Adaptation processes of wild waterfowl isolates of avian influenza during repeated passages in domestic chickens and ducks

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Key words
Low-pathogenic avian influenza virus, adaptation, transmission, hemagglutinin, tissue tropism

Aim of the study
Wild waterfowl represents a natural reservoir for low-pathogenic avian influenza viruses (LPAIV) which are shed into the environment and eventually transmitted to other species. LPAIV of subtypes H5 and H7 may further evolve to highly-pathogenic avian viruses (HPAIV) which cause a fatal disease in domestic poultry. The goal of this project was to decipher the molecular mechanisms which underlie the adaptation of LPAIV from wild waterfowl to domestic poultry.

Material and methods
Chickens were infected via the intratracheal route with LPAIV of either subtype H5 or H7. Sentinel birds were co-housed with the infected animals in order to monitor virus transmission. Seroconversion of the chickens was investigated by ELISA and hemagglutination inhibiting tests. In addition, oropharyngeal and cloacal swabs were collected and analyzed for presence of viral RNA by qRT-PCR. For virus reisolation, swab samples were inoculated into embryonated chickens eggs. Following extraction of RNA from the allantoic fluid and reverse transcription viral genomic sequences were determined. Virus reassortants were generated using RNA segments of A/chicken/Yamaguchi/7/04 (H5N1), A/duck/Hokkaido/Vac-1/04 (H5N1), and A/swan/Potsdam/62/81 (H7N7).

Results and significance
Following intratracheal inoculation with LPAIV nearly all chickens seroconverted indicating that domestic chickens are susceptible to infection with duck-origin H5 and H7 viruses. LPAIVs of subtype H5 were not shed from the animals, in contrast to the highly pathogenic A/chicken/Yamaguchi/7/04 (H5N1) which was transmitted very efficiently. Transmission did not occur when the HA proteolytic cleavage site of this virus was changed into a sequence motif characteristic for LPAIV. Likewise, H5 LPAIV containing different segments of A/chicken/Yamaguchi/7/04 (H5N1) did not gain transmission competence. These results suggest that H5 LPAIV may not replicate in domestic chickens to levels that allow efficient transmission. It is therefore likely that H5 HPAIV already evolve in wild-living hosts before they are transmitted to domestic poultry. In contrast to H5 LPAIV, all H7 viruses were shed from infected animals and could be re-isolated from swab samples. Three H7 isolates were also transmitted to sentinel birds without prior adaptation. All infected birds were free of clinical symptoms, demonstrating that infected birds may shed virus without showing symptoms of disease. To elucidate what the critical factor for transmission is, a panel of reassortants with genomic segments from A/swan/Potsdam/62/81 (H7N7) and A/duck/Hokkaido/Vac-1/04 (H5N1) was generated. It turned out that the HA segment of the H7N7 virus was a key factor for efficient transmission. This segment conferred transmission competence to an LPAIV with all other segments derived from the H5N1 LPAIV. Transmission appeared to be correlated with virus shedding from the cloaca. These findings suggest that LPAIV from wild-living birds differ in their ability to establish productive infections in domestic chickens. We have found evidence that the hemagglutinin might be an important determinant for this host tropism.

Publications, posters and presentations
Ludersdorfer, T. et al. (2013) A role of the viral RNA polymerase complex in adaption of a mallard-origin low-pathogenic avian influenza virus (H7N3) to domestic chickens. Poster presentation at the 23d Annual Meeting of the Society of Virology, 6 – 9 March 2013, Münster, Germany.

Ludersdorfer, T et al. Differential transmission of low-pathogenic avian influenza viruses in chickens: Role of the hemagglutinin in organ tropism and virus secretion (in preparation).

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