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Seroprevalence of Avian Influenza (H_5) in Broilers from Five Districts of Khyber Pakhtunkhwa, Pakistan

Ali Zaman[1,*], Abdul Haleem[1], Sajjad Ur Rahman[2], Adnan Amin[3] Asma Saeed[1], Sana Ullah[4] and Naimat Ullah[4]

[1]*Department of Biological Sciences, Gomal University, Dera Ismail Khan*
[2]*Institute of Microbiology, University of Agriculture, Faisalabad*
[3]*Department of Pharmacognosy, Faculty of Pharmacy Gomal University, Dera Ismail Khan*
[4]*Faculty of Veterinary and Animal Sciences, Gomal University, Dera Ismail Khan*

ABSTRACT

Avian influenza (H_5) is highly contagious infection of birds as well as humans which is zoonotic of significant economic and public health concern. From total 650 broilers sera were taken, around the five geographically distinct districts and analyzed through hemagglutination inhibition (HI) test for detection of H5 antibodies. Highest sero-prevalence of Avian Influenza (H5) was recorded in district Abbottabad 36.15% (47/130) followed by Dera Ismail Khan 33.08% (43/130), Tank 26.92% (35/130), Peshawar 21.54% (28/130) and Mansehra 16.92% (22/130). Statistical analysis confirmed a significant ($P<0.05$) difference of sero-prevalence between the districts. There was a significant ($P<0.05$) association of occurrence of the infection with the type of season, health status, vaccination, housing system, biosecurity and housing zones and non- significant ($P>0.05$) association with rearing system. To the best of our knowledge, this is the first sero-prevalence report of avian influenza (H5) in broilers in these districts of the province. Necessary measures are required to overcome the situation and improve vaccination and hygiene.

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Authors' Contribution
AH, SUR and AA devised and supervised the study plan. AZ, AS and SU collected the samples and performed the laboratory work. AZ, SU and NU analyzed the data and drafted the article.

Key words
Avian influenza, Broilers, Sero-prevalence, Risk factors, Pakistan.

INTRODUCTION

Avian influenza (H5) is a leading cause of mortality in birds around the globe thus ending up with an impact on public health and heavy economic loss up to 89% (Capua *et al*., 2000). The AI infection is common in wild birds in sub-clinical form and considered as a major source of death in commercial birds. The Orthomyxovirus (Orthomyxoviridae) is major cause of the infection (Huang *et al*., 2012). The virus has three genera (A, B and C) and divided into different subtypes based on the hemagglutinin (HA) and neuraminidase (NA) glycoproteins (Tong *et al*., 2013) which are categorised as low pathogenic AI (LPAI) or highly pathogenic AI (HPAI) viruses (World Organisation for Animal Health, 2009).

Among various infections, only Influenza virus type A is of bird origin has been implicated in endemic infections and outbreaks in poultry (Malik, 2009). The H5 and H7 AI viruses are reported as HPAI, which are reported for outbreaks in many parts of the world (Kalthoff *et al*., 2010). Also AI virus is associated in human infections, signifying a threat linked to this virus (Lin *et al*., 2000). The AIV presenting a severe threat to public health is normally transmitted from birds to humans. In Japan (2003-2004) after influenza outbreaks, the investigations proved that causative virus was H5N1 strain and affected birds were raised as pets. However, route of infection and dissemination of virus still remained unclear (Mase *et al*., 2005). An analysis based on molecular detection revealed that H9N2 viruses are still potential infectious for mammals (Lee *et al*., 2016).

Its high prevalence was observed in a recent study conducted in various regions of Pakistan (Abid *et al*., 2017). In Pakistan, five epidemic episodes of the infection have been reported and viruses of H7, H9 and H5 subtypes were primarily considered responsible for the infection (Naeem and Hussain, 1995). In one of such episodes in northern areas of Pakistan a high mortality (3.2 million) and decrease in egg production (10 to 75%) was recorded (Naeem *et al*., 2007). Likewise in another study, its prevalence was up to 48.7% in poultry workers that clearly depicts a critical situation regarding the zoonotic potential (Ahad *et al*., 2014).

Since the poultry industry in Pakistan contributes upto

* Corresponding author: alizaman@gu.edu.pk
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35% of the livestock net products (Naeem *et al*., 2007). A number of studies have reported its prevalence status in various parts of the country that are typically related to their geographical and seasonal parameters. In a recent study conducted in broilers in Quetta-Pakistan, a significant (P<0.05) high population was observed affected with H9 (Arif *et al*., 2015). Likewise in Faisalabad, the AI (H9) was detected from 9.4% of the study population (Shoaib *et al*., 2010).

To the best of our knowledge, this is assumed to be the first sero-prevalence report on broilers, of avian influenza (H_5) in the five districts of Khyber Pakhtunkhwa-Pakistan. Keeping in view the significance of avian influenza, current project was designed to study the status of sero-prevalence of AI in five major districts of Khyber Pakhtunkhwa with recognition of association of possible risk factors.

