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Short Communication

Seropositivity to Avian Influenza Virus Subtype H9N2 among Human Population of Selected Districts of Punjab, Pakistan

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ABSTRACT

The present study describes the seroprevalence of avian influenza subtype H9N2 among human population of three districts, Lahore (n = 197), Rawalpindi (n = 205) and Faisalabad (n = 119). Serum samples (n = 521) were collected from poultry workers (n = 351), retailers/butchers (n = 57), housewives (n = 53), general public (n = 60) and were assayed through haemagglutination inhibition test using known H9N2 virus. Of the total sera processed, 238 (45.7 %) were found seropositive to H9N2. Seropositivity was more frequent in district Faisalabad (55.5%, P = 0.009), population group; poultry workers (53.8%, P = 0.001), age group; 31 – 35 years (64.0%, P = 0.001), winter season (45.6%, P = 0.0049) and with geometric mean titer of 1:40 (P = 0.001). The study revealed that H9N2 is circulating in the environment and necessary measures related to public health should be adopted.

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Pakistan's poultry industry, of which Lahore, Rawalpindi, and Faisalabad are major production centers, has experienced sporadic infections with H7N3 and H5N1 subtypes of highly pathogenic avian influenza (HPAI) viruses since 1994 (Naeem et al., 2007). The first H9N2 AI virus outbreak in poultry was reported in 1998 (Naeem et al., 1999) and was closely related to viruses circulating in Hong Kong in 1997 (Cameron et al., 2000). The extensive co-circulation of H9N2 with other avian influenza viruses, including the highly pathogenic H5N1 and H7N3 subtypes, coupled with extensive vaccination, is likely to provide conditions suitable for generation of novel variants and re-assortment with increased epizootic and zoonotic potential (Iqbal et al., 2009; Xu et al., 2007). Such genetic reassortments are not uncommon and an epidemic resulting from mutation of low pathogenic avian influenza (LPAI) H7N3 to HPAI was reported in 2003 – 2004 (Naeem et al., 2007). Despite concurrent outbreaks and subsequent reporting of H5N1, H7N3, and H9N2 AI infections in poultry in Pakistan (Naeem et al., 2007), no sero-epidemiological study on human H9N2 infection has been conducted. Hence, serological prevalence of antibodies to H9N2 has been analyzed in poultry populated districts of Punjab province, Pakistan.

The presence of H9N2 among poultry farm workers (n = 283), retailers/butchers (n = 57), and homemakers (n =53) was investigated in three poultry rearing areas of Punjab Province where avian influenza had been reported (Iqbal et al., 2009; Naeem et al., 2007). Among the population groups studied, poultry workers had the history of work on farms where respiratory disease had occurred, however; no history was available for the other groups. Individuals in the general public (n = 60) who had rare or no known contact with poultry served as controls. A previous respiratory disease history, age and employment experience of each volunteer was recorded. Blood samples (3-5 mL in Venoject®, Belgium) were taken randomly in summer (July and August, n = 305) and winter (December-January, n = 216) from the vicinity of Lahore (n = 197), Faisalabad (n = 119) and Rawalpindi (n = 205) over a 2-year period (2009-10), and transported within 24 h at 4^{0}C to the laboratory. Serum was separated and stored at -20^{0}C till further use.

The heamagglutination inhibition test was performed (Hadipour *et al.*, 2010) using A/Ck/Pk/UDL-01/2008 H9N2 with 1% washed chicken RBCs. One part of each serum sample was diluted with three parts receptor destroying enzyme followed by incubation at 37 °C and subsequent enzyme inactivation at 56 °C for 30 min. Six parts of normal saline were added to obtain a dilution of 1/10. Samples with antibody titers ≤ 20 were considered negative; those with › 20 were considered seropositive to H9N2 virus ((Hadipour et al., 2010). Lack of immunological reactivity of H9N2 isolate with H3N2 (Influenza A/Perth/16/2009), H3 (Influenza Anti–A/Perth/16/2009), and N2 (A/Wyoming/3/2003/H3N2) antiserum confirmed seropositivity of studied sera to H9 only, and not due to cross reactivity of HA protein and/or steric inhibition of N2. Seroprevalence to H9N2 for each population group, district, and season and antibody titers (Geometric Mean titer, GMT) were analyzed with Pearson's Chi Square test (SPSS 18.0, Chicago, IL, USA). P ‹ 0.05 was considered significant.

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Poultry farm workers showed highest levels of percent seropositivity followed by butchers/retailers, homemakers, and the general public (56.5, 51.2, 22.4 and 15.3, P = 0.001). Percent seroprevalence was highest in the 31-35 year age group (64.0, P = 0.001, Figure), highest in Faisalabad district followed by Rawalpindi and Lahore (55.5, 45.6 and 39.6, P = 0.02). The percent seropositivity to homemakers was higher in Faisalabad than in Lahore and Rawalpindi (46.2, 16.7 and 4.5, P = 0.009) whereas, seropositivity to general public was higher in district Rawalpindi than in other two districts (20.0, 19.0 and 7.1, P = 0.549). Percent seropositivity was higher during winter than in summer (45. 6 and 28.3, P = 0.0049), and most of the sera sample showed high antibody titer with GMT, 1:40 (P = 0.001) (Table).

