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RESEARCH ARTICLE

Seroprevalence and Risk Factors Association of Avian Influenza in Desi Chicken (*Gallus domesticus*) in Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Avian influenza is extremely contagious illness of birds as well as humans. Sera were obtained from a total 400 Desi Chickens or household fowls (*Gallus domesticus*) from the five districts distinct geographically and analyzed through hemagglutination inhibition (HI) test for detection of antibodies. Maximum sero-prevalence of Avian Influenza (H5N1) was observed in district Tank 78.75% (63/80) followed by Dera Ismail Khan 63.75% (51/80), Peshawar 58.72% (47/80), Abbottabad 52.50% (42/80) and Mansehra 50% (40/80). It was confirmed through statistical analysis that there was a significant ($P<0.05$) difference of sero-prevalence among these districts. Similarly study also exposed significant ($P<0.05$) higher seroprevalence of the infection in winter 84% (168/200) and lower in summer 37.5% (75/200). The seroprevalence of the infection was significantly ($P<0.05$) more severe in sick 76.5% (153/200) than healthy 45% (90/200) desi chickens. Moreover, vaccinated birds 9% (18/200) were significantly at lower risk as compared to non-vaccinated 62.5% (125/200). The chickens kept in close housing system were significant highly prone to the infection 40.38% (86/213) as compared to ones kept in open housing system 30.48% (57/187). The same association was observed in case of biosecuirty ($P=0.000$), housing zones ($P=0.023$) and sex (0.000) with the sero-prevalance of infection but non-significant ($P=0.500$) with rearing systems.

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INTRODUCTION

Avian influenza is an acute infection of wild and domestic birds all around the world with an effect on public health and heavy financial loss as much as 89% (Capua *et al*., 2000). Sub-clinically, the infection is not unusual in wild birds and believed as a main cause of fatality in commercial birds. The aetiology of the infection is Orthomyxovirus (Orthomyxoviridae) (Huang *et al*., 2012). It has three genera (A, B and C) and classified into various subtypes on the basis of neuraminidase (NA) glycoproteins and hemagglutinin (HA) (Tong *et al*., 2013) which are considered as highly pathogenic AI (HPAI) and low pathogenic AI (LPAI) viruses (World Organization for Animal Health Avian Influenza, 2009).

Among numerous infections, bird originated Influenza virus type A is concerned with endemic infections in poultry (Malik, 2009). The H7 and H5 AI viruses are termed as HPAI, which are reported through various studies conducted in different parts of the globe (Kalthoff *et al*., 2010). The virus is also concerned with public health, indicative of a risk related to the virus (Lin *et al*., 2000). In Japan, the studies proved that causative agent strains were H5N1 and H9N2 and concerned with the illness of birds and pets (Mase *et al.*, 2005; Lee *et al*., 2016).

In a recent study conducted in different areas of our country, its maximum prevalence was reported (Abid *et al*., 2017). In Pakistan, viruses of H7, H9 and H5 subtypes were revealed in five epidemic episodes of the infection and were the main cause of the infection (Naeem and Hussain, 1995). Maximum mortality (3.2 million) with decreased egg production (10 to 75%) was recorded in one of such outbreak in northern areas of Pakistan (Naeem *et al*., 2007). Similarly, its prevalence was upto 48.7% in

poultry labours that clearly give a picture of a critical situation concerning the zoonotic potential as reported by another study (Ahad *et al*., 2014).

Since in Pakistan, the poultry industry contributions are upto 35% of net products of livestock (Naeem *et al*., 2007). Typically, significant variation in prevalence status in different parts of the country is related with their geographical and seasonal parameters. A significant (P<0.05) highest population of broilers was found affected with H9 in Quetta-Pakistan (Arif *et al*., 2015). Similarly, in Faisalabad, 9.4% of population was observed affected with AI (H9) (Sohaib *et al.*, 2010).

By recognizing the significance of the infection, current project was depicted to find out the status of the sero-prevalence in selected five districts of the province with inspection of certain potential risk factors.

MATERIALS AND METHODS

Study criteria: In the current study, the sero-prevalence was observed in five districts of Khyber Pakhtunkhwa i.e. D.I. Khan, Mansehra, Tank, Abbottabad and Peshawar. The blood samples were taken from a total of 400 Desi Chicken/household fowls (*Gallus domesticus*) and were analysed for AI virus type H5N1 through Hemagglutination Inhibition (HI) test (OIE, 2009).

Collection and storage of blood samples: Blood samples were taken from wing vein of Desi Fowls and were preserved in sterile vacutainers tubes. The samples in cold conditions were then taken to main laboratory of department of Biological Sciences, Gomal University, D.I. Khan for serum separation. At -20°C in low temperature freezer, the samples were kept for further use. Before subjecting for HI test, these samples were treated with a receptor-destroying enzyme to wash out the non-specific inhibitors.

