Mock	0/5
R65	5/5
R65-HA$_{R123S}$	0/5