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The Author

**ABSTRACT**

The purpose of this study is to determine the prevalence of avian influenza infection (bird flu) in Chittaging area and also in Bangladesh throughout the year. The data on the outbreak of Avian influenza was collected from the Department of Livestock Services (DLS). The highest mortality rate was found in Potia (23.7%) and lowest in Hathazari (2%). The mortality of layer bird was highest (13.4%) than broiler (1.3). There was also seasonal variance. Highly pathogenic (H5N1­) avian influenza viruses have swept through poultry populations across Bangladesh, and the parts of Chittagong. Their outbreaks are historically unprecedented in scale and geographical spread. Their economic impact on the agricultural sector of the affected region has been large. Influenza viruses are genetically unstable and their behaviors cannot be predicted, so the risk of further human cases persists. The human health implications have now gained importance, both for illness and fatalities that have occurred following natural infection with avian viruses, and for the potential of generating a re-assortment virus that could give rise to the next human influenza pandemic.

**Key words:** Avian influenza, Prevalence, Layer and Broiler, Chittagong.

**CHAPTER-I**

**INTRODUCTION**

Avian influenza (AI) is a zoonotic viral disease of birds that caused by the Avian Influenza Virus. Of all the viral diseases, the highly pathogenic avian influenza (HPAI) is considered as top ranking viral disease of poultry The economy of Bangladesh is agro based. About 21.77% of Gross Domestic products (GDP) come from agriculture sector of which livestock alone share 7.23%. **(*BBS, 2005-2006*)**.. This viral disease is responsible for serious economic losses every year to the poultry industry all over the world. Free-living birds may carry influenza viruses without becoming ill due to a natural resistance. In order to achieve the Millennium Development Goal (MDG), Bangladesh is committed to develop the poultry sector. The total poultry population, both backyard and commercial, accounts to approximately 246 million, providing 5400 million pieces of eggs annually and nearly 15% of total animal protein. This sector employs about 5 million people of the country and has experienced a long-term growth rate of about 4.5%, which is one of the highest in the economy and is believed to have accomplished a silent revolution in Bangladesh (***BLRI, 2008*).**

It is known that wild waterfowl present as a natural reservoir for these viruses and can be responsible for the primary introduction of infection into domestic poultry. The very virulent viruses cause highly pathogenic avian influenza (HPAI) with mortality in poultry as high as 100%. In the whole world there have been only 19 reported primary isolates of such viruses from domestic poultry since 1959. A severe epidemic occurred in Italy in 1999/2000 causing 413 outbreaks with 16 Million birds affected. Other AI viruses cause a much milder disease (low pathogenic avian influenza, LPAI). Clinical signs are much less evident or even absent and mortality is much lower.

Sometimes secondary infections or environmental conditions may cause exacerbation of LPAI infections leading to more serious disease. Evidence suggests that certain avian influenza virus subtypes of low pathogenicity may transformed into high pathogenicity, after circulation for some time in a poultry population, mutate into highly pathogenic virus strains

Avian influenza viruses do not normally infect humans. However, there have been instances of certain highly pathogenic strains causing severe respiratory disease in humans. In most cases, the people infected had been in close contact with infected poultry or with objects contaminated by their faeces.. World Health Organization (WHO) confirmed 442 cases of H5N1 in humans in Azerbaijan, Bangladesh, Combodia, China, Djibouti, Egypt, Indonesia, Iraq, Lao people’s Democratic Republic, Myanmer, Nigeria, Pakistan, Thailand, Turkey, Vietnam, leading to 262 deaths, as up to 24 September, 2009.

Since first report of outbreak in 2003, the H5N1 in poultry reached epidemic proportion with reports of serious outbreaks in several Asian countries including Vietnam, Thailand, Southkorea, Laos, Combodia, Indonesia, Japan and Malaysia (Ryan-Poirier et al., 1992; Nicolson et al., 2003). AI have the ability to affect different species of birds and mammal and the antigenic drift occur within the same strain of type A virus after a series of point mutation of amino acids at the distal region lf HA. ). Estimates of potential global mortality related to pandemic avian influenza are as high as 62 million deaths (***Katz, 1999***).

