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Background and objectives The main epidemiological reservoir for influenza A viruses is in wild aquatic birds. From this reservoir, some strains can cross the species barrier and evolve as new lineages in other susceptible hosts. Swine are among the sensitive species, with influenza A virus being one of its primary respiratory pathogens. Swine are also considered to be possible intermediary hosts enabling adaptation of avian influenza strains to humans. The objective of this project was to study the adaptation and genetic evolution of an avian influenza strain in swine respiratory cells.

Methods We first adapted an influenza virus field-strain isolated from a duck to swine respiratory cells by repetitive passaging and tested the consequences on growth kinetics in swine, avian, and human cells. We then sequenced the complete parental and adapted viral populations, combining Sanger sequencing and deep-sequencing approaches to study the genetic changes occurring both at the consensus and at the subpopulation levels. We finally set up a prototypic virus rescue system for reverse genetics study of that influenza strain and its swine cell-adapted descendent.

Results As a result of passaging, growth kinetics were improved in swine cells, while they remained mostly unchanged in duck fibroblasts and in a human bronchiolar epithelial cell line; passaging in swine cells also resulted in a marked growth kinetics improvement in chicken cells. The parental field sample was found to contain genes from co-circulating strains present as minority alleles. This enabled reassortment events involving segments 2, 7 and 8 upon culture in swine cells. These reassortments, together with two point mutations on segment 4, resulted in the selection of a near-homogeneous swine cell-tropic virus. Testing rescued viruses carrying combinations of the observed adaptive changes showed that, in the parental virus' genetic background, two non-synonymous point mutations on segment 4 could restore the adapted virus' fast growth phenotype. Reassortment of segment 2 alone, resulting in seven amino acid changes in PB1 and 11 amino acid changes in PB1-F2, seemed to achieve a similar effect.

Conclusions In the context of a naïve duck influenza virus strain, two amino acid changes away from the receptor binding site on the viral haemagglutinin could enhance growth in swine cells. Further, growth kinetics improvement in chicken cells following passaging in swine cells suggested a possible role for swine as an intermediary host for adaptation of an influenza strain from wild waterfowl to domestic chickens.