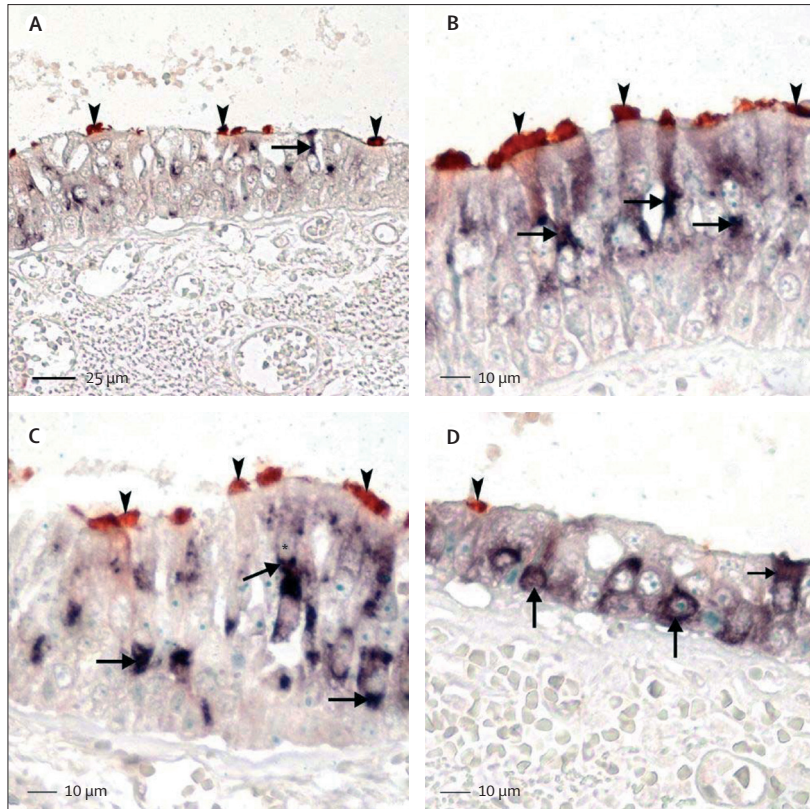


H5N1 infection of the respiratory tract and beyond: a molecular pathology study

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**Webfigure 2: Double labelling combining in-situ hybridisation (ISH, with nucleoprotein antisense probe) and immunohistochemistry (with tubulin-β antibody) on tracheal tissue from patient 2**  
 ISH signals seen with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (purple-blue) and immunohistochemical signals with 3-amino-9-ethylcarbazole (brown-red). (A) Positive ISH signals detected in cytoplasm (arrow) but not in nucleus of tubulin-negative, non-ciliated cell. Positive tubulin staining seen on apical surface of ciliated cells (arrowheads). (B) Both tubulin-positive signals (arrowheads) and ISH-positive signals (arrows) seen in ciliated cells. ISH signals seen in cytoplasm (arrows) but not nuclei of infected cells. (C) Viral genomic sequences (arrows) seen in cytoplasm of non-ciliated columnar epithelial cells (\*) and tubulin-positive ciliated epithelial cells (arrowheads). No ISH signals seen in nuclei. (D) Double labelling showing tubulin-negative non-ciliated cell with positive intracytoplasmic ISH signal (small arrow) and tubulin-positive uninfected cell (arrowhead) with no signal. Two ISH-positive putative basal cells are also seen (large arrows).