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**Burden of Avian Influenza A in Chicken on Retail Poultry Stalls of Lahore
District, Pakistan in 2009-2010**

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To the Editor: We read with interest To KK et al, in your Journal who reported seroprevalence of serum antibody titres against influenza A subtype H5N1, H9N2 and H7N9 amongst the workers in live poultry markets of Hong Kong (1).

Wet markets provide a favourable environment for interspecies transfer of AIVs, including with people, and thus for viral reassortment. These markets have therefore been hypothesized to be “a missing link in the epidemiology of avian influenza viruses (AIVs)” (2). A cross-sectional survey of live bird retail stalls (LBRs) in Lahore District of Pakistan was conducted during December 2009-February 2010, to estimate the burden of AIVs circulating in these LBRs and association of the characteristics of these stalls with AIVs prevalence. The target population was consisted of all LBRs throughout Lahore District (9 towns and 1 cantonment area). Information about Lahore District and LBRs was obtained from the office of Lahore City District Government and from the Punjab Livestock and Dairy Development Department (PLDDD).

As no complete list of elementary units (LBRs) in different towns of Lahore was available, a list of administrative towns of Lahore with the approximate total number of LBRs in each town was obtained from the PLDDD and used as the sampling

frame for the current study. Two-stage cluster sampling was conducted with towns of Lahore District as primary sampling units (PSU) and individual LBRS as elementary units. Clusters (i.e. PSU) were selected with probability proportional to size with replacement, such that a given PSU could be sampled more than once, whereas elementary units were sampled without replacement (3).

The sample size was calculated using C-Survey software, version 2.0 (<http://www.ph.ucla.edu/epi/csSurvey.html>). Out of nine towns of Lahore, eight PSU were selected randomly (eight PSU from seven towns); one town (Allama Iqbal town) was selected twice due to replacement. Within the selected PSU, thirty-five LBRSs were selected as elementary units systematically. All birds in a LBRS were treated as a single flock because they were kept under same conditions. At each LBRS, individual swabs were obtained from the oropharyngeal tracts of five apparently healthy live birds (broiler and indigenous chicken) selected at the arbitrary choice of the stall owner and pooled. Each pool was then considered as a single sample.

Supportive epidemiological information about sampling locations and dates, species of birds, management practices, biosecurity measures etc. was collected using a questionnaire survey of stall owners/managers. Point estimates of the weighted proportions for AIVs prevalence and their 95% confidence intervals were calculated using the survey package in R software (4). Chi-squared tests with Rao and Scott second order corrections were used to assess the association between stall characteristics and prevalence of AIV.

In all, 280 (35x8) oropharyngeal swab sample pools were collected from 1400 birds and were classified as either positive or negative for AIV (H9, H5, H7) by qRT-PCR. Swabs were stored in viral transport medium and characterization was carried out at

the OIE and National Reference Laboratory for Avian Influenza and Newcastle Disease, FAO Reference Centre for Animal Influenza and Newcastle Disease, Istituto Zooprofilattico Sperimentale delle Venezie, Padua, Italy. First, qRT-PCR was performed using primers and probes targeting the matrix (M) gene of type A influenza virus. All positive influenza A samples were analysed further by using qRT-PCR protocols for HA gene subtyping (5).

A total of 34 pool swab samples were positive by qRT-PCR for type A influenza (M gene). Out of these 34, 28 samples were positive for H9 while no sample was positive for H5 and H7 subtypes (Figure). Large-scale surveillance would be required to capture low-prevalent AIVs (H5 and H7).

The overall prevalence of AIV subtype H9 was 10.0% (95% CI: 5.61%-14.39%), which fell between the 2.5% prevalence estimates reported from China (6) and that of 16.5% from Bangladesh (7) in 2008-2010. Variation in the prevalence estimates from different locations could be due to live bird market characteristics, sampling methods or regional circulation level of virus. In the current study, 82.4% (28/34) of AIV positive pools belonged to subtype H9. In Korea, approximately 56% (9 out of 16 isolates) of the influenza viruses isolated from chicken, ducks, and doves from LBMs in 2003 were H9N2 viruses (8).

The highest prevalence was seen in Ravi town (17.1%; 95% CI: 4.5%-29.8%) followed by Data Gunj Bakhsh town (14.3%; 95% CI: 2.5%-26.0%) (Table Supplementary Appendix). Data Gunj Bakhsh and Ravi town are the most populated town of Lahore with a congestion level of 25.33 and 21.6 persons per square meter respectively (9). This suggests that surveillance efforts for this virus should be concentrated on areas of high population density.

We visited 280 stalls during the survey and 99.6% told us that they remained opened 7 days a week. Majority of the stalls (43.25%) kept one cage for birds (range 1-8 cages) with a capacity of 30 birds (12.5% stalls) and average turn over of 87 birds sold/day (range 10-1000). Only 33.2% stalls had other breeds of poultry (indigenous or mix of exotic breeds) with commercial broiler birds on their stalls and they were significantly associated with prevalence of AIV H9 ($p < 0.01$). Amongst the 280 stalls visited, 3 had ducks, 2 had guinea fowl and quails, 1 had geese and peafowl on their stalls, while none had turkey, pheasants, partridge or other pet birds on their stalls. The major source of the purchase of birds for these stalls was from dealers or wholesaler (66.4%) and was significantly associated with AIV H9 prevalence ($p = 0.001$). 96.1% respondent owned the stalls. The average number of people visiting the stalls on daily basis was 59 (range 12-700) and only 33.2% stall owners reported that government personnel visited their stalls.

Our study confirmed the circulation of H9 on LBRs in Lahore and supported the hypothesis that H9 is circulating and possibly perpetuating in these LBRs. A major aim of this study was to provide baseline data for large-scale surveillance of LBRs in Pakistan to facilitate control of AIVs in different compartments of the poultry production system.

Conflict of Interest

The authors declare no conflict of interest.

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Figure 1. Spatial distribution of live bird retail stalls, positive and negative for AIV H9N2 subtype

Table. Town level prevalence of H9N2 subtype in live bird retail stalls of Lahore district.

Town	No. of Sampled LBRS	No. of Positive LBRS	Point prevalence estimate	95% CI
Ravi	35	6	17.14	4.47-29.81
Data Gunj Bakhsh	35	5	14.28	2.52-26.04
Samanabad	35	3	8.57	0.83-17.98
Gulberg	35	1	2.85	2.7-8.45
Allama Iqbal	70	9	12.85	4.95-20.75
Aziz Bhatti	35	1	2.85	2.7-8.45
Wagha	35	3	8.57	0.83-17.98

