

Short Communication

**RETROSPECTIVE STUDY ON HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1
OUTBREAKS DURING 2006 -2008 IN ICT, PAKISTAN**

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ABSTRACT

Here we present the findings of the retrospective data collected from April 2006 to June 2008 regarding highly pathogenic avian influenza virus H5N1 outbreaks in commercial poultry farms. The results showed that most of these outbreaks affected 45 weeks old layers and breeder farms. The cumulative morbidity was 57-95% and mortality was 5-43%. The highest culling percentage was recorded in layers (95.29%). Mortality, morbidity, case fatality was significantly ($P < 0.05$) higher in layers than breeders and broilers. While culling rate was higher in broilers. Seasonal impact was recorded on incidence of outbreaks in commercial poultry farms in the study area i.e. most of the outbreaks occurred in the months of April, May and June in the year 2006, 2007 and 2008. It was concluded that the enormous losses due to 2006 -2008 outbreaks were due to high rate of mortality and morbidity mostly in adult birds especially in layer and breeder farms when they were in or near to egg laying stage of life. To combat and prevent future epidemics, a regular sero-surveillance programs should be conducted on regular basis to identify HPAI viral strains in Pakistan.

Key words: HPAI, ICT, Pakistan, surveillance, outbreaks, Poultry.

INTRODUCTION

Avian influenza (AI) outbreaks in poultry sector imparted huge economic losses throughout the world due to high mortalities, mass culling and international trade restrictions (Stegeman *et al.*, 2004; Brown, 2010). These outbreaks are caused by the avian influenza viruses, classified as low or highly pathogenic viruses (HPVs). Highly Pathogenic Avian Influenza (HPAI) is caused by the single stranded RNA viruses from the family of Orthomyxoviridae (Holmes, 2010). Asian lineage HPAI H5N1, first discovered in domestic geese in China in 1996 (Duan *et al.*, 2008). The recent spread of HPAI type A (H5N1) viruses globally among poultry industry is unparalleled. AIV infections can be devastating, causing huge losses worldwide in poultry industry (Capua and Alexander, 2002).

According to Food and Agricultural Organization (FAO) (FAO, 2004) four sectors of the poultry production systems exist (i.e. Industrial integrated production system with the high level biosecurity; commercial production system with high to moderate biosecurity level; commercial production system with minimal to low biosecurity and backyard production system of poultry with minimal biosecurity). Pakistan has all the four sectors of production system mentioned above. In Pakistan, poultry sector contributes 35% of total livestock production. It's the second major

industry, with turnover of US\$ 2 billion per annum. It has proved to be the most vibrant segments of agriculture in Pakistan. 1.5 million people directly or indirectly are estimated, having been benefitted in terms of income and employment from this sector (Naeem *et al.*, 2007).

AI outbreaks on the commercial and backyard poultry sector have devastating impact on poultry industry in Pakistan and numerous outbreaks of AIV i.e. subtype H7N3 (1995, 1998 and 2001-2002 outbreaks), H9N2 (1998 outbreak, which is currently endemic in the country) and H5N1 (2006-2008 outbreaks) (Naeem *et al.* 1999; Abbas *et al.* 2010; Aamir *et al.*, 2009; Siddique *et al.*, 2012) have been reported. In spite of endemicity and huge economic losses in this sector from AIV, particularly subtype H5N1 and H9N2, there is lack of epidemiological information regarding these outbreaks in Pakistan. Emergence of highly pathogenic avian influenza (HPAI) H5N1 virus in Pakistan in April 2006 to June 2008 caused huge economic losses to poultry farms and presented a potential risk to public health. The epidemiological information from this study will be practical to plan prompt strategies for control and prevention of HPAI and LPAI virus infections in future.

MATERIALS and METHODS

Study Design: A cross-sectional survey was conducted in Islamabad Capital territory (ICT) in order to assess the

trends and patterns of HPAIV H5N1 outbreaks that occurred during the period of 2006 -2008. We used and analyzed the data collected during active disease surveillance by National reference Laboratory for Poultry Diseases (NRLPD) at Animal Health program, NARC, Poultry Research institute (PRI) and livestock department of ICT.

Study Population: Birds in all commercial poultry farms (broiler, breeder and layer) of Islamabad constituted the target population. All broilers (1000000); breeders (600000) and layers (1500000) in 170 commercial poultry farms of ICT were selected as study population.

Sampling technique: Selection of samples was carried out by multistage sampling. At first stage of sampling, all (12) union councils of ICT were taken as a sampling frame whereas one union council was taken as sampling unit. At second stage, all (43) villages/jurisdictions of 4 selected union councils were taken as sampling frame whereas one village was taken as sampling unit. At third stage all birds (187520) in 35 commercial poultry farms (broiler, breeder and layer) of 8 selected villages were taken as sampling frame whereas one bird was taken as sampling unit.