MATERIALS AND METHODS

Study criteria

In present study, five districts of Khyber Pakhtunkhwa were selected namely D.I. Khan, Tank, Mansehra, Abbottabad and Peshawar. The blood samples were randomly collected in winter and summer from 1650 birds to detect avian influenza by Hemagglutination Inhibition (HI) test through the titration of AI virus type H5 (OIE, 2009).

Table I.- Sero-prevalence of avian influenza (H_5) in broilers in five districts of Khyber Pakhtunkhwa, Pakistan.

Districts	No. of samples	Positive	Prevalence	P-value	X^2 – value
D.I. Khan	130	43	33.08%	0.002	16.656
Tank	130	35	26.92%		
Abbottabad	130	47	36.15%		
Mansehra	130	22	16.92%		
Peshawar	130	28	21.54%		

Collection and storage of blood samples

Blood samples were collected in the EDTA coated vacutainers tubes from wing vein of commercial broilers by using a disposable sterile syringe. The contents of the vacutainers tube were mixed by gentle tapering to avoid rupture of suspended erythrocytes. The samples were then brought to laboratory of Department of Biological Sciences, Gomal University, D.I. Khan in cold conditions for serum separation. The serum samples were then stored at −20°C in low temperature freezer for further use.

All serum samples were analysed quantitatively with known antigen H_5 (control positive) obtained from Poultry Research Institute (PRI) Rawalpindi-Pakistan by Hemagglutination Inhibition (HI) test to check the prevalence of Avian Influenza (OIE, 2009; Allan *et al*., 1974).

Statistical analysis

Statistical comparison between variables of various factors was examined by Chi-square test. A P-value of 0.05 was assessed to specify statistical significance. Data analysis was carried out using SPSS 20 (IBM, Armonk, NY, USA).

RESULTS

Sero-prevalence of avian influenza in five districts

Out of total 650 samples, maximum had antibody titer at 1:4 and minimum at 1:32 whereas none of the samples had the titer above 32. In current study, overall sero-prevalence of avian influenza (H_5) in five ecologically different districts of Khyber Pakhtunkhwa-Pakistan was 33.08%, 26.92%, 36.15%, 16.92% and 21.54% in Dera Ismail Khan, Tank, Abbottabad, Mansehra and Peshawar, respectively. Statistical analysis through chi-square test revealed a significant (P< 0.05) difference of sero-prevalence of the infection among the studied districts. It confirms significant (P<0.05) highest sero-prevalence of the infection in Abbottabad (36.15%) and lowest in Mansehra (16.92%) (Table I).

Risk factors and the sero-prevalence of avian influenza

The current study also highlighted the association of various factors with the sero-prevalence of avian influenza (H_5) in broilers. Season directly affects the occurrence of avian influenza in broilers. In summer (14.46%), lower cases of avian influenza (H_5) were observed as compared to winter (39.38%). Statistical analysis showed a significant (P< 0.05) difference between these seasons. Maximum sero-prevalence was observed in sick broilers (37.11%) and minimum in healthy ones (22.59%). Statistically there was a significant (P< 0.05) difference between them. Vaccination at appropriate time has significantly impact on prevention of the infection. In this study we observed considerable lower sero-prevalence in vaccinated broilers (6.15%) and higher in non-vaccinated (47.69%). Statistical analysis confirmed a significant (P< 0.05) difference between these two groups. Next housing system severely affects the occurrence of avian influenza (H5) in broilers. Maximum sero-prevalence of the infection was observed in close housing system (32.74%), as compared to open (20.70%). Significant (P< 0.05) difference between them denoted a considerable association with sero-prevalence of the infection in broilers (Table II).

Table II.- Association of various risk factors with the sero-prevalence of avian influenza (H5).

Risk factors	Determinants	No. of samples	Positive	Prevalence	P-value	X^2–value	Odds ratio	95% C.I
Season	Winter	325	128	39.38%	0.000	51.304	3.84	2.62-5.62
	Summer	325	47	14.46%				
Health status	Sick	194	72	37.11%	0.000	14.596	2.023	1.4-2.91
	Healthy	456	103	22.59%				
Vaccination status	Vaccinated	325	20	6.15%	0.000	142.51	0.072	0.04-0.12
	Non-vaccinated	325	155	47.69%				
Housing system	Open	314	65	20.70%	0.001	11.95	0.536	0.38-0.76
	Close	336	110	32.74%				
Rearing system	Floor	322	97	30.12%	0.068	3.32	1.382	0.97-1.95
	Cage	328	78	23.78%				
Bio-security	Present	319	23	7.21%	0.000	123.73	0.092	0.06-0.15
	Absent	331	152	45.92%				
Housing zones	Middle	205	87	42.44%	Ref			
	Vent	224	36	16.07%	0.000	36.389	3.85	2.45-6.05
	Fans	221	52	23.53%	0.000	17.299	2.396	1.58-3.63