Recently Bangladesh is facing a series of outbreak of AI following emergence of HPAI virus (H5N1) at different location throughout the country. Avian Influenza (AI) posing a great threat to the growing poultry industry.Avian influenza is a disease of viral etiology that ranges from a mild or even asymptomatic infection to an acute, fatal disease of chickens, turkey, guinea fowls and other avian species, specially migratory water fowl **(Clavijo et al., 2003).** Bangladesh was free from Highly Pathogenic Avian Influenza till March, 2007. The presence of the disease was declared on 22nd March of 2007 after detection of the disease in Biman Poultry complex. However, according to the report submitted to OIE the index case was Naz Poultry Farm of Jamalpur district where outbreak started back in 05 February, 2007 **(OIE Report, 2007)**. The National Reference Laboratory for AI at Bangladesh Livestock Research Institute indicated AI subtype H5virus from 5 samples on 15 march, 2007 from a breeder farm of Savar area and from Jamalpur area. All the 5 samples were confirmed by the National Institute of Animal Health, Bangkok on 21 March 2007 to be positive for AI subtype H5N1. After that, HPAI out breaks have been reported from different parts of Bangladesh with an increasing frequency till mid May 2007. By late May, the frequency of outbreaks declined. In the recent years what are the trends of Avian Influenzea outbreaks in Bangladesh is not known well. The present study is therefore undertaken with the following **objectives:**

1. To know the prevalence of Avian Influenzea outbreak in Chittagong District; and
2. To know the variation of Avian Influenzea outbreak throughout the year.

**CHAPTER-II**

**REVIEW OF LITERATURE**

* 1. **Etiology**

Influenza viruses (family *Orthomyxoviridae*) are classified into three types, A, B, and C, based on the antigenicity of viral nucleoproteins (CN) and matrix proteins (M). Influenza viruses are further classified into different subtypes based on the hemagglutinin (HA) and the neuraminidase (NA) surface glycoproteins. . On the basis of the antigenicity of these glycoproteins, influenza A viruses currently cluster into sixteen H (H1 - H16) and nine N (N1 - N9) subtypes. These clusters are substantiated when phylogenetically analysing the nucleotide and deduced amino acid sequences of the HA and NA genes, respectively **(Fouchier, 2005).** *Antigenic shift* denotes a sudden and profound change in antigenic determinants, i.e. a switch of H and/or N subtypes, within a single replication cycle. This occurs in a cell which is simultaneously infected by two or more influenza A viruses of different subtypes.Since the distribution of replicated viral genomic segments into budding virus progeny occurs independently from the subtype origin of each segment, replication-competent progeny carrying genetic information of different parental viruses (so-called *reassortants*) may spring up **(*Webster and Hulse, 2004, WHO 2005*).** While the pandemic human influenza viruses of 1957 (H2N2) and 1968 (H3N2) clearly arose through reassortment between human and avian viruses, the influenza virus causing the 'Spanish flu' in 1918 appears to be entirely derived from an avian source **(*Belshe 2005*).** AIV is categorized into two groups based on its virulence. Excellent reviews on the structure and replication strategy of influenza viruses have been published recently **(*Sidoronko and Reichl, 2004*).** Importantly, LPAIVs can serve as progenitors to HPAIVs. Therefore, early detection and control of LPAIV infection are be economically significant. Avian influenza (AI) / (or bird flue) is a “notifiable” highly infectious disease affecting many species of birds, including chickens, ducks, turkeys and geese**.** It can affect commercial and pet birds. There are various subtypes of bird flu, but the various subtypes that are concerned at the moment are the deadly H5N1 strainthat cause generalised rather than respiratory disease belong to either the H5 or H7 subtypes. However, not all H5 and H7 viruses are virulent for poultry. This occurs in a cell which is simultaneously infected by two or more influenza A viruses of different subtypes. Since the distribution of replicated viral genomic segments into budding virus progeny occurs independently from the subtype origin of each segment, replication-competent progeny carrying genetic information of different parental viruses (so-called *reassortants*) may spring up ***(Webster and Hulse, 2004, WHO 2005)***. The pathogenicity of Avian Influenza (AI) viruses is correlated to the ability of trypsin to cleave the hemagglutinin molecule into two subunits. Highly pathogenic strains of H5 and H7 viruses have several amino acid residues at the cleavage site. Trypsin sensitivity and amino acid sequencing can be used diagnostically to determine whether or not an isolated virus is potentially pathogenic. (www.fao.org).

