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TO INVESTIGATE THE SERO-PREVALENCE OF AVIAN INFLUENZA ANTIBODIES IN BACKYARD POULTRY OF HAZARA DIVISION

Naqash Khalid[1*], Hajira Mehmood[1], Mohsin Ali[1], Riaz Ali[1], Yasir Amin[1], Muhammad Athar Abbas[2], Muhammad Ayaz[1]

[1]Poultry Research Institute Jaba, Khyber Pakhtunkhwa, Pakistan
[2]National Reference Laboratory for Poultry Diseases ASI NARC Islamabad, Pakistan

Abstract. Avian Influenza (AI) is a contagious viral disease cosmopolitan that affects the chickens of all ages with variable mortality ranging from 30% to 100%, depending on the pathogenicity of the AI virus. The current study was conducted to investigate the sero-prevalence of Avian Influenza Antibodies in backyard poultry population of Hazara Division. For this purpose, a total of 500 blood samples were collected in clot activator vacutainer with the help of sterile syringes. These samples were sent to National Reference Laboratory for Poultry Diseases (NRLPD) for antibody titration through Haemagglutination inhibition (HI) test. HI test was conducted for Avian Influenza antibodies according to the protocol described by (Allan, Lancaster, & Toth, 1978), by using 4HA units of Avian Influenza Virus H5, H7 and H9 serotypes. From the obtained results, it was found that from total of 500 serum samples, 11.8% samples were had HI titer of MT (log_2)=6.00 to MT (log_2)=11.00 and was considered as positive for sero-prevalence of Avian Influenza in backyard poultry population of Hazara Division. Majority of the samples had HI titer of less than MT (log_2)= 5.00 and were considered as negative for sero-prevalence of Avian Influenza. Out of the positive samples, maximum sero-prevalence 81.35% was recorded antibodies for AIV-H9 subtype while 18.64% was recorded antibodies for AIV-H5 subtype and no antibodies were found for AIV-H7 subtype. Area wise sero-prevalence was recorded and found that from total of positive samples, Maximum sero-prevalence 52.54% was recorded from District Mansehra, followed by 30.51% was recorded from District Abbottabad and minimum sero-prevalence 16.94% was recorded from District Haripur. Month wise sero-prevalence was recorded and observed that from total of positive samples, maximum sero-prevalence 18 samples (30.51%) were found sero-prevalence in the month of January, followed by February in which 14 samples (23.72%) were found sero-prevalence, followed by March in which 12 samples (20.33%) were found sero-prevalence, followed by December in which 09 samples (15.25%) were found sero-prevalence and 06 samples (10.16%) were found sero-prevalence in the month of April. From the current study it was concluded that the antibodies of Avian Influenza Viruses were present in the backyard poultry population of Hazara Region especially in District Mansehra. Avian Influenza H9 subtype was most prevalent in the present study. Winter season was ideal for propagation of Avian Influenza Virus in the area.

Keywords: *Avian Influenza, Sero-prevalence, Backyard Poultry, Hazara Region and Haemagglutination Inhibition (HI).*

Corresponding Author: *Naqash Khalid, Poultry Research Institute Jaba, Khyber Pakhtunkhwa, Pakistan, e-mail: dr.naqash092@gmail.com*

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1. Introduction

Avian influenza viruses are classified in the family Orthomyxoviridae, genus Influenza virus A. The surface is covered by two types of glycoprotein projections; rod shaped trimers of haemagglutinin (HA) and mushroom shaped tetramers of neuraminidase (NA). The HA is the major antigen that elicits antibodies which protect against death and clinical signs. These antibodies are HA subtype specific and can last

for periods greater than 35 weeks (Brugh & Stone, 2003). Influenza A virus is classified on surface glycoprotein which are designated as hemagglutinin (HA) and neuraminidase (NA). So far 16 HA and 9 NA have been identified in wild migratory birds and in poultry around the globe (Khan *et al*., 2012). The H9N2 were frequently isolated from China. The H9N2 subtype was isolated in Hong Kong from 2 children who are suffering from respiratory disease. In Hong Kong, it also showed that blood donors had neutralizing antibody against H9N2 suggestive that it had infection before blood donation. In one study it shows that H9N2 avian influenza virus can replicates in murine respiratory tract without prior adaptation. Recently the virus has been isolated from an immunocompromised patient. From northern area of Pakistan low pathogenic H9N2 was isolated from a poultry flock. Antibodies against serotype H9N2 was demonstrated in 7 wild bird species. The H9N2 infections in human were reported around Rawalpindi and Islamabad. In Netherlands in 2003 H7N7 causes infection in 89 human with one case of fatality (Ahad *et al*., 2013; Koopmans *et al*., 2004).