Using known antigen H5N1 as control positive obtained from Poultry Research Institute (PRI) Rawalpindi-Pakistan, the serum samples were analyzed through Hemagglutination Inhibition (HI) test as per recommendations of previous studies (Allan *et al.*, 1974; Sastry, 1989; OIE, 2009; Su *et al.*, 2013).

Statistical analysis: The whole data was analyzed through SPSS.20 (IBM, Armonk, NY, USA). Variables of different factors were compared statistically using chi-square test with level of significance 0.05.

RESULTS

Sero-prevalence of avian influenza: In current study, maximum samples were positive at antibody titer 1:4 and minimum at 1:32 while not any sample was observed positive at the titer above 32. Overall sero-prevalence of avian influenza was 52.5, 63.75, 78.75, 50 and 58.72% in five ecologically varied districts of Khyber Pakhtunkhwa-Pakistan in Abbottabad, Dera Ismail Khan, Tank, Mansehra and Peshawar respectively. Chi-square test confirmed a significant (P<0.05) difference of sero-prevalence of the infection among the districts. It revealed significant (P<0.05) highest sero-prevalence of the infection in Tank (78.75%) and lowest in Mansehra (50%) (Table 1).

Table 1: Sero-prevalence of Avian Influenza (H5N1) in Desi Chicken (*Gallus domesticus*) in selected districts of Khyber Pakhtunkhwa, Pakistan

Districts	No. of samples	Positive	Prevalence	*P-value*	X^2–*value*
Dera Ismail Khan	80	51	63.75%	0.002	17.467
Tank	80	63	78.75%		
Abbottabad	80	42	52.50%		
Mansehra	80	40	50.00%		
Peshawar	80	47	58.72%		

Association of various risk factors with the sero-prevalence of avian influenza: The present study also enlightened the association of various factors with the occurrence of avian influenza in Desi Chicken. Seasonal conditions impose direct affects on the occurrence of AI in house-hold chicken. Minimum cases were observed in summer (37.5%) as compared to winter (84%). A significant (P<0.05) difference was observed among different types of seasons for sero-prevalence of the infection through statistical analysis. Higher sero-prevalence was found in sick chicken (76.5%) and lower in healthy ones (45%). Statistically a significant (P<0.05) difference was noticed between them. The infection can be prevented by vaccination at appropriate time because in current study we examined considerable minimum sero-prevalence in vaccinated chickens (9%) and maximum in non-vaccinated (62.5%). Statistically a significant (P<0.05) difference was observed between the both groups. Housing system severely affects the occurrence of avian influenza in Desi Chickens. Maximum sero-prevalence of the infection was observed in close housing system (40.38%) as compared to open (30.48%). Significant (P<0.05) difference between them denoted a considerable association with sero-prevalence of the infection in the chickens (Table 2).

Table 2: Association of various risk factors with the sero-prevalence of Avian Influenza (H5N1) in Desi Chicken

Risk factors	Determinants	No. of samples	Positive	Prevalence	*P-value*	X^2–*value*	Odds Ratio	95% C.I
Season	Winter	200	168	84.00%	0.000	90.68	8.75	5.45-14.06
	Summer	200	075	37.50%				
Health status	Sick	200	153	76.50%	0.000	41.61	3.98	2.59-6.11
	Healthy	200	090	45.00%				
Vaccination status	Vaccinated	200	018	09.00%	0.000	124.61	0.059	0.034-0.104
	Non-vaccinated	200	125	62.50%				
Housing system	Open	187	057	30.48%	0.039	4.244	0.647	0.428-0.980
	Close	213	086	40.38%				
Rearing system	Floor	184	069	37.50%	0.500	0.454	1.151	0.764-1.735
	Cage	216	074	34.26%				
Bio-security	Present	200	005	15.50%	0.000	192.52	0.012	0.005-0.029
	Absent	200	138	56.00%				
Housing zones	Middle	149	047	31.54%	Ref			
	Vent	120	054	35.00%	0.023	5.133	0.563	0.342-0.927
	Fans	131	042	41.22%	0.926	0.009	0.976	0.59-1.62
Sex	Male	188	042	22.34%	0.000	27.769	0.316	0.204-0.489
	Female	212	101	47.64%				

Rearing system was next risk factor for sero-prevalence of the infection in the house-hold chickens. The sero-prevalence was lower in the flock kept in cages (34.26%) as compared to ones reared on floor (37.5%), while through statistical analysis there was observed a non-significant ($P>0.05$) difference between them indicating the non-significant association of the sero-prevalence with rearing system. The bio-security performs a major role in avoidance of the infection in house-hold domestic chickens. Lower infection (15.5%) was observed in the chickens which were reared inappropriate bio-security as contrast to ones where it was not present (56%). Statistically a significant ($P<0.05$) difference observed between them confirmed marked association of the infection with the biosecuirty. Housing zones were observed as key factor related with the sero-prevalence of the infection in the chickens. The birds kept in middle area (31.54%) had minimum sero-prevalence of the infection as compared to ones kept in vent area (35%). A statistically significant difference ($P<0.05$) of sero-prevalence among the different housing zones, confirmed that housing zone has marked association with the occurrence of the infection (Table 2).