**2.2 History**

Highly pathogenic avian influenza (HPAI) a subtype H5N1 is a deadly zoonotic pathogen since the first reported human case in Hong Kong Special Administration Region (SAR) in 1997, caused by H5N1 virus of Poultry origin (***De Jong et al., 1997; Claas et al., 1998; Subarrao et al., 1995)*** between 1997 and 2008. Three hundred seventy eight human cases were reported in 14 countries with 238 deaths (***WHO, 2007***). It appears that the threat H5N1 influenza virus poses to both poultry and public health has intensified with widening the spread of virus in domestic and wild avian species in 61 countries in the span of four gears-from 2003 to 2007 developing country, such as Indonesia, the numbers of human cases increase. Human-to-human transmission of the virus has been very inefficient so far; however, its incidental entries to humans might lead to the emergence of a mutant or reassortant H5N1 virus with increased transmissibility among humans, and that hypothetical new virus might initiate a global influenza pandemic. With an average growth rate of about 20%, the commercial population in Bangladesh is expanding fast according to National Influenza and pandemic influenza preparedness and Response plan Bangladesh (NAIHPIPRPB. 2006).The national declaration that Bangladesh is a highly pathogenic Avian Influenza (HPAI) affected country has come on 22nd march 2007; this proclamation declined prices of poultry and their products. Thus, HPAI in poultry in Bangladesh might have significant negative impact to the national economy should future outbreaks go on uncontrolled. Before Bangladesh two SAARC (South Asian Association of Regional Co-Operation) countries: India and Pakistan (***OIE, 2007***), and one neighbor country to Bangladesh-Myanmar, reported the occurrence of HPAI in chickens (***OIE, 2007***).

**2.3** **. Ordinary host**

Wild aquatic birds, notably members of the orders *Anseriformes* (ducks and geese) and *Charadriiformes* (gulls and shorebirds), are carriers of the full variety of influenza virus A subtypes, and thus, most probably constitute the natural reservoir of all influenza A viruses **(Webster, 1992, Fouchier, 2003).** While all bird species are thought to be susceptible, some domestic poultry species - chickens, turkey, guinea fowl, quail and pheasants - are known to be especially vulnerable to the sequelae of infection **(Gorman, 1992).**

**2.4** **Transmission**

**2.4.1 Transmission to Birds**

* Routes of bird-to-bird transmission include:
  + Airborne transmission if birds are in close proximity
  + Direct contact with contaminated respiratory secretions or fecal material
* Vertical transmission is not known to occur
* Other factors that contribute to spread within and between flocks include the following:
  + Broken contaminated eggs in incubators infecting healthy chicks (***OIE 2002***)
  + Movement of infected birds between flocks
  + Movement of fomites such as contaminated equipment, egg flats, feed trucks, and clothing and shoes of employees and service crews (USDA: Avian influenza; Swayne 2008)
  + Contact with infected wild birds and waterfowl
  + Fecal contamination of drinking water
  + Garbage flies (suspected of transmitting the virus during the 1983-1984 epidemic in Pennsylvania) (***Swayne 2008***)

The disease is highly contagious. One gram of contaminated manure can contain enough HPAI virus to infect 1 million birds (USDA: Avian influenza

**2.4.2 Transmission to Humans**

Transmission of avian influenza viruses to humans, leading to the development of clinically overt disease is a rare event. The risk of direct transmission of the H5N1 virus from birds to humans seems to be greatest in persons who have close contact with live infected poultry, or surfaces and objects heavily contaminated with their droppings. **(http://www.who.int/csr/don/2005\_08\_18/en/).**

**2.4.3 Transmission to other Mammals**

Avian influenza viruses have been transmitted to different mammal species on several occasions. Here, following cycles of replication and adaptation, new epidemic lineages can be founded. In the U.S., a triple reassortant (H3N2) between the classical H1N1, the human H3N2 and avian subtypes is circulating (Olsen, 2002). Other subtypes of presumably avian origin (e.g. H1N7, H4N6) have been found mainly anecdotally in swine (***Brown, 1997***). A H9N2 virus of avian provenance is moderately prevalent in swine populations in the East of China (***Xu, 2004).*** In addition to swine, marine mammals and horses have been shown to acquire influenza A viruses from avian sources (***Ito, 1999).***

**2.5. Pathogenicity of HPAI**

Pathogenicity is a general viral property in influenza A viruses is a polygenic trait and depends largely on an 'optimal' gene constellation affecting host and tissue tropism, replication efficacy and immune evasion mechanisms, amongst others (Swayne and Suarez, 2000). Usually, H5 and H7 viruses are stably maintained in their natural hosts in a low pathogenic form. From this reservoir, the viruses can be introduced by various pathways into poultry ﬂocks.

Following a variable and indecisive period of circulation (and, presumably, adaptation) in susceptible poultry populations, these viruses can saltatorily mutate into the highly pathogenic form (Rohm, 1995).