Sample size was calculated according to recommendations of Thrusfield (2005).

$$n = \frac{1.96^2 P_{\text{expected}} (1 - P_{\text{expected}})}{d^2}$$

n = sample size required

P_{expected} = prevalence expected

d = Required absolute precision

By specifying expected prevalence and desired absolute precision as 5% each, the estimated/ desired sample size was n = 73. 73 birds were selected from each farm for confirmation of H5N1 outbreak in that farm. Consequently, 70 farms were selected from 08 villages in 4 UCs of ICT.

Randomization and data collection: Out of total 12 union councils, 4 union councils including Kuri, Tarlai Kalan, Sihala and Chirah were randomly selected. From these four union councils, a total of 8 villages (2 from each union council) including Chak Shahzad, Kuri, Ara, Cherah, Dhok Kazim, Tarlai Kalan, Jandala and Sihala (Fig-1) were randomly selected for present study. Required information about birds (broilers, layers & breeders) of all commercial poultry farms was recorded through a phenotypic questionnaire (available on request). One Performa was filled for one farm.

Temporal and spatial distribution of samples: Temporal and spatial distribution of samples was mapped using the geographical coordinates of the outbreak locations and boundary map of Islamabad (Fig-1). For temporal distribution, first detection date of the outbreaks was used (Fig-2).

Statistical Analysis: All data collected at the farm level was entered into an excel spreadsheet for data summary and management (Excel 2010, Microsoft Corporation) and analyzed using STATA-11 (STATA Corporation, Texas). The incidence rate for morbidity, mortality, case fatality and culling rate for chickens of each production system was calculated. Descriptive statistical analysis of the data was performed using SPSS software (version 16.0).

RESULTS AND DISCUSSION

Table-1 shows the farms profile, including types of chickens reared (i.e. layers, breeders and broilers), poultry production system categories, age of birds and incidence rates of morbidity, mortality, case fatality and culling rate in commercial poultry farm chickens infected with HPAIV (H5N1). Whereas Figure 1 portrays the spatial allocation of the different chicken farms infected in the study area belonging to FAO poultry production system 2 and 3.

Epidemiological tools were used to investigate the trend and patterns of morbidity, mortality and case fatality for these outbreaks. Data analysis from different layer farms showed average flock size of 5097 birds in ICT area from April 2006 to June 2008. Most of the outbreaks occurred in 45 weeks old birds. The mean cumulative morbidity and mortality rates were 28.27% (range 57-95%) and 8.98% (range 5-43%) respectively. The highest culling percentage recorded in layers (95.29%), while, the average culling percentage in broilers was 83.69 %. This was practiced to control and contain the disease spread in the study area as reported by respondents. Mass culling was enforced and implemented by Government authorities and ban was imposed on the bird movement and sale to control the spread of outbreak. These results were in accordance to those of (Sims *et al.*, 2002; Hulse *et al.*, 2005). The morbidity rate was 63-96%, mortality was 4-37% and culling percentage was 62.96% in breeder farms while morbidity and mortality rate of 81-88% and 12-19% with culling percentage of 95.29% was recorded in layers respectively. The case fatality rate in the present study was much higher as compared to the previous studies (Vong *et al.*, 2006) reporting 29% case fatality. The higher morbidity, mortality and case fatality rates in our study may be attributed to the changes in the virulence of prevalent H5N1 strains or susceptible production system in Pakistan. Here most of the poultry production systems are of category 3 and 4 according to OIE standards. Other factors influencing higher case fatality rate were high density of poultry farms in the study area contributing as an important factor in spread of disease. These results are in line with the findings of (Chaudhry *et al.* 2015). Our findings revealed the highest morbidity and mortality rates in layers followed by broilers and least in breeders.

These findings are very much aligned with the results of (Biswas *et al.*, 2009) reporting the same pattern and trend of HPAI outbreaks in poultry farms. The highest morbidity rate recorded in the present study for nondescript layer farms were strongly in consent with those of (Ayaz *et al.*, 2010). It may be due to the fact that no vaccination is practiced in backyard poultry against avian influenza. High morbidity, mortality and case fatality associated with present outbreaks of HPAIV H5N1 suggest that the virus is becoming even more dangerous for poultry birds, resulting in even higher economic losses to the poultry industry. Appropriate mitigation strategies are required and a regular surveillance activity is needed to monitor the activity and dynamics of the virus in poultry birds.

