ABSTRACT

Prevalence of Avian Influenza (AI) in poultry population in Hazara region of North West Frontier Province of Pakistan was studied in broiler breeder stocks (4 millions), layers (0.5 millions), broilers (2.5 millions) and non-descript captive layers (0.5 million), for prevalence of AI viruses (AIV) during a period of one year. Five hundred flocks were sampled between May 2004 through April 2005. The isolation and identification was conducted. Virus was isolated using embryonating eggs and the allantoic fluid of egg containing dead or dying embryo was recovered and tested for HA (Haemagglutination) activity. The virus was typed through (HI) Haemagglutination inhibition test using antisera. The data were analyzed statistically through chi square test. An incidence of AIV in 8.8 % of the flocks was recorded; highest in non-descript layers (16.7 %) followed by broilers, breeders and commercial layers (9%, 6.8 % and 4 %, respectively). Prevalence of H7 subtype was highest in non-descript layers (13 %) followed by 5 % in breeders, 4 % in layers, 3.8 % in broilers. H9 was highest in broilers (5%) followed by 1.8 % in breeders, 0.25 % in layers. No highly pathogenic Avian Influenza (HPAI) infection was recorded during study period. The economic loss due to low pathogenic Avian Influenza (LPAI) was estimated to as Rs. 2.2 billion per annum (US$ 1 = Pak Rs. 62.6). It was concluded that AI was found in low pathogenic form causing huge population of the region was probably the predisposing factor of transmitting the disease to the commercial flocks.

Key Words: Avian Influenza, AI, economics, Pakistan, serotypes

INTRODUCTION

Hazara region, an area of 17000 sq km, is northeastern region of KP, Pakistan comprises five districts. Over four million broiler breeder stock is annually reared in this region, in addition to 2.5 million broilers, 0.5 million commercial layers and 3.00 million rural poultry. This region is also rich in wild fauna and also lies on the route of migratory birds specially the ducks, coming from Russia and stay at Tarbela Lake during their migration. This scenario is quite suitable for the propagation of Avian Influenza viruses in the area. 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 (Swayne and Suarez , 2000; Suarez and Schultz, 2000; Compiletti et al. 2004; Tollis and DiTrani, 2002; Suarez, 2000; Fouchin et al., 2004). Capua et al.,(1999) reported the presence of Avian Influenza H7N1 outbreak in Italy, where low pathogenic strain causing 2-10 percent mortality mutated to high pathogenic Avian Influenza infection resulting in a mortality of 70-90 %. Some of the major outbreaks occurring in other places of the HPAI of H7 or H5 serotypes in recent years included Mexico (H5), Pakistan (H7), China (H5), Australia (H7), Vietnam (H5), Hong Kong (H5), North Korea (H7) and Thailand (H5) (Yamaguchi, 2000).
Avian Influenza was first recognized in Pakistan during 1995 when highly pathogenic outbreak destroyed the broiler breeder stocks in Hazara region killing two million birds. The cause of the outbreak was H7N3 subtype. Another outbreak of HPAI H7 serotype in commercial layers in southern parts of the country in 2003 resulted into heavy losses (60-80 % mortality). The disease appeared in Hazara region among broiler breeders in April 2004 resulting into high mortality ranging between 40-50 percent. Since than the HPAI serotypes have mutated into LPAI form but the disease is still endemic in the area. The present study was, therefore, designed to study the prevalence and economic importance of Avian Influenza viruses in poultry population of Hazara region.

MATERIALS AND METHODS

Sampling source, materials and data collection: Three main districts of Hazara region namely, Mansehra, Abbottabad and Haripur were included in this study. A total of 500 flocks, including broilers, broiler breeders and layers, suffering from respiratory tract infection, were sampled over a period of one year between May, 2004 through April, 2005. Isolation and identification were conducted in National Reference Laboratory for Poultry Diseases, National Agricultural Research Center, Islamabad. From each affected flock samples including trachea, lungs, spleen, and local swabs were collected from birds and 10 blood samples from the live birds. Complete data of each flock was collected on a proforma. This included flock history of disease vaccination, treatment forward and backtrack record of infection.

Virus isolation: The organs were brought to the laboratory in ice boxes, the swabs were placed in transport medium (brain-heart-infusion broth, Oxoid, UK). This material was processed for virus isolation using embryonating eggs as recommended by WHO (1982) and further typed using the method described by Beard (1989). The organs were cooled, saturated in phosphate buffer saline, centrifuged at 800 x g for 10 minutes at 10oC. The supernatant was passed through 0.2 µ filter and the filtrate was inoculated into allantoic cavity of 9 days old embryonated egg. The eggs were incubated at 35 to 37°C for 3 to 5 days. The allantoic fluid of egg containing dead or dying embryo was recovered and tested for Haemagglutination (HA) activity. At the end of the first incubation, if there was no HA activity, the same was subjected to second inoculation and incubation for five days. A negative to HA at second incubation was considered negative and discarded. The HA positive allantoic fluid was subjected to Haemagglutination inhibition (HI) test using Newcastle disease Virus (NDV), Avian Influenza (AIV) H7 and H9 antisera using standardized positive and negative control sera.