In order to gain infectivity, influenza A virions must incorporate HA proteins which have been endoproteolytically processed from a HA0 precursor to a disulphide-linked HA1,2 dimer (Chen, 1998). The newly created N-terminus of the HA2 subunit harbors a fusogenic peptide, composed of a highly lipophilic domain (Skehel, 2001). This domain is vitally required during the fusion process of viral and lysosomal membranes because it initiates the penetration process of viral genomic segments into the host cell cytoplasm.

**2.6. Important Clinical Signs**

## Clinical Features in Domestic Birds

The clinical signs of HPAI are severe and result in high mortality rates in many species of birds, especially domestic fowl. In its highly pathogenic form, the illness in chickens and turkeys is characterised by a sudden onset of severe symptoms and a mortality that can approach 100 % within 48 hours **(Swayne and Suarez, 2000).**The symptoms following infection with low pathogenic AIV may be as discrete as ruffled feathers, transient reductions in egg production or weight loss combined with a slight respiratory disease **(Capua and Mutinelli, 2001).**

|  |
| --- |
| Clinical Features of Highly Pathogenic Avian Influenza in Domestic Birds |
| **CHICKENS** |
| Incubation Period : 3-7 days |
| Clinical signs   * Sudden death * Severe depression with ruffled feathers * Inappetence * Drastic decline in egg production,soft shell egg etc. **(Elbers, 2005).** * Edema of head and neck (Swayne, 2008) * Swollen and cyanotic combs and wattles (***Swayne, 2008***) * Petechial hemorrhages on internal membrane surfaces * Excessive thirst * Watery diarrhea that begins as bright green and progresses to white * Swollen and congested conjunctiva with occasional hemorrhage * Diffuse hemorrhage between hocks and feet (***Swayne, 2008)*** * Respiratory signs are dependent on tracheal involvement * Nasal and ocular discharge * Mucus accumulation (varies) * Lack of energy * Coughing/sneezing * Ncoordination * Nervous system signs such as paralysis,torticolis etc.  **(Kwon, 2005).** |
| Complications   * Cessation of egg production, and eggs laid immediately prior to infection often soft-shelled and misshapen * Surviving birds are in poor condition and resume laying only after a period of several weeks |
| Case-fatality rate   * Can be as high as 100% * Death may occur prior to any symptoms or as late as a week after symptoms, though it is frequently within 48 hr |
| **Fig-1: Lesions in AI infected chicken**  ***G:\Avian Influenza\Picture of AI\AI Clinical Signs (14).jpg*** |

Fig 1: Hen infected with H5N1 on a layer farm showing ecchymotic

hemorrhagic discoloration at a leg shank



Fig 2: Tracheas with muco-hemorrhagic materials from a H5N1



Fig 3: Brain haemorrage in AI infected chicken



Fig 4: Cyanotic comb & wattle in AI infected chicken

## 2.7. Pathological Characteristics

HPAI can be recognized by the high mortality rate in affected flocks as well as by the clinical signs. Characteristic necropsy lesions, listed below, also can help make the diagnosis. The lesions in chickens and turkeys are highly variable and resemble those found in other systemic avian diseases.

* Birds that die peracutely and young birds may have few or no lesions.
* In other cases, the sinuses may be swollen, and the comb and wattle are often edematous, hemorrhagic, congested and/or cyanotic.
* There may be subcutaneous edema on the head and neck, edema and diffuse subcutaneous hemorrhages on the feet and shanks, fluid (which may contain blood) in the nares and oral cavity, and congestion, swelling and hemorrhages of the conjunctivae.
* Hemorrhagic tracheitis can be seen in some birds; in others, the tracheal lesions may be limited to excess mucoid exudates **(Elbers, 2004).**
* The lungs may be reddened from hemorrhages and congestion, and they may exude fluid when cut.
* Petechiae may be noted throughout the abdominal fat, on serosal surfaces and on the peritoneum, and they can sometimes be found in the muscles. **(Mutinelli, 2003a).**
* Hemorrhages may also be seen on the mucosa and in the glands of the proventriculus, especially with junction of the gizzard and often with errosion and in the intestinal mucosa.
* The kidneys can be severely congested and they are sometimes plugged with urate deposits.
* The ovaries may be hemorrhagic or degenerated, with areas of necrosis.
* The peritoneal cavity often contains yolk from ruptured ova, which may cause severe airsacculitis and peritonitis.
* Lesions in ducks may be similar to those seen in chickens though not as marked, or they may be absent altogether

**2.7. Laboratory Diagnosis**

**2.7.1. Collection of Specimens**

Specimens should be collected from several fresh carcasses and from diseased birds of a ﬂock. Ideally, adequate sampling is statistically backed up and diagnosis is made on a ﬂock basis.