Rearing system was another key factor for occurrence of the infection in broilers. Maximum cases of the infection were observed in the flock reared in cages (30.12%) as compared to ones kept on floor (23.78%). But statistical analysis revealed a non-significant ($P > 0.05$) difference between them by confirming non-significant association of the sero-prevalence with rearing system. Bio-security plays a chief role in prevention of the infection in broilers. The sero-prevalence of the infection was minimum in the birds which were reared in appropriate bio-security (7.21%) as compared to ones where it was absent (45.92%). A significant ($P< 0.05$) difference between them revealed a marked association of the infection with the biosecuirty measures. Housing zones was observed as a magnetic factor associated with the sero-prevalence of the infection in the birds. A lowest sero-prevalence of the infection was observed in the birds near to vent area (16.07%) and highest in ones kept in middle area (42.44%). Statistical analysis revealed a significant difference ($P< 0.05$) of sero-prevalence among these variant housing zones, thus presented a marked association of the housing zone with the occurrence of the avian influenza (H_5) (Table II).

DISCUSSION

Avian influenza (H_5) is highly pathogenic infection of the birds as well as humans. Due to direct and frequent exposure to chickens, poultry workers remain at high risk to the infection (Monne *et al*., 2013; Capua and Catolli, 2013; Turner *et al*., 2017). A number of factors directly influence the occurrence of the infection. These factors include season, location, specie, vaccine failure due to improper storage conditions, immune status, unsatisfactory hygienic conditions, poor supply of clean and fresh water, lack of booster administration, overcrowded stress, harsh climatic conditions. These factors potentiate the occurrence of the infection (H5) in poultry (Le *et al*., 2013; Chang *et al*., 2014).

The current study was planned with the investigation of certain factors that directly or indirectly influence the occurrence of avian influenza (H_5) in the broilers. The location wise significant ($P< 0.05$) variation in present study is in line with the conclusions of Fatima *et al*. (2017) who studied it in five districts (Mansehra, Haripur, Abbottabad, Islamabad and Rawalpindi) of Pakistan. The study confirmed significant ($P< 0.05$) highest sero-prevalence of the infection in Abbottabad and lowest in Mansehra. This location wise difference in sero-prevalence of the infection was also endorsed by a number of studies conducted aboard (Aly *et al*., 2008; Sun *et al*., 2014; Osman *et al*., 2015).

Our results exposed maximum sero-prevalence of the infection in broilers in winter as compared to summer. The significant ($P< 0.05$) higher variation in both seasons is in line with the findings of Turner *et al*. (2017). This highest sero-prevalence in winter might be due to lower temperature and humidity conditions which not only enhance the survival rates of the virus but also its transmission (Fatima *et al*., 2017). In current study significant ($P< 0.05$) maximum sero-prevalence of the virus was observed in sick broilers as compared to healthy. It might be due to maximum infection of the sick broilers with Influenza virus (H_5). The findings are contrary to the conclusions of Turner *et al*. (2017) which might be due to his smaller and uneven sample size from the sick birds.

The vaccination has a key effect on prevention and

control of the infection. The vaccinated broilers have specific antibodies which fight against the virus antigens, thus diminishes its consequences (Capua and Catolli, 2013). Significant ($P< 0.05$) lower sero-prevalence in vaccinated broilers is in line with the conclusions of a numbers of recent past studies (Monne *et al*., 2013; Capua and Catolli, 2013). It was revealed through the current study that broilers kept in close type of housing system were more positive for the infection as compared to kept in open housing system. It might be due to the soggy and humid conditions of the close housing system that favours the escalation of the infectious agents. The findings are endorsed by Monne *et al*. (2013) and Akhter *et al*. (2017).

Different rearing systems influence severely on the occurrence of the infection in broilers. In current study, a highest number of positive cases were observed in broilers kept in farms provided with floor as compared to ones with cages but statistically there was non-significant ($P< 0.05$) difference. It means that propagation and dissemination of the virus is not affected by the type of rearing system. The findings are in line with the conclusions of Turner *et al*. (2017). The significant ($P < 0.05$) lower sero-prevalence of the infection in the broilers reared in appropriate biosecurity emphasis on the importance of biosecurity. This lowest sero-prevalence might be due to provision of virus free environment provided to the broilers through appropriate biosecurity measures. The finding is in line with the conclusions of Capua and Catolli (2013). In present study, a significant ($P< 0.05$) highest sero-prevalence of the infection observed in the broilers reared at middle area might be due to damp and stagnant air where the conditions of temperature and humidity are optimum for invading the birds and production of the infection. A significant ($P< 0.05$) lowest sero-prevalence of the infection in the birds reared at vent area might be due to availability of fresh air which provided little appropriate conditions for viral growth and induction of the infection.

CONCLUSIONS

The study highlights different factors associated with the sero-prevalence of avian influenza in Khyber Pakhtunkhwa, Pakistan. The current research confirms an evidence of association of the factors (*i.e.* season, vaccination status, housing system, rearing system, biosecurity and housing zones) with the sero-prevalence of the infection. Negligence of these factors would secure its occurrence. Hence by focus on appropriate management in specific seasons, housing system, rearing system and biosecurity measures may lessen the cases of avian influenza (H_5).

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Statement of conflict of interest

Authors confirm no conflict of interest concerning article publication.

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