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Impact of the polybasic cleavage site within the HA of a recent German H7N7 virus on its pathogenicity in different poultry species

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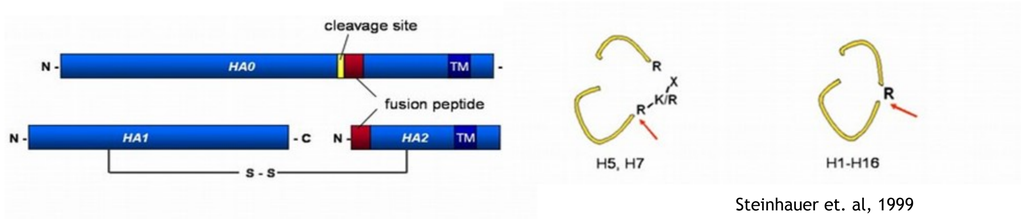
Abstract

Virulence of avian influenza viruses (AIVs) is largely dependent on the amino acid sequence of the hemagglutinin cleavage site (CS). Low pathogenic AIV (LPAIV) carrying an HA with a monobasic CS which is activated by trypsin-like proteases in the respiratory and intestinal tracts cause only local infections with mild clinical signs, if any. Some H5 and H7 subtypes exhibit high pathogenicity (HP) by acquisition of a polybasic CS after circulation of LP precursors in terrestrial poultry. The polybasic CS of HPAIV is cleaved by ubiquitous furin-like proteases causing systemic infections and high mortality. In 2015, in an outbreak in poultry in Germany LP and HP H7N7 viruses could be isolated on the same farm indicating precursor-progeny relationship. Here, we investigated the pathogenicity of these LP and HP viruses in chickens, Muscovy ducks and turkeys via oculonasal and intravenous inoculation and analyzed virulence determinants by reverse genetics. Muscovy ducks showed no or mild clinical signs, while turkeys and chickens died after infection with HPAIV. Insertion of a polybasic CS into the HA of the LP virus (LP-poly) increased pathogenicity for chickens and turkey drastically, but was not sufficient for 100% mortality. LP-poly was less virulent in turkeys than in chickens exhibiting IVPI values of 2.8 and 1.9, respectively. Thus, virulence determinants differ between these two species. Our results contribute to understand the pathobiology and evolution of recent LP and HP H7N7 viruses in different poultry species.

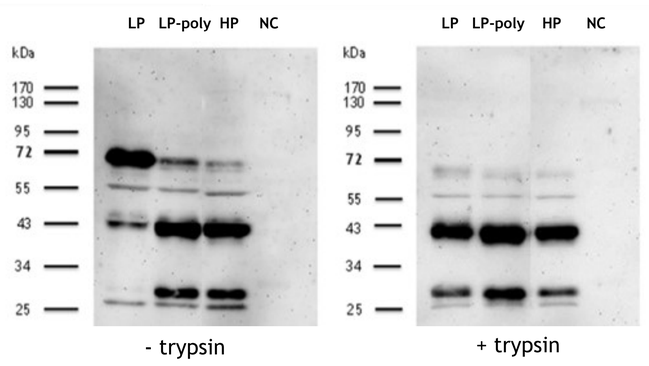
Introduction



Steinhauer et. al, 1999

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 restriced 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

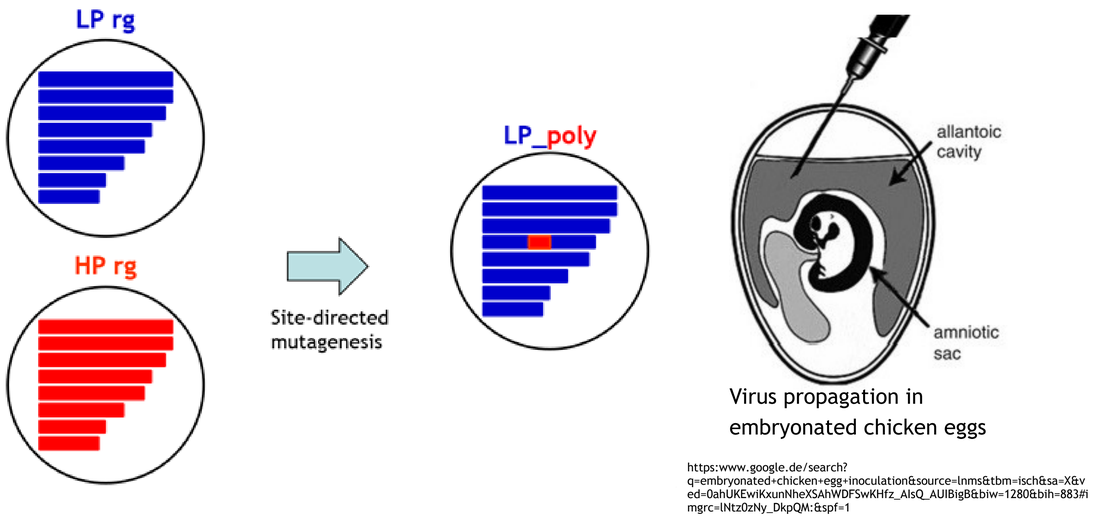
HA Cleavability



MDCK II cells were infected with an MOI of 1 and incubated for 24 hours, in the presence (right) or absence (left) of trypsin. Western Blot analysis shows that the HA of the LP Virus can´t be cleaved efficiently without trypsin.