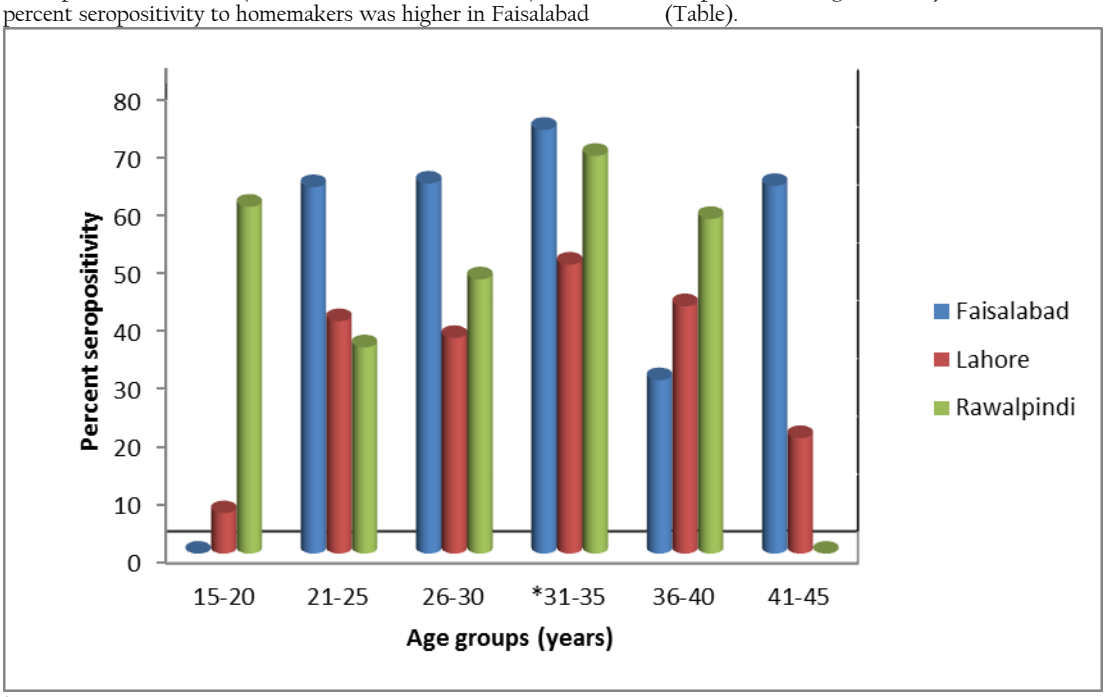

Figure: Seropositivity of H9N2 to different age groups among districts

*P = 0.0001

District	Population group	Serum samples examined (n)	GMT*						
			1:20	1:40	1:80	1:160	1:320	1:640	1:1280
Lahore	Poultry Workers	144	79	16	13	9	26	01	-
	Retailers/Butchers	21	12	5	01	-	02	01	-
	Homemakers	18	15	02	-	01	-	-	-
	General Public	14	13	01	-	-	-	-	-
Rawalpindi	Poultry Workers	139	62	19	14	20	21	03	-
	Retailers/Butchers	19	08	03	07	01	-	-	-
	Homemakers	22	21	-	01	-	-	-	-
	General Public	25	20	02	02	-	01	-	-
Faisalabad	Poultry Workers	68	19	17	09	10	13	-	-
	Retailers/Butchers	17	08	02	04	02	01	-	-
	Homemakers	13	07	-	-	02	04	-	-
	General Public	21	17	03	01	-	-	-	-

Table: Geometric mean antibody titers (GMT) to H9N2 among sample groups in the districts surveyed

*P = 0.001

Large scale H9N2 LPAI virus infection outbreaks occurred in poultry around the world in 1990s (Iqbal et al., 2009), and it is endemic in many parts of the Asia, Middle and Far East, making it a risk factor for humans. The higher seropositivity in poultry workers, butchers, and age group 31-35 years, and homemakers, compared to controls, may be due to frequent occupational contact (Hadipour et al., 2010; Cheng et al., 2002). Poultry isolates of H9N2 viruses in Asia possess human influenza virus-like receptor specificity, which increases the potential for human infection (Mikhail et al., 2001). Higher seropositivity to H9N2 AI virus in winter in humans may correlate with increased prevalence of H9N2 virus in birds (Xu et al., 2005); however, further investigation is needed to ascertain the validity of these results. Owing to poor biosecurity practices, sale of live birds and direct contact with outdoor-reared domestic poultry as opposed to indoor commercial poultry facilities in Lahore and Rawalpindi may be the reason for increased seropositivity in Faisalabad (Jia et al., 2009).

The results indicated seropositivity to H9N2 LPAI among human in major poultry rearing areas of Punjab Province, Pakistan. So far there have been no documented clinical cases of H9N2 virus infection, however, the possibility of mild or sub-clinical infection in human cannot be excluded and thus, increased public health surveillance is invaluable. Further isolation of H9N2 from human and subsequent

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molecular characterization should be done to better elucidate epidemiology as well as adaptability of virus.

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