Poultry keeping is the dominant form of poultry production in the developing countries. Investment in poultry sector in Pakistan is about one billion US$ and egg availability is increasing 4% annually. Every family in rural areas and every 5th family in urban areas is associated with poultry production activities in one way or the other (Sadiq, 2004). Infectious diseases are one of the main factors constraining the poultry sector. In Pakistan, poultry industry is facing various diseases such as Newcastle disease (ND), Infectious bronchitis (IB), Infectious bursal disease (IBD), Egg drop syndrome (EDS), Hydropericardium syndrome (HPS) and Avian influenza (AI). These diseases are causing high economic losses in terms of high mortality, morbidity, stress, decreased egg production and hatchability all over the world including Pakistan (Swayne & Suarez, 2000). Avian influenza is a contagious viral disease, worldwide in distribution. It affects the chickens of all ages with variable morbidity and mortality. With the highly pathogenic AI viruses, morbidity and mortality rates are very high (50–89%) and can reach 100% in some flocks (Capua *et al*., 2000).

From northern area of Pakistan low pathogenic H9N2 was isolated from a poultry flock. Antibodies against serotype H9N2 was demonstrated in 7 wild bird species (Khawaja *et al.*, 2005). The H9N2 infections in human were reported around Rawalpindi and Islamabad (Bashir *et al*., 2003). Natural infection of human being infected with H7N7 virus associated with conjunctivitis can readily be transmitted either directly from birds to human or by seals. It has been reported antibodies against Influenza A (H3) and (H1) cross react with avian influenza virus A (H9N2) that infect human being. However sensitivity of HI test for detecting antibodies against H5N1 in human being against H6N5, H11N6, H13N6, equine A (H7N7) influenza viruses could be improved by using horse RBC instead of turkey RBC (Ahad et al., 2013). 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 (Huang *et al*., 2012).

From the literature reviewed, it was cleared that the sero-prevalence of Avian Influenza (AI) was first reported from the Province Khyber Pakhtunkhwa of Pakistan, especially from broilers. In the light of the importance of AI, the present study was designed to investigate the status of sero-prevalence of AI from Hazara Division of Khyber Pakhtunkhwa.

2. Material and methods

Study Area:

The present study was designed to isolate the sero-prevalence of Avian Influenza (AI) from Hazara Division of Pakistan. Three major districts were selected to be included in the study that is District Mansehra, District Abbottabad and District Haripur.

Samples collection:

Total numbers of 500 blood samples were randomly collected from unvaccinated backyard poultry population of Hazara Division of Khyber Pakhtunkhwa. Samples were collected throughout the year starting from July, 2019 to June, 2020. These samples were collected by the technical team of Poultry Research Institute Jaba, Mansehra. Blood samples were collected from the wing vein of the chickens. For the collection of sample, sterile syringe of 5ml was used and blood was collected in EDTA coated vacutainers tubes. These tubes were then kept in thermos cooler, containing ice packs, before shifting to the Poultry Research Institute Jaba, Mansehra.

Serum Separation:

Serum was separated from the collected blood samples by using centrifuge machine. For serum separation, the tubes containing blood were centrifuged at 2500rpm for 15 minutes at room temperature. After centrifugation, the supernatant fluid (serum) was separated and was collected in the serum collection tubes with the help of pipette.

Antibody Titration:

The tubes were labeled according to the tubes were sent to the National Reference Laboratory for Poultry Diseases (NRLPD) Islamabad, maintaining cold chain protocol, for Antibody Titration against Avian Influenza Virus (AIV) through the protocol described by (Allan et al., 1978). All serum samples were analyzed quantitatively with the different antigens H9, H7 and H5 in NRLPD, through Haemagglutination inhibition (HI) test (OIE, 2009; Zaman *et al.,* 2018) to evaluate the prevalence of AI in the selected Districts of Khyber Pakhtunkhwa.

Statistical Analysis:

Obtained results were subjected to the excel worksheet. Difference among the subtypes of Avian Influenza Virus, selected Districts and Months were expressed in the percentage to analyze the variance.

Results:

From the obtained results, it was found that out of total 500 serum samples, 59 (11.8%) samples had HI titer of MT (log_2)=6.00 to MT (log_2)=11.00 and was considered as positive for sero-prevalence of Avian Influenza in backyard poultry population of Hazara Division. Majority of the samples had HI titer of less than MT (log_2)= 5.00 and were considered as negative for sero-prevalence of Avian Influenza as shown in table 1.

Table 1. Total Sero-prevalence of Avian Influenza virus

	Total Samples	**MT(log_2)=0.00-2.00**	**MT(log_2)=3.00-5.00**	**MT(log_2)=6.00-8.00**	**MT(log_2)=9.00-11.00**
AIV-H5	500	342	147	02	09
AIV-H7	500	500	00	00	00
AIV-H9	500	219	233	11	37

Out of the positive samples, maximum sero-prevalence 48 samples (81.35%) were recorded antibodies for AIV-H9 subtype while 11 samples (18.64%) were recorded antibodies for AIV-H5 subtype and no antibodies were found for AIV-H7 subtype as shown in table 2.