Total economic losses were grouped into five categories of mortality, production losses, low hatchability, culling of day-old chicks and treatment cost. The data on total population of a group, current price per bird, per egg and day old chick, and cost on treatment was considered.

Statistical Analysis

The data was maintained in computer based files and analyzed through SPSS-10 software adopting the procedures recommended by Steel and Torrie (1982). Chi square test was used for comparing means for various poultry types, i.e. broilers breeders, layers, rural poultry. Seasonal incidence of the disease was expressed in percentages for assessing the financial losses.

RESULTS AND DISCUSSIONS

Out of the 500 poultry flocks examined, 8.8% were found positive for AI viruses as shown in Table I. Prevalence of the disease was highest in non-descript layers (16.7%) followed by broilers (9.0%), breeders (6.8%) and layers (4.0%). Among the Avian Influenza isolates, highest incidence of H7 was recorded in non-descript layers (13%), followed by breeders (5%), commercial layers (4%) and broilers (3.8%). Incidence of H9 was found highest in broilers (5%) whereas 0.25% broiler flocks were found carrying both the types. The area wise distribution of Avian Influenza entity is summarized as given under Table II. The data indicates the highest incidence of AIV in flocks of district Abbottabad (9.75%) followed by Mansehra (8.4%) and Haripur (6.8%). Highest incidence of H7 (5.7%) was recorded in broilers of district Abbottabad. AIV H9 was highest (5.4%) in district Mansehra where as combine infection of H7 and H9 was recorded only in poultry of Mansehra district. The data indicates a total loss of 2.2 billion rupee annually due to LPAI in poultry population (Table 2). The highest loss of Rs 800 million in breeder stocks and 150 million in broiler birds due to direct mortality whereas loss of Rs 400 each of low hatchability and culling of day old chicks have been indicated.

Figure 1 shows the highest incidence of AIV H9 (47% of flocks) in Hazara region during July, followed by 20 percent during May and June and less than 7% during rest of the months. The highest incidence of AIV H7,
recorded as 29% of the flocks during the month of October, followed by November and December as 9% approximately. The combine incidence of H7 and H9 was recorded in 20% of the flocks of Hazara region during the month of May only.

Table-I Prevalence (%) of Avian Influenza virus (AIV) across various types of poultry populations

<table>
<thead>
<tr>
<th>Type of poultry</th>
<th>Positive (%)</th>
<th>H7**</th>
<th>H9**</th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breeders</td>
<td>6.77 b</td>
<td>5.08 b</td>
<td>1.69 b</td>
<td>0.00</td>
</tr>
<tr>
<td>Broilers</td>
<td>9.05 b</td>
<td>3.77 b</td>
<td>5.03 a</td>
<td>0.25</td>
</tr>
<tr>
<td>Layers</td>
<td>4.00 b</td>
<td>4.00 b</td>
<td>0.00 b</td>
<td>0.00</td>
</tr>
<tr>
<td>Non-descript layers</td>
<td>16.67a</td>
<td>16.67a</td>
<td>0.00 b</td>
<td>0.00</td>
</tr>
<tr>
<td>District</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manshehra</td>
<td>8.43 a</td>
<td>2.41a</td>
<td>5.42a</td>
<td>0.60</td>
</tr>
<tr>
<td>Abbottabad</td>
<td>9.75 a</td>
<td>5.69a</td>
<td>4.06a</td>
<td>0.00</td>
</tr>
<tr>
<td>Haripur</td>
<td>6.82 a</td>
<td>4.55a</td>
<td>2.27a</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Percentages in the same column within the group followed by different letters are significantly different (P<0.05)

** Virus strains

Table-II Economic losses due to low path AIV infection in commercial poultry of Hazara region.

<table>
<thead>
<tr>
<th>Poultry Type</th>
<th>Mortality</th>
<th>Production Loss</th>
<th>Low hatchability</th>
<th>Culling of day old chicks</th>
<th>Treatment cost</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>(Rs.m)</td>
<td>%</td>
<td>(Rs.m)</td>
<td>%</td>
<td>(Rs.m)</td>
</tr>
<tr>
<td>Broiler Breeder</td>
<td>4.0</td>
<td>20 800.0</td>
<td>15 93.0</td>
<td>10 400</td>
<td>10 400</td>
<td>64.0</td>
</tr>
<tr>
<td>Layers</td>
<td>0.5</td>
<td>15 113.3</td>
<td>25 9.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Broilers</td>
<td>8.0</td>
<td>25 150.0</td>
<td>20 90.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>12.5</td>
<td>- 961.3</td>
<td>- 192.0</td>
<td>- 400</td>
<td>- 400</td>
<td>108.0</td>
</tr>
</tbody>
</table>

