



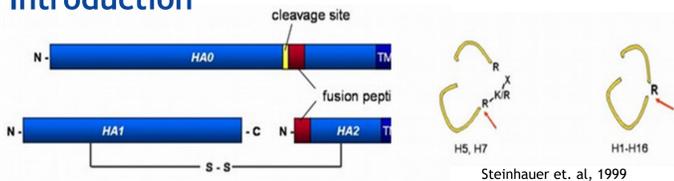
# Different genetic constellations for high virulence, transmission and tropism of highly pathogenic H7N7 avian influenza virus in turkeys and chickens

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## Abstract

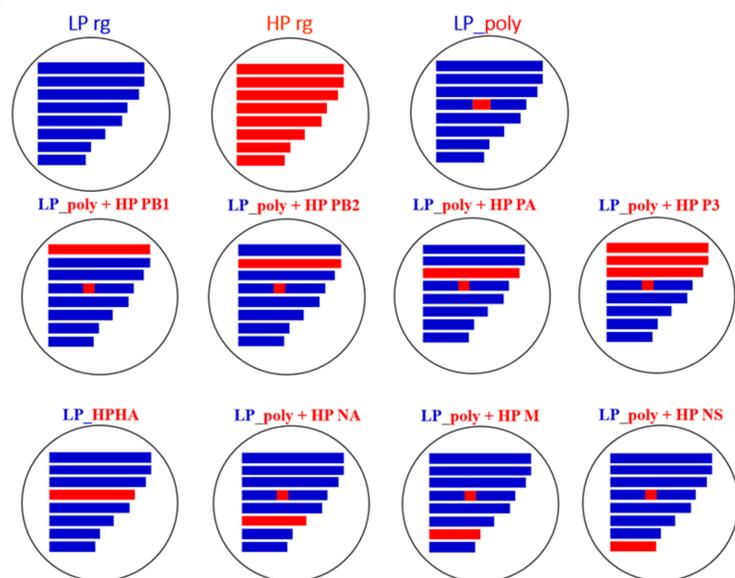
Highly pathogenic (HP) avian influenza viruses (AIV) cause severe mortality in chickens (*Gallus gallus domesticus*) and turkeys (*Meleagris gallopavo*), although turkeys are more sensitive than chicken. HP AIV evolve from low pathogenic (LP) progenitors after mutations in the hemagglutinin cleavage site (HACS) and other gene segments. In 2015, an HPAIV H7N7 and the putative LP ancestor were isolated from the same chicken farm in Germany. In addition to acquisition of a polybasic HACS, mutations in all gene segments were observed. The aim of this study was to investigate the impact of mutations in different segments in addition to the polybasic CS on virulence, transmission, replication and tissue tropism in chickens and turkeys. Using reverse genetics, different virus reassortants with an LP backbone carrying the polybasic CS and single or multiple HP segments were generated. Interestingly, viruses carrying the HP NS or M gene segments or NS segment were as virulent and transmissible as the HPAIV in turkeys and chickens, respectively. All viruses were detected in the brain, although the endothelial tropism was exclusively found in chickens. The HACS and to lesser extent NA are major determinants for the endotheliotropism. The HPAIV has lower NA activity and higher polymerase activity compared to the LPAIV H7N7. Together, this study showed two major differences between chickens and turkeys: more genetic constellations to confer high virulence and transmission in turkeys than in chickens and neurotropism in turkeys vs. endothelial tropism in chickens.

## Introduction



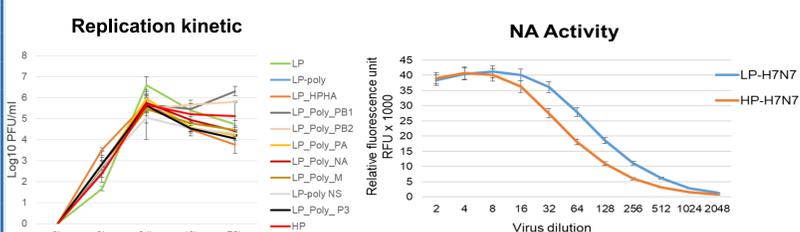
The hemagglutinin is first generated as HA0 and has to be cleaved into HA1 and HA2 for its activity. Depending on the structure of the cleavage site different enzymes can activate this protein. The monobasic cleavage site of the HA from LPAIV is cleaved by trypsin-like proteases restricted to the intestinal and the respiratory tract and therefore these viruses cause mild clinical signs, if any. Ubiquitous furin-like proteases activate the cleavage site of the HA of HPAIVs leading to severe clinical signs and up to 100% mortality. The standard method for virulence investigation of AIVs is to determine the intravenous pathogenicity index (IVPI). An IVPI exceeding 1.2 specifies high pathogenic AIVs.