Table 2. Subtype wise Sero-prevalence of Avian Influenza Virus

Subtype	**Total Samples**	**Positive**	**Prevalence Percentage**
AIV-H5	500	11	18.64%
AIV-H7	500	00	00
AIV-H9	500	48	81.35%

Area wise sero-prevalence was recorded and found that from total of positive samples, Maximum sero-prevalence 31 samples (52.54%) was recorded from District Mansehra, followed by 18 samples (30.51%) was recorded from District Abbottabad and minimum sero-prevalence 10 samples (16.94%) was recorded from District Haripur as shown in table 3.

Table 3. Area wise Sero-prevalence of Avian Influenza Virus

District	**Positive samples**	**Positive Percentage**
Mansehra	31	52.54
Abbottabad	18	30.51
Haripur	10	16.94

Month wise sero-prevalence was recorded and observed that from total of positive samples, maximum sero-prevalence 18 samples (30.51%) were found sero-prevalence in the month of January, followed by February in which 14 samples (23.72%) were found sero-prevalence, followed by March in which 12 samples (20.33%) were found sero-prevalence, followed by December in which 09 samples (15.25%) were found sero-prevalence and 06 samples (10.16%) were found sero-prevalence in the month of April, for Avian Influenza Viruses from July, 2019 to June, 2020 as seen in table 4.

Table 4. Month wise sero-prevalence of Avian Influenza Virus from Hazara Region

Month	Sero-prevalence	Sero-prevalence percentage
July	00	00
August	00	00
September	00	00
October	00	00
November	00	00
December	09	15.25 %
January	18	30.51 %
February	14	23.72 %
March	12	20.33 %
April	06	10.16 %
May	00	00
June	00	00

3. Discussion

Avian influenza Virus (AIV) is highly pathogenic infection of the chickens, Avian species as well as humans. Due to direct and frequent exposure to chickens, poultry workers remain at high risk to the infection (Zaman *et al*., 2018). The current study was designed to investigate the sero-prevalence of Avian Influenza (AI). According to the current study, total 11.8% serum samples were found sero-prevalence for AI. Out of these positive samples, 81.35% samples were had sero-prevalence for AIV-H9 subtype and 18.64% samples were had sero-prevalence for AIV-H5. No sero-prevalence was found for AIV-H7 subtype in this study. These results had an agreement with the results of (Aamir *et al.,* 2007), according to which Avian influenza H9N2 is prevalent in poultry industry in Pakistan since last 2 decades. Although it is low pathogenic yet it is the leading cause of the respiratory infections resulting in great economic losses in terms of reduced egg production, weight loss and high morbidity. (Aly *et al*., 2008; Feare, 2007) stated that since 2003 the H5N1 avian influenza virus has spread easily between various wild and domestic bird species in many locations worldwide. The wide spread of the H5N1 virus from the East in wild birds in 2006 including non-migratory species in many countries and infection of some domestic poultry has also been recorded.

According to the findings of the current study, the maximum sero-prevalence was recorded from District Mansehra (52.54%), followed by District Abbottabad (30.51%) and minimum sero-prevalence was recorded from District Haripur (16.94%). This may be due to the reason that District Mansehra is known to be a central District, an ideal location for commercial poultry production and majority of poultry breeders, layers and broilers farms on commercial level were present in the District, which on the other hand act as a risk factor for backyard poultry population of District Mansehra. These results were similar to the study conducted by(Muhammad et al., 2010) in which the prevalence of Avian Influenza Viruses were reported from Hazara Region of Khyber Pakhtunkhwa. According to which the maximum isolation was recoded from District Mansehra of Hazara Region. Another study conducted by(Naqash *et al.,* 2020) in which AI was isolated and they found majority of the isolation from District Mansehra as compared to District Abbottabad and Haripur of the Hazara Region.

Results of the current study showed that sero-prevalence was found in the month of December, January, February, March and April but no sero-prevalence was found in

the other months of the year. These results show that the AI isolation was recorded in the colder weather but no isolation was recorded in the summer season. Our results has an agreement with the results of (Zaman *et al*., 2018) in which maximum sero-prevalence of the infection in broilers of AI was recorded in winter as compared to summer. 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). Another study conducted by(Aly *et al*., 2008) in which data collected during the period from February to December 2006, it is obvious that phase I (early outbreaks from February to May) recorded the highest incidence (963 flocks/cases), while in phase II (summer season from June to October) 23 cases were reported, and in phase III (early winter during November and December) 38 cases were found. This illustrates that the climatic conditions are critical for increasing incidence of cases reported during the winter season. It is obvious that the geographic region, time of year, and the environment have a role in the incidence and distribution of the disease(Aly *et al*., 2008).

4. Conclusion

From the current study it was concluded that the antibodies of Avian Influenza Viruses were present in the backyard poultry population of Hazara Region especially in District Mansehra. Avian Influenza H9 subtype was most prevalent in the present study. Winter season was ideal for propagation of Avian Influenza Virus in the area.

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