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Striking variations in pathogenesis and virulence determinants of H7N1 in two closely related galliforms

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Introduction

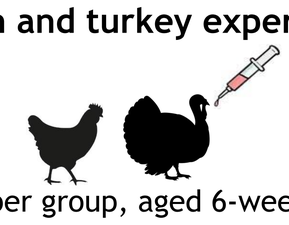
In chickens and turkeys, the transition of low pathogenicity (LP) avian influenza virus (AIV) of H5 and H7 subtypes to high pathogenicity (HP) AIV is accompanied mainly by changing the hemagglutinin monobasic cleavage site (CS) to a polybasic motif (pCS). For as yet unexplained reasons, turkeys show higher morbidity and mortality following HPAIV infection than chickens. Here, we compared the pathogenesis of H7N1 virus using recombinant LP, HP and LP H7N1 carrying pCS (LP_poly) in turkeys and chickens, including the host response.

Methods

Table 1. Recombinant viruses used:

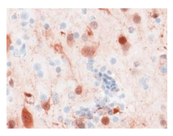
H7N1 Virus	Cleavage site	Other gene segments	Virus titre MDCK-II cells (log_{10} pfu/ml)
Lp (A/chicken/Italy/473/1999)	PEIPKG - - - - R/G	LP	8.0
Lp_poly	PEIPKGSRVRR/G	LP	7.8
Hp (A/chicken/Italy/445/1999)	PEIPKGSRVRR/G	HP	6.8

Chicken and turkey experiments



n= 10 per group, aged 6-weeks, oculonasal infection, 200µl, $10^{5.0}$ PFU

Pathology workflow
- Autopsy day 4, sampling
- Viral antigen distribution
- Viral RNA load in tissues



Transcriptome analysis workflow on brain tissue, 4 days post infection (dpi)
- Libraries: Collibri Stranded RNA Library Prep Kit for Illumina Systems; sequenced on a NextSeq v2
- Functional enrichment analysis using g:Profiler and QuickGo, Volcano plots generated by VolcaNoseR

Results

Survival

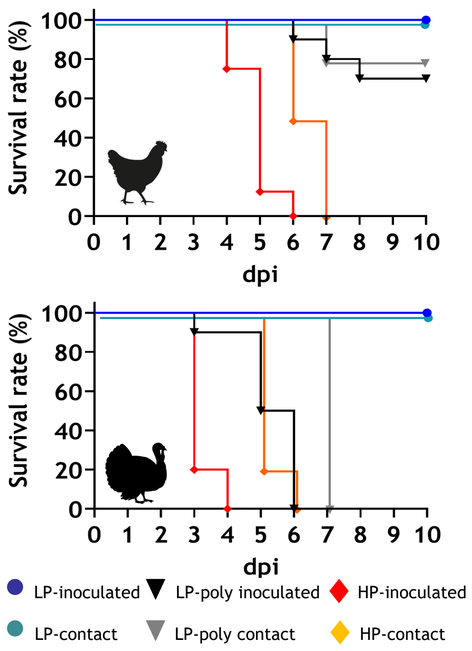


Fig.1. The pCS is the main determinant of virulence in turkeys but not in chickens

Virus detection by RT-qPCR in tissues (brain)

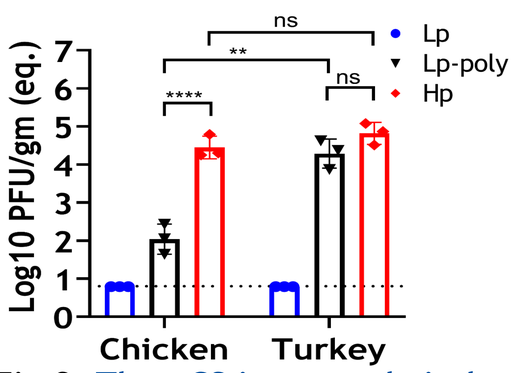


Fig.2. The pCS increased viral replication of LP in all organs, i.e. in the brain of turkeys (lung, pancreas, spleen not shown)

AIV tissue tropism in Chickens

AIV tissue tropism in Turkeys

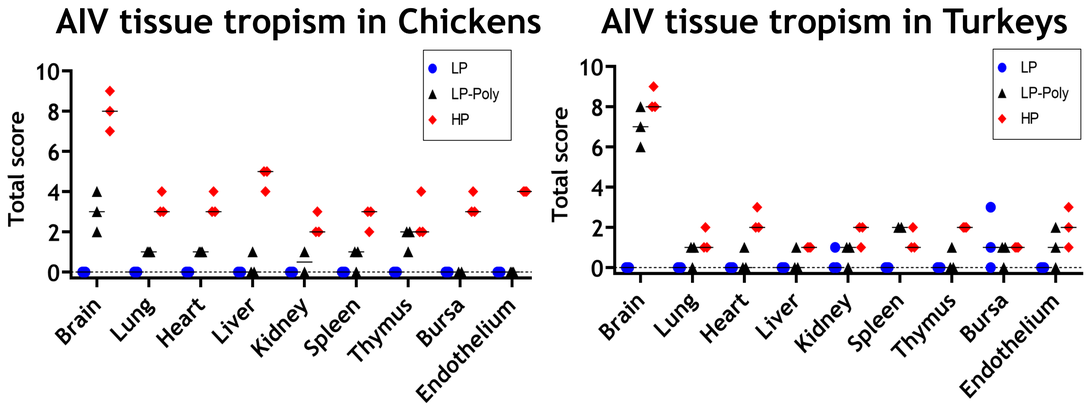


Fig. 3. The pCS mediates distinct neurotropism in turkeys and enables endotheliotropism in turkeys but not in chickens.