(NC = Negativ control)

Generation of 3 Viruses



Virus propagation in embryonated chicken eggs

https:www.google.de/search?q=embryonated+chicken+egg+inoculation&source=lnms&tbm=isch&sa=X&ved=0ahUKEwiKxunNheXSAhWDFSwKHfz_AIsQ_AUIBigB&biw=1280&bih=883#imgrc=lNtz0zNy_DkpQM:&spf=1

Three viruses were generated to investigate the influence of the cleavage site within the hemagglutinin on virus pathogenicity. The two parental viruses LP (blue bars) and HP (red bars) were generated by using reverse genetics. Site-directed mutagenisis lead to a LP virus carrying the polybasic cleavage site of the HP virus, designated as LP-poly. The rescued viruses were propagated in 9 to 11 day-old embryonated chicken eggs and characterized *in vitro*.

Experimental Design

Intravenous inoculation

- To determine intravenous pathogenicity index (IVPI)
- 8 to 10 animals per group
- Infection dose: HA > 2^4, diluted 1/10
  100µl per animal

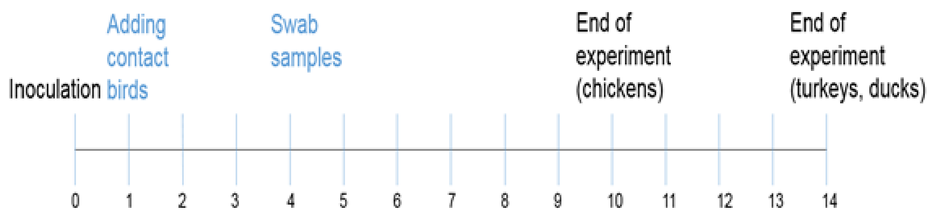
Oculonasal inoculation

- Natural infection route
- 6 to 10 inoculated, 4 to 5 contact birds per group
- Infection dose: 1x10^5 PFU/bird
  200µl per animal

Scoring

- 0= healthy
- 1= mild clinical signs (diarrhea, ruffled feathers...)
- 2= severe clincal signs or more than one
- 3= dead

Observation period



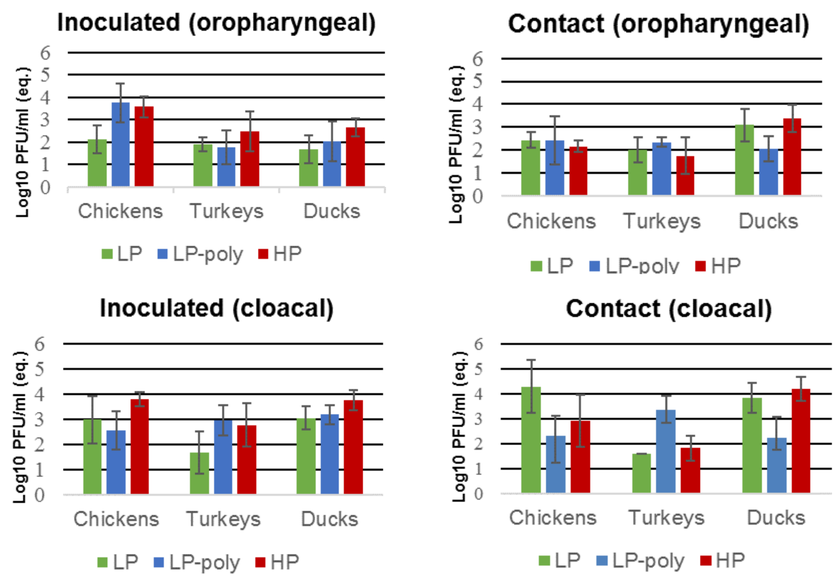
IVPI

Results of the Animal Trials

Virus	Chickens				Turkeys				Ducks			
	Dead/ Inoculated	Dead/ Contact	PI	IVPI	Dead/ Inoculated	Dead/ Contact	PI	IVPI	Dead/ Inoculated	Dead/ Contact	PI	IVPI
LP	0/6	0/4	0.02	0.14	0/10	0/5	0,09	0.04	0/10	0/5	0	0
LP_poly	5/6	3/4	1.81	2.98	3/10	2/5	0,93	1.88	0/10	0/5	0	0.2
HP	6/6	4/4	2.2	2.96	10/10	5/5	2,4	2.77	0/10	0/5	0,14	0.53

While the LP virus showed no or mild clinical signs in all species the HP virus killed all chickens and turkeys. LP_poly killed almost all chickens except for two. Interestingly only five turkeys died afterLP_poly infection. Thus, virulence determinants in chickens and turkeys seem to be different. The ducks were resistent against all three viruses. The intravenous pathogenicity index (IVPI) confirmed the data from the oculonasal infection experiment.

Virus Shedding (RT-qPCR)



Real Time RT-PCR was performed with oral and cloacal swab samples from day 4 post inoculation. The chickens shed the viruses the most whereas orophryngeal and cloacal swab samples were comparable except for the LP-poly virus which showed higher titers in the oropharyngeal swabs. Virus titers measured in turkey and duck swab samples were mostly

Summary

Polybasic cleavage site increased virulence of LPAIV in chickens and turkeys, however other gene segments are required for full virulence of the current German H7N7 virus, particularly in turkeys

Virulence determinants in chickens and turkeys are different

Muscovy ducks showed only mild to moderate depression after HP infection but shed all three viruses to moderate titers