Antigenic characterisation of haemagglutinin proteins derived from different avian influenza virus subtypes.

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Introduction:
The spontaneous emergence of HP H5N1 and H7N7 and the high mutation rate of influenza viruses point out that a correct prediction of new HPAVs is impossible. A comprehensive surveillance and antigenic knowledge of all circulating isolates between the 6 haemagglutinin and 9 neuraminidase subtypes will lead to a more efficient preparedness for now highly pathogenic strains, irrespective of the subtype they will be. In this study, we present a first set of data about the repertoire of subtype-specific and intersubtype-conserved epitopes of haemagglutinin proteins derived from different avian influenza virus subtypes (A/huefde duck/Switzerland/554A/96N1 (HP), A/duck/Cz56/44H6) (LP), A/mallard/ Switzerland/WW4061/66/2006 (H12N2) (LP) relevant for further examination in differential diagnostics and multivalent vaccination approaches.

Genetic relationship of HA full-length coding region among all 16 subtypes of influenza virus.

Results: Purification of baculovirus-expressed recombinant haemagglutinins. The purified proteins were used to immunise rabbits.

Results: Epitope reactivity for each membrane shown as integrated intensity (arbitrary units) or normalised in %, (highest value set as 100%).

Results: Identification of HA subtype-specific epitopes, based on the subtraction of the heterologous from the homologous sera signals. HS H4 H12

Results: Semi-quantitative analysis of the reactivity of linear epitopes on PepSpot membranes with their corresponding (homologous) rabbit antiserum.

Results: Membrane/Serum HS/H5 H4/H4 H12/H12

Discussion:

- as shown by peptide scanning, linear epitopes are present in H1A and H2A ectodomain
- H4 shows most epitopes with homologous serum
- the strongest reactive linear epitopes are subtype-specific (compared with the alignment to the right) and their localisation is not inter subtype-conserved

References:

Methods: Make-up of recombinant haemagglutinin.

1: cloning of truncated HA ORF in pFASTBAC
2: fusion peptide
3: HA1 ectodomain
4: purification w/ Ni-NTA FPLC (imidazole gradient)
5: immunisation of rabbits
6: peptide scanning using -jet Peptide Technologies GmbH
-Lowery Infrared Imaging System (LI-COR Biosciences)
-polyclonal sera of rabbits (2. bleeding)

Results: Infra Red signals