* US$ 1 = Rs.62.6

Prevalence of AIV across poultry types

In the present study, highest incidence of AIV was recorded in non-descript poultry population kept at commercial forms followed by broilers, broiler breeders and commercial layers. The affected flocks showed low production. It was observed that mortality due to AIV in breeder was 20% and drop in egg production was 15%. On the other hand 25% mortality was recorded in broiler flocks due to H7 or H9 infection During the study period no highly pathogenic outbreak was recorded which indicates that virus with low pathogenicity had been circulating in the region. Swayne and Suarez (2000) reported that most AIV strains are mildly pathogenic and produce either sub-clinical infections or respiratory and/or reproductive disease in a variety of domestic and wild bird species. While investigating pathogenicity of the disease, no differences in lesion distribution patterns or in sites of AIV replication were evident to explain the variation in mortality rates for different HP AIVs, but HP AIVs that produced the highest mortality rates had more severe necrosis in heart and pancreas (Swayne, 1997). The ability of individual HP AIVs to produce low or high mortality rates could not be explained by changes in sequence of the hemagglutinin-connecting peptide alone, but probably required the addition of other undetermined genomic changes. The highest incidence of AIV in 16.7 percent flocks of nondescript poultry is alarming. These birds, usually the crossbred progeny of different rural breeds, are kept in farms up to the age of 8-10 weeks and than sold out in villages and towns. People keep these birds for egg production on non-scientific lines. By carrying Influenza viruses, these birds may be a major source of spread of virus in the region.

Seasonal and area effect

Location had no significant effect on prevalence of the disease (Table I), however, higher incidence was recorded in Abbottabad, followed by Manshehra and Haripur. The difference may be due to the cooler weather than the other two districts. The colder weather helps the virus in existing longer in infected materials i.e., droppings, poultry shops, wastes and water.

Incidence of H7 was highest in district Abbottabad and H9 was highest in Manshehra. The distinct impact of weather was observed in this study. Temperature varies from -5 to 35 °C in the region during winter to summer respectively. The extremes of the weather remain for short period and weather remains pleasant for most of the year. Precipitation up to 15 mm is received mostly in monsoon and winter fall which maintain the weather pleasant. Relatively high incidence during warmer month may be due to the reason that the farming is increased to its maximum during summer months and activity of wild fauna as well as migratory birds is also at their maximum,
hence, possibility of the spread of virus is increased during this season. All Influenza virus subtypes and most HA/NA combinations have been detected in the bird reservoir and poultry, whereas relatively few have been detected in other species (Olsen, et al., 2006). Although many wild bird species may harbor Influenza viruses, birds of wetlands and aquatic environments such as the Anseriformes (particularly ducks, geese, and swans) and Charadriiformes (particularly gulls, terns, and waders) constitute the major natural LPAI virus reservoir (Webster et al., 1992).

**Economic losses**

Significant economic losses may be directly attributed to this disease in the form of mortality, treatment cost, low hatchability and high culling rates. Lister (2004) reported a loss of US $ 350 million of 1983-84 during the Pennsylvania outbreak destroying 17 million birds; 200 million Euro of 1999-2000 Italy outbreak destroying 14 million birds; and 750 million euro of Netherlands outbreak destroying 30 million birds. Wright (2004) reported a loss of US $ 250 million as a result of an outbreak in Chile during the year 2002 destroying 12000 broiler breeder and depopulation of rest of the flocks reducing production up to 40 %. Qureshi (2000) reported a loss of 8 billion as a result of HPAI outbreak during 1995 destroying 4 million breeder stocks in Pakistan. He also recorded production losses of 40-80 % as a result of LPAI infection in broiler flocks in Pakistan. Present study reports low pathogenic form of the diseases with relatively lower losses. The economic losses due to low pathogenic Avian Influenza in broiler breeder birds are multifaceted. It starts from direct mortality to poor growth resulting into reduced production and poor quality eggs. The persistent viraemia affects the health of the males by causing fever, reducing feed intake, affecting spermatogenesis and libido. Sluggish, females not intend to receive males, hence, poor fertility leads to low hatchability and increased culling of day old chicks. The losses in broiler birds are due to respiratory complications, impaired immunity, vaccine reactions and suffocation due to pulmonary ascitis. Poor feed conversion and rejection of diseased birds is another way of loss in broiler production.

Fig. 1. The figure indicates that the highest incidence of AIV (47% of flocks) was recorded in warmer month of July, followed by 40 percent in May and 33% during October. Incidence had been less than 20% in rest of the months.

![Seasonal Effect on Avian Influenza Prevalence](image)

**CONCLUSION AND RECOMMENDATIONS**

It was concluded that AI is found in low pathogenic form causing huge economic losses and the viruses associated are H7 and H9. Cold stress is identified as the predisposing factor and rural poultry is found as a risk factor for spreading the disease in the region.
REFERENCES