## Viruses



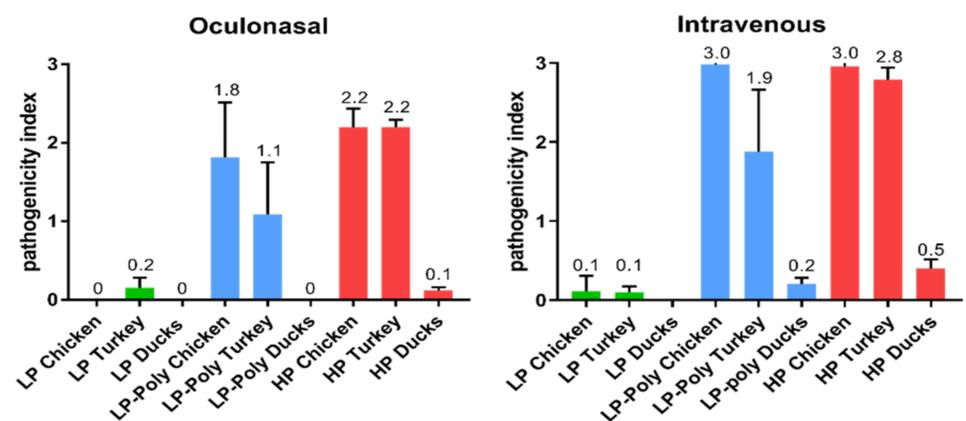
Using reverse genetics eleven viruses have been generated, characterized *in vitro* and tested *in vivo*. The parental LP and HP viruses originated from an outbreak of H7N7 in Germany in 2015 where both viruses have been isolated from one chicken farm.

## In vitro characterisation



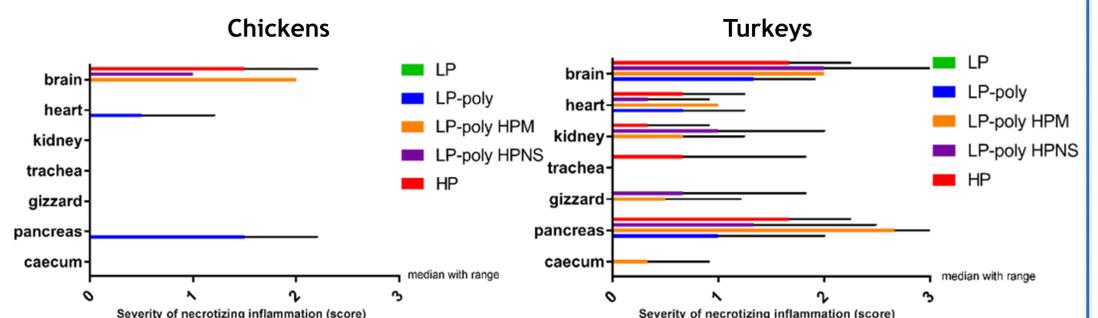
Replication kinetic was determined on primary chicken embryo kidney cells (CEK). All viruses showed their replication peaks at 24h post infection except for LP-poly HPPB1 and LP-poly HPPB2. There were no significant differences between these viruses. The MUNANA-Assay revealed higher NA-activity of the LPNA compared with HPNA.

## Results of the Animal Trials



The LP virus showed no or mild clinical signs in all species while the HP virus killed all chickens and turkeys. Interestingly, LP-poly was more virulent in chickens than in turkeys for both routes of inoculation. Thus, virulence determinants in chickens and turkeys seem to be different. The ducks were resistant against all three viruses. The intravenous pathogenicity index (IVPI) confirmed the data from the oculonasal infection experiment.

Virus	Oculonasal Inoculation			
	Chickens		Turkeys	
	Dead/ inoculated	Dead/ contact	Dead/ inoculated	Dead/ contact
LP-poly HPP3	6/6	2/4	/	/
LP-poly HPPB1	/	/	2/10	2/5
LP-poly HPPB2	/	/	0/10	2/5
LP-poly HPPA	6/6	0/4	1/10	3/5
LP-poly HPNA	6/6	1/4	1/10	2/5
LP-poly HPM	6/6	1/4	10/10	5/5
LP-poly HPNS	6/6	4/4	10/10	5/5
HP	6/6	4/4	10/10	5/5



Histopathological examinations revealed necrotizing inflammation in several organs in turkeys. In both species the brain showed high inflammation while nervous clinical signs like seizures were more prominent in turkeys. Endothelial tropism was only observed in chickens.

## Summary

Polybasic cleavage site alone was enough to induce high virulence in chickens, but not in turkeys

In chickens and turkeys, H7N7 carrying HP-NS segment or carrying HP-NS or HP-M segments were as virulent and transmissible as HPAIV, respectively

Pathobiology in chickens and turkeys varies, since endothelial tropism was observed exclusively in chickens and neurotropism in turkeys