

## Prevalence of Avian Influenza Virus in Poultry Population of District Mansehra

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### Abstract

The purpose of the present study was to isolate the Avian influenza viruses from poultry population of Mansehra district. For this purpose, 792 numbers of samples from Mansehra district were analyzed on Avian influenza virus (AIV) isolation. Total number of 70 isolates (8.83%) was recovered out of 792 swabs and tissue samples. The majority isolation of AIV subtypes H9N2 (n = 57; 7.19%) followed by H5N8 (n = 13; 1.64%) was isolated from different chicken species. From the positive samples, overall prevalence among different chickens species was as follows: broilers (n = 62; 88.57%), commercial layers (n = 05; 7.14%), backyard chickens (n = 03; 4.28%). Month wise isolation of AIV recorded and it was observed that from the total of positive isolates, maximum isolation was recorded in the month of January (44.28%) followed by February (21.42%), March (12.85%), December (11.42%) and April (07.00%) while the other months of the year were found no isolation of Avian influenza viruses from July, 2017 to June, 2018. From the results of the current study it was concluded that the commercial broilers were most liable for AIV infection especially in the moist atmosphere. Low pathogenic AIV is mostly found in the results of the current study which cause economic losses.

**Keywords:** Avian Influenza; Broilers; Commercial Layers; Backyard Chickens and Adoptability

### Introduction

The poultry sector is considered amongst the most vibrant sectors of agriculture in the country. Directly and indirectly, more than 1.5 million people are likely to have benefited in terms of income and employment from this sector [1]. Avian influenza (AI) is highly transmissible viral infection caused by different subtypes of influenza viruses. Based on the antigenic differences of the structural proteins, such as nucleoprotein (NP) and matrix protein (M1), virus is classified into three types: A, B and C. However, only type A influenza virus is further classified into different subtypes which are based on the antigenicity of two transmembrane glycoproteins termed, hemagglutinin (H) and neuraminidase (N). So far, amongst aquatic wild birds, in total sixteen H (H1- H16) and nine N (N1-N9) serotypes have been reported [2,3]. Avian influenza virus (AIV) being highly species-specific mainly infects birds. Wild aquatic birds like geese, waterfowl, shorebirds and wild ducks serve as the natural reservoirs for these viruses. Due to mutagenic nature of AIV, they pose consistent threat and may cross specie specific barriers [4]. Highly pathogenic avian influenza (HPAI) is an extremely contagious, multi-organ system disease of poultry leading to high mortality and is caused by some of the H5 and H7 subtypes of type-A Influenza virus. However, most Avian Influenza virus

strains are mildly pathogenic and produce either sub-clinical infections or respiratory or reproductive diseases in a variety of wild and domestic bird species [5]. In 1998 an outbreak of low pathogenic avian influenza virus (H9N2 subtype) has occurred in Iranian poultry industry. There is increasing evidence that H9N2 avian influenza viruses are endemic in chickens and other land based poultry, such as quail, pheasant, chukar and other minor domestic poultry in many Asian and European countries [6]. Typically, significant variation in prevalence status in different parts of the country is related with their geographical and seasonal parameters. Highest population of broilers was found affected with H9 in Quetta-Pakistan Similarly, in Faisalabad, 9.4% of population was observed affected with AI (H9) [7]. After reviewing the data from the previous studies, avian influenza virus is remained prevalent in the area, so the present study was designed to investigate the prevalence of avian influenza virus in the poultry population of district Mansehra.

