Unexpected virulence of natural occurring avian influenza virus H3N1 is associated with N1-mediated plasminogen-recruitment

Christian Grund, Jacob Schön, Jürgen Stech, Martin Beer and Timm Harder

Friedrich-Loeffler-Institut, Greifswald – Insel Riems, Germany

Corresponding Author: Christian.grund@fli.de

Abstract

In 2019, several H3N1 low pathogenicity avian influenza viruses (LPAIV) caused serious outbreaks in gallinaceous poultry in Belgium over five months, associated with unusually high mortality of up to 60%. Initial pathology and infection studies in chicken suggested that those strains spread systemically and displayed neurotropism, rather pointing to a high pathogenicity phenotype (HPAIV) (Steensels et al., 2020; de Wit et al., 2020).

We further addressed the tissue tropism of two representative H3N1 LPAIV isolates (Schön et al., 2021). Surprisingly, intravenous infection of chicken with either of the two Belgian H3N1 isolates induced neuronal manifestation in one of ten inoculated chickens. Neurotropism could be reproduced for one isolate (A/ck/BE/1940/2019) by intracerebral inoculation of day-old chicks resulting in an ICPI of 1.73, accompanied by productive virus infection in neurons and endothelial cells of the brain. Remarkably, A/ck/BE/1940/2019 replicated efficiently in a permanent chicken liver cell line (LMH) without external trypsin supplementation. Together, those results indicated that despite a monobasic hemagglutinin cleavage site, proteolytic HA activation was independent from tissue-restricted serine peptidases. However, luciferase reporter cleavage assays revealed that the second pathway of proteolytic activation accessible for the HPAIV, the subtilisin-like intracellular proteases, was not utilized for HA cleavage. Interestingly, the trypsin-independent virus replication was inhibited by the plasmin inhibitor 6-aminohexanoic acid. Correspondingly, in silico analysis of the genome recognized a deletion of a strongly conserved N-glycosylation site at position 130 in the N1 protein, which, together with a C-terminal lysine residue, has been reported to facilitate plasminogen recruitment by the mouse brain-passaged laboratory strain A/WSN/33 (H1N1), and is associated with neurovirulence in mice.

Our findings provide novel evidence that some LPAIV field strains of the N1 subtype recruit plasminogen for proteolytic activation of the HA. The potential emergence of such strains displaying increased pathogenic potential through non-canonical protease recruitment, point to an additional virulence mechanism. Beyond its relevance to naturally occurring viruses in poultry, it may establish zoonotic potential.

References:

Steensels M, et al., 2020. Emerg Infect Dis. Aug;26(8):1899-1903. doi: 10.3201/eid2608.191338.

de Wit et al., 2020. Avian Pathol. 2020 Jun;49(3):286-295. doi: 10.1080/03079457.2020.1731423. Epub 2020 Mar 12.

Schön et al., 2021.PLOS Pathogens 17(4): e1009490. https://doi.org/10.1371/journal.ppat.1009490