Investigating 2006-2008 outbreaks due to HPAI H5N1 most of the cases were recorded in the month of April, May and June in layers, breeders as well as broilers farms in the year 2006, 2007 and 2008. It may be due to the higher rate of migratory and wild birds activities in these months and most of birds having breeding season. Therefore, in search of proper nesting places, the commercial farms remains the best choice for sparrows, pigeons and other wild bird species that may play a vital role in the spread of influenza viruses. The temporal distribution of outbreaks has also been depicted in Figure 2 that occurred during March 2006 and May 2007. Majority of the outbreaks was observed during late spring to early summer during 2006 and 2007. The data suggests a seasonal pattern of outbreaks of HPAIV H5N1 in these flocks. Though this study was limited to ICT only, but such a seasonal pattern is important consideration for designing a national control strategy.

Lack of awareness regarding the disease amongst the farmers, free movement of scavenging birds adds on to the problem. Duck keeping with backyard chicken is reservoir for viral strains asymptotically. The highest exposure rate to HPAI and other LPAI virus make them highly susceptible. The higher morbidity and

mortality rates in broiler farms may be attributed to the low biosecurity measures in these farms. The present study thus highlights the need for an improvement in the levels of biosecurity in local poultry farms. Majority of infected broiler farms were dependent largely upon temporary workers. They were considered having a connection chain between the commercial poultry and infected backyard flocks and vice versa. Same workers were working at the same day in more than one farm visiting other farms in the same premises without practicing any biosecurity measures.

Higher mortality rate in layers may be due to the prolonged exposure as compared to broilers as reported by (Alexandar *et al.*, 1995). The higher mortality rate may also be due to reoccurrence of outbreaks in the study area and exposing the birds again to the HPAI makes them more susceptible to be affected. Low morbidity rate in breeder farms in the present study was in agreement to the findings of (Ayaz *et al.*, 2010).

Exploring age of birds at the outbreak showed that most of the outbreaks in layers and breeders occurred at 5-45 week of their life stage while in broilers mostly outbreaks were recorded during 35-45 days. It might be due to the increasing susceptibility of birds with increase in age, due to the negative associated impact on immune status and housing stress, ultimately rising the exposure opportunity for viruses (Alexandar *et al.*, 1995).

The present study highlights the impact of H5N1 on the local poultry sector in ICT. The impact of HPAIV in terms of high morbidity and mortality, rapid transmission, and transboundary nature requires a regular surveillance of poultry birds for this disease. The present study highlights these facts. In addition, a seasonal pattern may be present in these outbreaks. The farm biosecurity may be critical in preventing the transmission of these outbreaks. Overall, the study suggests a number of possible policy issues for the control of HPAIV infection in poultry sector.