### Materials and Methods

#### Sampling area, source and materials used

District Mansehra was selected to include in the study. District Mansehra is popular for the poultry production on commercial level because of its cool weather. A total of 792 samples, including backyard chickens, broilers, and commercial layers, were sampled throughout a period of one year from July 2017 to June 2018. Serum samples, swabs and tissues samples were collected in this period. The samples of the chickens from commercial poultry were collected from non-vaccinated chickens and the backyard poultry samples includes the birds of Desi, Aseel, Golden and Fayoumi breed chickens with mix age from 2 months to 56 months of chickens. Samples were collected by the technical team of Poultry Research Institute Jaba Mansehra, maintaining the cold chain protocol. From each affected flock, samples including trachea, lungs, spleen and cloacal swabs were collected from birds and blood samples from the live birds. Complete data of each flock was collected on a Performa. This included flock history of disease vaccination, treatment forward and backtrack record of infection. From total of 792 samples, 604 (76.26%) samples were collected from commercial broilers, 110 (13.88%) samples were collected from backyard chickens and 78 (9.85%) samples were collected from commercial layers. After collection, the samples were stored in a thermos cooler containing ice bags, maintaining the cold chain protocol, before shifted to Poultry Research Institute (PRI) Jaba Mansehra and through PRI Jaba these collected samples were sending to the National Reference Laboratory for Poultry Diseases (NRLPD), National Agriculture Research Center (NARC) Islamabad, on weekly interval for the isolation and identification of the virus.

#### Isolation of the virus

Tissues were processed and blended to prepare a 20% suspension in PBS (pH 7.2) solution containing penicillin ( $2 \times 10$  IU/L) Gentamycin ( $2 \times 10$  IU/L) and Streptomycin (200 mg/L). The whole material was centrifuged at 2000 rpm for 10 minutes at 10°C. The supernatant was filtered through 0.2  $\mu$ m syringe filter (Biotech) and inoculated into 9-days-old embryonated chicken eggs. The eggs were incubated at 37°C for 2 days. The Cloacal swabs were placed in the glycerol viral transport medium. The tubes containing swabs were vortexed, centrifuged at 2000 rpm and the supernatant was also processed for egg inoculation. After incubation of 2 to 3 days, the eggs were chilled for 3 to 4 hours and then the allantoic fluid was harvested for test through Hemagglutination (HA) activity. Hemagglutination positive allantoic fluids were additionally tested using reference antisera of AIV subtypes (H5, H7 and H9) by using standard protocols of Hemagglutination Inhibition (HI) test as described in literature [8].

#### Statistical analysis

For comparison among viral subtype, species and months the data recorded was compiled in excel worksheet and was expressed in percentage for assessing the difference among the selected parameters.

## Results

Total of 70 isolates (8.83%) were recovered out of 792 swabs and tissue samples. Positive samples were subjected to HI test for AIV subtype confirmation (i.e. H5, H7 and H9) by using their specific antigen. Avian Influenza (AI) subtypes H5N8 and H9N2 were isolated from the samples through in ovo inoculation. The results revealed that from total of 70 positive samples, 57 (7.19%) samples were found positive for AI subtype H9N2 and 13 (1.64%) isolates were found positive for AI subtype H5N8 (Table 1).

Strain	No. of Positive Samples	Positive percentage
H9N2	57	7.19%
H5N8	13	1.64%

**Table 1:** Strain-wise isolation of AIV in Hazara Division during 2017-18.

The chickens were considered AI positive if tissue samples, tracheal swabs or cloacal swabs tested were positive through HI assay. Among the positive samples, overall prevalence among different chickens species was as follows; broilers (n = 62; 88.57%), commercial layers (n = 05; 7.14%), backyard chickens (n = 03; 4.28%) (Table 2).

Specie	No. of Positive Samples	Positive percentage
Broilers	62	88.57%
Commercial Layers	05	7.14%
Backyard Chickens	03	4.28%

**Table 2:** Species-wise isolation of AIV in Hazara Division during 2017-18.

Month wise isolation of AIV recorded and it was observed that from the total of positive isolates, maximum isolation was recorded in the month of January (44.28%) followed by February (21.42%), March (12.85%), December (11.42%) and April (07.00%) while the other months of the year were found no isolation of avian influenza viruses from July, 2017 to June, 2018 (Table 3).

