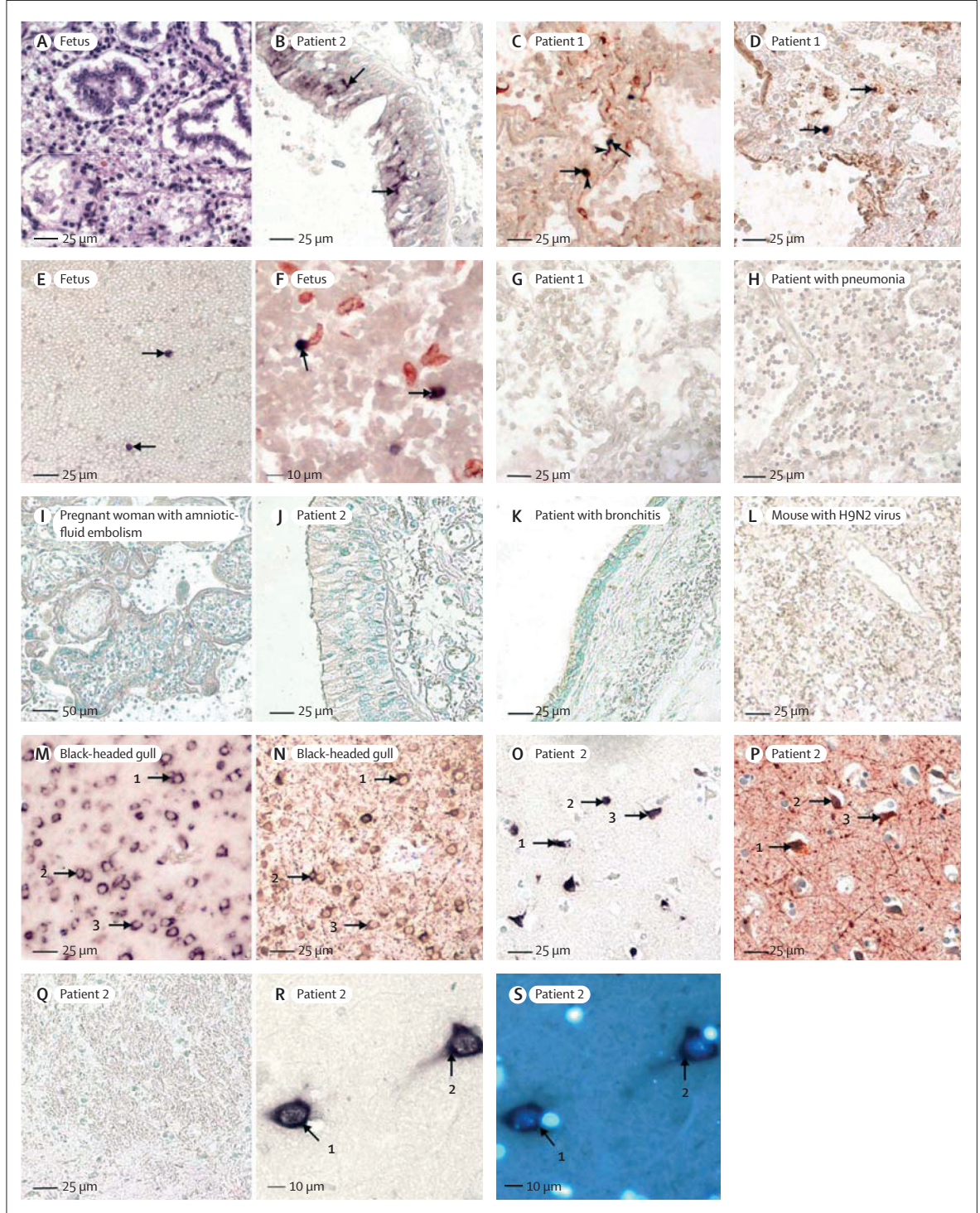


H5N1 infection of the respiratory tract and beyond: a molecular pathology study

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Webfigure 1: In-situ hybridisation (ISH) with sense and antisense probes to haemagglutinin and nucleoprotein, double labelling, consecutive sections, and positive and negative controls

ISH signals seen with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (purple-blue) and immunohistochemical signals with 3-amino-9-ethylcarbazole (double labelling; brown-red). (A) Haematoxylin and eosin staining of fetal lung tissue showing little or no damage, by contrast with severe damage in adult cases. (B) Positive ISH signals (with nucleoprotein antisense probe) in trachea, localising to cytoplasm of epithelial cells (arrows), with no evidence of nuclear staining, counterstained with methyl-green. (C) Double labelling combining ISH (with nucleoprotein sense probe) and antibodies for cytokeratin (AE1/AE3) showing both brown-red intracytoplasmic signals (cytokeratin, arrowheads) and dark-blue signals for viral genome (arrows) in nuclei of the same lung cells, establishing that infected cells are epithelial cells. (D) Double labelling with ISH (with nucleoprotein sense probe) and surfactant antibody showing dark-blue nuclear stain (H5N1 viral genome) and brown-red cytoplasmic signal of surfactant (type II pneumocytes) in one lung cell (arrows). (E) Positive ISH signals (with haemagglutinin sense probe) in mononuclear cells (arrows) in one chamber of fetal heart. (F) Double labelling (with nucleoprotein sense probe and CD68 antibody, brown-red) showing positive ISH and CD68 staining in same fetal liver cells (arrows), identifying them as Kupffer cells. (G) No positive ISH signal in H5N1-infected lungs with use of unrelated probe (antisense probe against fragment of polymerase gene [R1AB] of SARS-CoV). (H) No positive ISH signal (with nucleoprotein antisense probe) in lungs of patient with viral infectious pneumonia who died of respiratory failure (SARS-CoV infection). (I) No positive ISH signals (nucleoprotein antisense probe) in placenta of a pregnant woman who died of amniotic fluid embolism. Counterstained with methyl-green (negative control). (J) No positive ISH signal in trachea with use of unrelated probe. (K) No positive ISH signal (with nucleoprotein antisense probe) in trachea of a patient who died from purulent bronchitis. (L) No positive signal (with nucleoprotein antisense probe) in lungs of a mouse infected with H9N2 virus. (M) Brain tissue of black-headed gull was confirmed as infected with H5N1 by viral isolation and used as a positive control for probes, showing positive ISH signals with nucleoprotein antisense probe in cytoplasm of neurons. (N) Immunohistochemistry with neurofilament antibody (arrows) on consecutive section from webfigure 1M showing identical cells (numbered arrows) to be positive for H5N1 and neurofilament (ie, confirming cells to be neurons). (O) Positive ISH signals (with nucleoprotein antisense probe) in brain cells. (P) Immunohistochemistry of consecutive section to webfigure 1O with neurofilament antibodies, showing ISH-positive cells to be brain neurons. (Q) No positive ISH signal in brain with use of unrelated probe (antisense probe against fragment of polymerase gene [R1AB] of SARS-CoV). (R) ISH signals on brain tissue mainly located in cytoplasm of neurons, with slight staining of nuclei. (S) Section from webfigure 1R counterstained with Hoechst stain to identify nuclei of neurons (light blue); positive ISH signals for H5N1 mainly localised in cytoplasm.