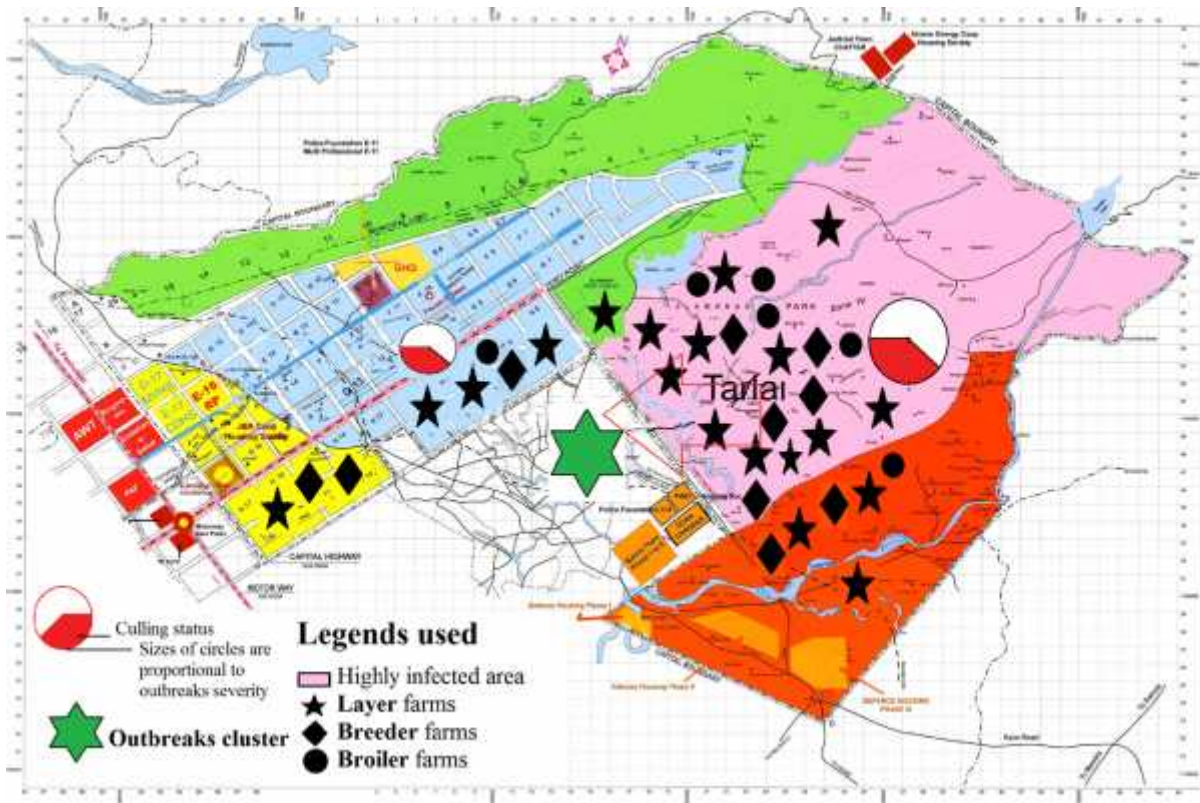


Fig 1. Spatial distribution of April 2006-May 2007 HPAI subtype A H5N1 outbreaks in commercial poultry farms in ICT, Pakistan.

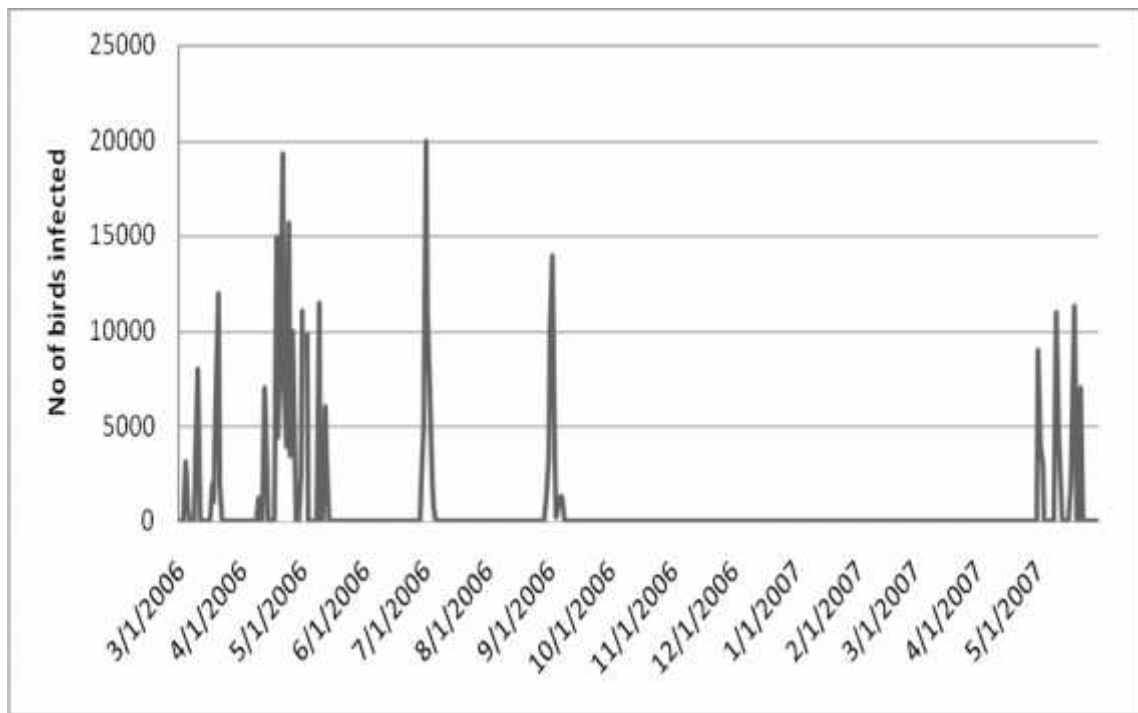


Fig-2. Temporal distribution of HPAI subtype A H5N1 March 2006-May 2007 outbreaks in ICT commercial poultry farms in Pakistan.

Table 1. Epidemiological characteristics of HPAI subtype H5N1 2006-2007 outbreaks and affected farms in ICT Pakistan.

Farm status	No of farms with outbreaks	Mean flock size (Min-Max)	Mean age in weeks (Min-Max)	Morbidity in number	Mortality in number	Case fatality in number	Total birds culled (Min-Max)
Layers	19	5097 (1600-14000)	29(4-45) weeks	1441	458	653	74143 (11600-1040)
Breeders	10	4910 (1500-15000)	30(5-45) weeks	799	200	271	38790 (1200-10569)
Broilers	6	6928 (3000-12000)	42.5(35-45) days	506	94	119	34791(2640-9752)

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