Next-generation sequencing based transcriptome analyses

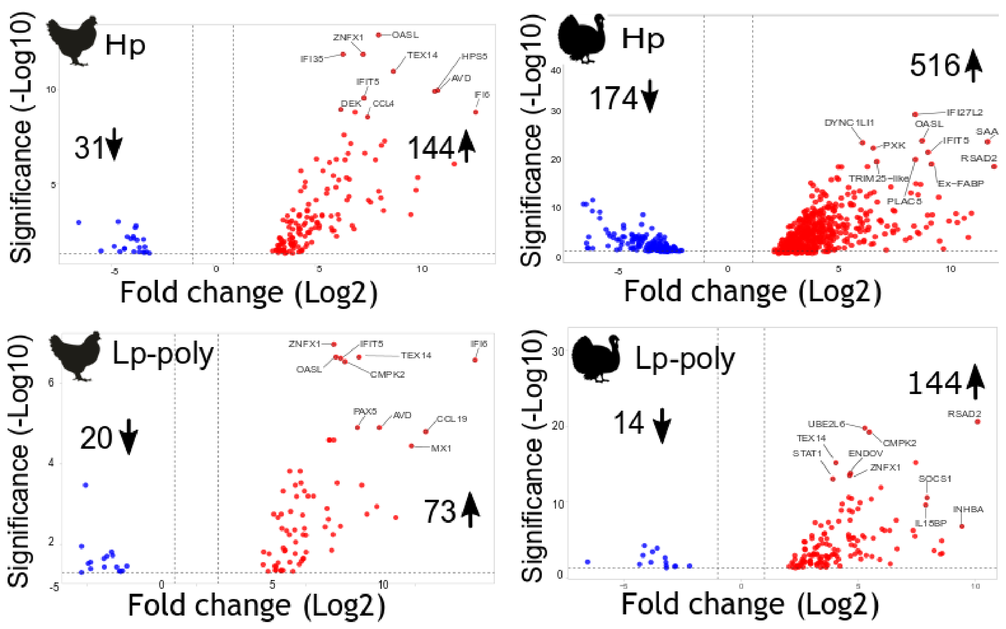


Fig. 4. The number of differentially expressed genes is higher in turkeys

Enrichment analysis for differentially expressed genes

Hp

response to interferon gamma (type II IFN)	GO:0034341	1.226×10^{-7}
cellular response to interferon gamma	GO:0071346	3.810×10^{-7}
response to virus	GO:0009615	7.385×10^{-7}
type I interferon signaling pathway	GO:0060337	1.687×10^{-6}
cellular response to type I interferon	GO:0071357	1.871×10^{-6}
response to type I interferon	GO:0034340	3.080×10^{-6}
defense response to symbiont	GO:0140546	6.816×10^{-6}
defense response to virus	GO:0051607	6.816×10^{-6}
cytokine-mediated signaling pathway	GO:0019221	1.043×10^{-5}
response to biotic stimulus	GO:0009607	1.151×10^{-5}
cellular response to cytokine stimulus	GO:0071345	1.569×10^{-5}
innate immune response	GO:0045087	1.986×10^{-5}

Fig. 5. In chickens, Hp infection led to enrichment of GO terms involved in the response to virus and the innate immune response (e.g., IFN, cytokines)

Hp

RNA metabolic process	GO:0016070	4.304×10^{-8}
mRNA metabolic process	GO:0016071	6.915×10^{-7}
nucelic acid metabolic process	GO:0090304	8.059×10^{-7}
nucelobase-containing compound metab.process	GO:0006139	5.227×10^{-6}
RNA processing	GO:0006396	1.396×10^{-5}
gene expression	GO:0010467	1.422×10^{-5}
mRNA processing	GO:0006397	1.462×10^{-5}
positive regulation of cellular process	GO:0048522	1.648×10^{-5}
positive regulation of biological process	GO:0048518	1.804×10^{-5}
regulation of RNA metabolic process	GO:0051252	3.287×10^{-5}
heterocycle metabolic process	GO:0046483	4.448×10^{-5}
cellular aromatic compound metab. process	GO:0006725	8.466×10^{-5}
regulation of mRNA metabolic process	GO:1903311	8.821×10^{-5}
Pos.regulation of macromolecule metab.process	GO:0010604	1.064×10^{-4}
chromatin organization	GO:0006325	1.686×10^{-4}

Fig. 6. In turkeys, Hp infection induced the responses of GO terms associated with RNA and cell metabolism

Full length paper: Blaurock et al. Evidence for Different Virulence Determinants and Host Response after Infection of Turkeys and Chickens with Highly Pathogenic H7N1 Avian Influenza Virus. J Virol. doi: 10.1128/jvi.00994-22.

Discussion

- H7N1 HPAIV: high virulence in both galliforms & lesion-associated viral antigen in all organs
- LP-poly: low virulence in chickens, high virulence in turkeys
- LP-poly in chickens: expanded the tissue tropism of LP but not to the same extent as HP, no endotheliotropism
- LP-poly in turkeys: tissue tropism and severity of lesions comparable with HP, including endotheliotropism
- Transcriptome analysis: turkeys and chickens showed a different host response, particularly from genes involved in RNA metabolism and immune response

→ The variable pathogenesis, virulence determinants and host responses after infection with HPAIV H7N1 may explain the high vulnerability of turkeys to HPAIV

Acknowledgment

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