DISCUSSION

Avian influenza is extremely pathogenic infection of the birds with zoonotic significance. Poultry labour is highly prone to the infection owing to direct and common exposure to chickens (Catolli, 2013; Monne *et al.,* 2013; Capua and Turner *et al.,* 2017). Different factors potentiate the occurrence of the infection like location, season, species, vaccine failure due to unsatisfactory storage and hygienic conditions, immune status, poor supply of fresh and clean water, stress, harsh environmental conditions and lack of booster dose. These factors potentiate the occurrence of the infection in poultry (Le *et al.,* 2013; Chang *et al.,* 2014).

In current study, certain factors were studied to investigate their direct or indirect influence on the sero-prevalence of avian influenza in the house-hold chickens. In present study, significant ($P<0.05$) variation based on location (Table 1) is endorsed by Fatima *et al.* (2017) who made investigations in five districts (Haripur, Mansehra, Abbottabad, Rawalpindi and Islamabad) of Pakistan. This location based difference in sero-prevalence of the infection was also in line with the conclusions of a number of studies conducted aboard (Aly *et al.,* 2008; Sun *et al.,* 2014; Osman *et al.,* 2015).

Season of a year has deep impact on occurrence of the infection. Significantly ($P<0.05$) highest sero-prevalence of the infection in winter and lowest in summer in house-hold chicken observed in current study is endorsed by Turner *et al.* (2017). The highest cases observed in winter might be due to fall in environmental conditions (temperature and humidity) which not only potentiate survival rates but also dissemination of the virus (Fatima *et al.,* 2017). Health status of flock has a magnetic effect on sero-prevalence of the infection. Current study recorded significant ($P<0.05$) higher sero-prevalence in sick as compared to healthy chickens. These observations are different to the findings of Turner *et al.* (2017). This contrast might be owing to small and uneven sample size from the sick birds.

The infection can be prevented and controlled through vaccination at appropriate time. The vaccinated chickens contain particular antibodies which play central role in fight against the virus antigens and prevent the disease occurrence (Capua and Catolli, 2013). Significant ($P<0.05$) maximum infection in non-vaccinated chickens is in concurrence with the reports of a numbers of researchers (Capua and Catolli, 2013; Monne *et al.,* 2013). Different housing systems impose their different effects on occurrence of the infection. It was observed in present study that Desi chickens reared in open type of housing system were significantly ($P<0.05$) less prevalent to the infection than those reared in close housing system. The highest sero-prevalence in close housing system might be due to the damp and sticky surroundings of the close housing system which enhance the intensification of the pathological agents. The result is in agreement with the conclusions of Monne *et al.* (2012) and Akhter *et al.* (2017).

Study of effect of various rearing systems on occurrence of the infection in chickens was also part of our study. Maximum sero-prevalence observed in chickens reared in floored pens is in line with the findings of Turner *et al.* (2017) but there was non-significant ($P>0.05$) difference between both types of rearing systems. It means that type of rearing system does not affect the proliferation and spreading of the infection. Current study declared that biosecuirty significantly affects the occurrence of the infection. Significant ($P<0.05$) minimum sero-prevalence in chicken reared in proper biosecuirty highlights its importance. The finding is in line with the conclusions of Capua and Catolli (2013) and Zaman *et al,* (2018). Impact of housing zones has a certain influence on sero-prevalence of the infection. Significant ($P<0.05$) maximum sero-prevalence was recorded in chickens kept near to fan area zone. Soggy and stagnant air of the area might be the genuine cause which provides optimum conditions for infecting the birds. The birds kept at middle area had significant ($P<0.05$) minimum sero-prevalence that might be due to accessibility of fresh atmosphere with minimum contamination with the infectious agent.

Conclusions: In this study, effect of various factors was studied linked with the sero-prevalence of avian influenza in Khyber Pakhtunkhwa, Pakistan. The current investigation gives an evidence of relationship of the factors (i.e. housing system, season, rearing system, biosecuirty, vaccination status and housing zones) with the sero-prevalence of the infection. Inattention to these issues would enhance its occurrence.

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Authors contribution: Abdul Haleem and Sajjad Ur Rahman devised and supervised the study plan. Ali Zaman and Naimat Ullah collected the samples and performed laboratory work, analyzed the data and drafted the article.

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