Month	No. of Positive Samples	Positive percentage
August	00	00
September	00	00
October	00	00
November	00	00
December	08	11.42%
January	31	44.28%
February	15	21.42%
March	09	12.85%
April	07	07.00%
May	00	00
June	00	00
July	00	00

**Table 3:** Month-wise isolation of AIV in Hazara division during 2017-18.

### Discussion

AIV is responsible for economic losses in the form of mortality, morbidity and low production was reported in Pakistan in recent years, it is of utmost importance to isolate the AIV from backyard as well as commercial poultry of Pakistan. The present study was designed to isolate the AIV from poultry population of District Mansehra. Ayaz, *et al.* [9] indicated the existence of Avian Influenza H9N2 in Hazara Region of Pakistan especially in the Native poultry and Desi Golden chickens in isolated areas which was alarming. In the present study, overall isolation of AIV from District Mansehra from July 2017 to June 2018 was 8.83% out of the 792 number of randomly collected samples in the present study. According to the literature Pakistan has experienced several AI outbreaks of AIV serotypes including H7N3, H9N2 and H5N1 were reported from Pakistan since 1995 [10]. From 2003-04 an epizootic of high pathogenic avian influenza H7 N3 and later on H5N1 during 2006-08 affected the major poultry of the region [9].

In the present study, the majority isolation of AIV subtypes H9N2 (7.19%) followed by H5N8 (1.64%) was isolated from different chicken species of Mansehra District. These multiple episodes of infections with LPAI (low pathogenic) and HPAI (high pathogenic) outbreaks caused massive economic losses in the poultry industry of Pakistan and subsequently directed the initiation of AIV surveillance throughout the country. The present study was conducted to determine the sero-prevalence of AIV subtypes AIV H5, AIV H7, AIV H9. Isolation and identification of these viral subtypes in various non vaccinated bird species was performed throughout the year 2013 [11]. Similarly, Fournié, *et al.* [12] isolated H9N2 from water sources of live poultry markets. H9N2 were mostly isolated in the present study from samples collected in colder months of the year i.e. December, January and February.

In the present study, majority effected species of chickens, from the positive isolates were commercial broilers (88.57%) followed by Commercial Layers (7.14%) and Backyard Chickens (4.28%). These results have an agreement with the results of Kausar, *et al.* [11] in which the distribution of AIV among different non-vaccinated bird species remained as follow; broilers (n = 39; 93%), desi poultry (n = 2; 4.7%) and 3 day old layer (n = 1; 2.3%). Furthermore, 6.5% of the AIV H9N2 samples were confirmed by viral culture isolation (also positive for HA antibody). In context with the rural/backyard poultry shows the endemicity of the virus. However, results found in another study conducted by Muhammad, *et al.* [5] revealed that prevalence of the AIV in Hazara region of North West Frontier Province of Pakistan was highest in non-descript layers (16.7%) followed by broilers (9.0%), breeders (6.8%) and layers (4.0%) [5]. In resemblance with the results of our study, the study conducted by Sharif, *et al.* [13] revealed that 53% AIV were isolated from the non-vaccinated commercial poultry (broiler), 7% from non-vaccinated wild birds and 40% were reported from backyard poultry during the whole year [13].

From the results of current study in round the year, month wise isolation of the AIV revealed that maximum isolation from the positive isolates was recorded in the month of May (41.79%) followed by February (22.38%), December (11.94%), April (11.94%) and June (11.94%). These results were closely related with the results of Kausar, *et al.* [11] according to which month wise prevalence of the AIV H9N2 was as follows (May, n = 15; 36%), followed by (January, n = 8; 19%) and (December, n = 7; 17%). Similarly, in the study of Fereidouni, *et al.* [14] the highest incidence of AIV was in the month of February and November. Another study conducted by Monne, *et al.* [15] in which samples collected from the first wave of the LPAI epidemic (late September 1999 to January 2000) and from the second wave (August 2000 to February 2001), these results were closely related with the results of the present study however due to the moist atmosphere, some isolation may be recorded in the summer season in various studies.

### Conclusion

From the results of the current study it was concluded that the commercial broilers were most liable for AIV infection, while some isolates were also be recorded from the native poultry of District Mansehra. Though the isolates from native poultry were low pathogenic type but still the risk of mutation of the Virus prevails which is alarming. The AIV mostly isolated in the months when temperature was low which cause high economic loses specially in winter season.

### Author's Contribution

Naqash Khalid conducted the research and contributed in the collection of data and write up. Muhammad Ayaz supervised the research work, study designing, write-up of research article and helped at each step of the work. Hajira Mehmood and Maila Syed contributed in sample collection and data recoding. Muhammad Athar Abbas contributed in the Laboratory diagnosis. Yasir Amin and Malik Mohsin Ali contributed in the sample collection.

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