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 seroprevalence 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 seroprevalence 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) de
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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 labourers 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 analysed 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 | Value | Value X<sup>2</sup>–value | Value P<sup>2</sup>–value | Odds Ratio | 95% C.I |
|--------------------|----------------|---------------------|-------|-----------------------------|-------------------------|------------|--------|
| Dera Ismail Khan   | 80             | 51                  | 63.75%| 0.002                       | 17.467                  |            |        |
| Tank               | 80             | 63                  | 78.75%| 0.000                       | 21.126                  |            |        |
| Abbottabad         | 80             | 42                  | 52.50%| 0.004                       | 4.072                   |            |        |
| Mansehra           | 80             | 40                  | 50.00%| 0.193                       | 1.076                   |            |        |
| Peshawar           | 80             | 47                  | 58.72%| 0.002                       | 17.647                  |            |        |

**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 | Value | Value X<sup>2</sup>–value | Value P<sup>2</sup>–value | Odds Ratio | 95% C.I |
|-------------------|--------------|----------------|----------|------------|-------|-----------------------------|-------------------------|------------|--------|
| **Season**        | Winter       | 200            | 168      | 84.00%     | 0.000 | 98.68                      | 8.75                    | 5.45-14.06 |        |
|                   | Summer       | 200            | 75       | 37.50%     | 0.000 | 38.15                      | 3.75                    | 1.99-7.11  |        |
| **Health status** | Sick         | 200            | 153      | 76.50%     | 0.000 | 41.61                      | 3.98                    | 2.96-11.7  |        |
|                   | Healthy      | 200            | 90       | 45.00%     | 0.000 | 27.76                      | 2.77                    | 1.28-5.94  |        |
| **Vaccination status** | Vaccinated | 200            | 18       | 9.00%      | 0.000 | 124.61                     | 0.59                    | 0.034-10.4 |        |
|                   | Non-vaccinated | 200        | 125      | 62.50%     | 0.000 | 124.61                     | 0.59                    | 0.034-10.4 |        |
| **Housing system** | Open         | 187            | 57       | 30.48%     | 0.039 | 4.244                      | 0.64                    | 0.428-0.980|        |
|                   | Close        | 213            | 88       | 45.00%     | 0.000 | 124.61                     | 0.59                    | 0.034-10.4 |        |
| **Rearing system** | Floor        | 184            | 69       | 37.50%     | 0.000 | 45.00                      | 1.15                    | 0.764-1.735|        |
|                   | Cage         | 201            | 74       | 36.26%     | 0.039 | 4.244                      | 0.64                    | 0.428-0.980|        |
| **Bio-security**  | Present      | 200            | 94       | 47.00%     | 0.000 | 192.52                     | 0.012                   | 0.005-0.029|        |
|                   | Absent       | 200            | 104      | 52.00%     | 0.000 | 192.52                     | 0.012                   | 0.005-0.029|        |
| **Housing zones** | Middle       | 149            | 47       | 31.54%     | 0.000 | 35.00                      | 0.93                    | 0.352-0.927|        |
|                   | Vent         | 120            | 54       | 45.00%     | 0.039 | 5.13                       | 0.56                    | 0.342-0.972|        |
|                   | Fans         | 131            | 42       | 32.00%     | 0.000 | 5.009                      | 0.976                   | 0.59-1.62  |        |
| **Sex**           | Male         | 188            | 74       | 40.00%     | 0.000 | 124.61                     | 0.59                    | 0.034-10.4 |        |
|                   | Female       | 212            | 101      | 47.64%     | 0.000 | 27.769                     | 0.316                   | 0.204-0.489|        |
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 in 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 biosecurity. 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 biosecurity significantly affects the occurrence of the infection. Significant (P<0.05) minimum sero-prevalence in chicken reared in proper biosecurity 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, biosecurity, vaccination status and housing zones) with the sero-prevalence of the infection. Inattention to these issues would enhance its occurrence.

**Acknowledgements:** We are grateful to Department of Biological Sciences, Faculty of Veterinary and Animal Sciences; Gomal University, Dera Ismail Khan, Institute of Microbiology, Agriculture University of Faisalabad for financial and technical support for completing the project.

**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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