Investigating the presence of stalk directed avian influenza antibodies in highly exposed occupational workers in live bird markets in Bangladesh

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Introduction and Hypothesis

Repeated exposure to influenza has shown to induce broader cross-reactive antibodies, including haemagglutinin (HA) stalk-directed, alleviating disease by promoting antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP).

Hypothesis: those in persistent proximity to poultry and repeatedly exposed to avian influenza viruses (AIVs) have increased cross-protective immunity.

Methods

1. Acquisition of human sera:
   a) Pilot survey (Feb/Mar 2022): poultry workers (n=204), fruit and vegetable sellers (n=87).
   b) Cohort study (Feb/Mar 2023): poultry workers, n=415, fruit and vegetable sellers, n=212.
2. Generation of chimeric HA soluble proteins (insect cell expression) and viruses (reverse genetics (RG)).
3. Identification of stalk-directed antibodies by ELISA.
4. Future investigation of neutralisation capabilities by microneutralisation.

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Results

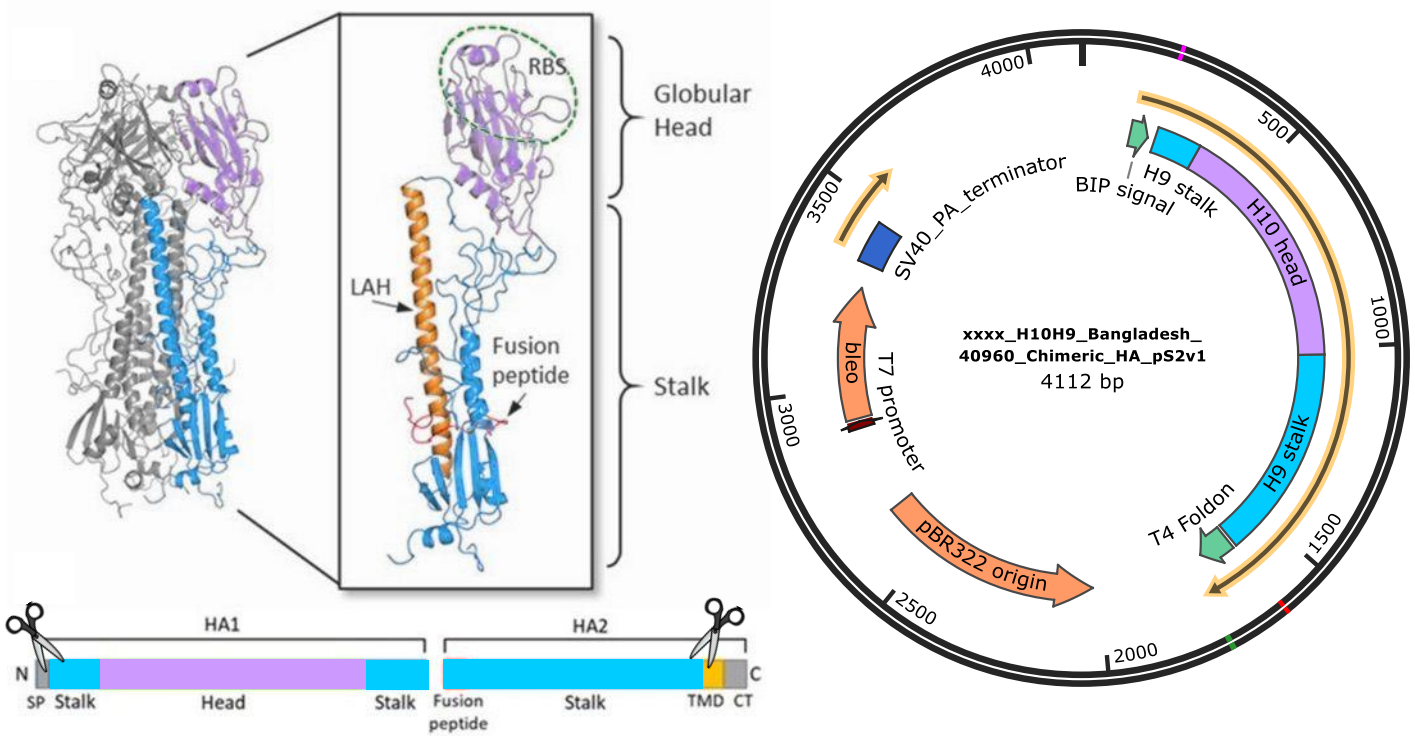

***Figure 1**: Chimeric haemagglutinin (HA) design for expression in insect cell system. H10/H9 HA used as example. Globular head (purple), stalk domain (blue). Signal peptide (SP), transmembrane domain (TMD) and cytoplasmic tail (CT) removed for expression.*

Stalk-targeting antibodies have greater cross-reactivity between human and avian influenza viruses (IV).

To identify stalk-targeting antibodies of humans in the poultry sector exposed to endemic avian IVs, chimeric proteins were engineered as H10(head domain)-H5(stalk domain) and H10(head domain)-H9(stalk domain) using recently isolated H5N1 (clade 2.3.2.1a) and H9N2 (G1 lineage) sequences. The globular head of HA was replaced at the cystine bridge at the stalk superior. Whole H5 and H9 proteins were constructed to identify antibody presence and to compare to the chimeras.

H10 was selected as a redundant head as the human infection rate is incredibly low and targeting antibodies unlikely.

Results Continued

	Positive (Pilot Study)	
Protein	N	%
H5	165	68.75
H9	89	37.08
H10/H9	25	10.42

***Table 1**: Positive serum samples to H5, H9 and chimeric H10/H9 proteins by ELISA. Negative sera deemed with values <0.38.*

- Presence of targeting antibodies to whole H5 and H9 proteins and H10H9 chimeric proteins were identified by indirect ELISA.
- Sera obtained from UK donor bank were used as “negative”, providing background cross-reactivity (no AIV exposure but have human IV antibodies).
- There was greater positive binding to H5 (68.75% of samples), despite the dominance of H9N2 in Bangladesh. May be due to potential cross-reactivity of H5 to H1, hence increased binding.
- N=11 (4.58% of positive) sera had greatest values to H10/H9, greater than H5 and H9 whole HAs. These individuals likely to carry antibodies targeting H10.

Conclusions and Ongoing Work

- 68.75% of sera were positive in direct binding to H5, whereas only 37.08% were to H9.
- 10.42% of samples were positive to H10/H9 chimeric protein but may be skewed due to binding to H10 head.
- Some individuals potentially exposed to H10. Consequently, re-engineered chimeric proteins/viruses to incorporate H13 head.
- Cohort study samples to begin testing.