When sampling birds suspected of HPAI, safety standards must be observed to avoid exposure of the sample collectors to potentially zooanthroponotic HPAIV **(Bridges, 2002)**. Guidelines have been proposed by the CDC **(CDC, 2005).** For virological assays, swabs obtained from the cloaca and the oropharynx generally allow for a sound laboratory investigation. The material collected on the swabs should be mixed into 2-3 ml aliquots of a sterile isotonic transport medium containing antibiotic supplements and a protein source (e.g. 0.5 % [w/v] bovine serum albumin, up to ten percent of bovine serum or a brain-heart infusion).

**2.7.2. Transport of Specimens**

Swabs, tissues and blood should be transported chilled but not be allowed to freeze. If delays of greater than 48 hours are expected in transit, these specimens should be frozen and transported on dry ice. In all cases, transport safety regulations (e.g. IATA rules) should be punctiliously observed to avoid spread of the disease and accidental exposure of personnel during transport. It is highly advisable to contact the assigned diagnostic laboratory before sending the samples and, ideally, even before collecting them.

**2.7.3. Diagnostic Procedure**

**Identification of the Agent**

Once specimens have been collected and processed, the OIE Manual recommends the following for identification of avian influenza (OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals):

* The preferred method of growing avian influenza A viruses is by the collection of nasopharyngeal swab from trachea and cloaca and inoculation via allantoic cavity of embryonated specific pathogen free (SPF) fowl eggs, or specific antibody negative (SAN) eggs of 9 to 11 days' incubation.
  + Eggs should be incubated at 35°C to 37°C for 4 to 7 days.
  + Eggs containing dead or dying embryos as they arise within 24 hours, and all eggs remaining at the end of the incubation period, should first be removed from container ane chilled to 4°C and the allantoic fluids should then be tested for hemagglutination (HA) activity chicken erythrocyte. (Palmer et al.,1975)
* Detection of HA activity indicates a high probability of the presence of an influenza A virus or of an avian paramyxovirus. Fluids that give a negative reaction should be passaged into at least one further batch of eggs.
* Cell culture: Primary cynomolgus or Rhesus monkey kidney cell (PMKG) are susceptible to variety of respiratory viruses including Influenza Viruses. But they are not effective for their cost and the presence of spuma viruses.
* Several methods are available to confirm the presence of influenza A virus; these include:
  + Agar gel immunodiffusion (AGID) tests that demonstrate the presence of the nucleocapsid or matrix antigens
  + Various enzyme-linked immunosorbent assays (ELISAs)
  + Reverse-transcription polymerase chain reaction (RT-PCR) using nucleoprotein-specific or matrix-specific conserved primers; the presence of subtype H5 or H7 influenza virus can be confirmed by using H5- or H7-specific primers
  + Complement Fixation Test.

### Developing Techniques for the Diagnosis

In addition to the tests mentioned above, new diagnostics have become available in recent years, including:

Antigen detection tests

* + The commercially available Directigen Flu A Kit (Becton Dickinson Microbiology Systems), which is an antigen-capture enzyme immunoassay system, has been used for detecting the presence of influenza A viruses in poultry, particularly in the United States. The kit uses a monoclonal antibody against the nucleoprotein and should therefore be able to detect any influenza A virus.
  + The main advantage of the test is that it can demonstrate the presence of avian influenza within 15 minutes. The disadvantages are that it may lack sensitivity, it has not been validated for different species of birds, subtype identification is not achieved, and the kits are expensive.

**Differential Diagnosis**

Acute fowl cholera (Pasteurellosis) and other septicaemic diseases Bacterial cellulites of the comb and wattles. Less severe forms of HPAI can be clinically even more confusing. Rapid laboratory diagnostic aid, therefore, is pivotal to all further measures **(Elbers, 2005).**

Low pathogenic avian influenza must be differentiated from other respiratory diseases or causes of decreased egg production including:

1. Acute to subacute viral diseases such as

* Infectious bronchitis
* Infectious Laryngotracheitis
* Lentogenic Newcastle Disease
* Infections by other Paramyxoviruses

1. Bacterial diseases sucs as

* Mycoplasmosis
* Infectious Coryza
* Ornithobacteriosis
* Turkey Coryza
* Respiratory form of Fowl Cholera

1. Fungal disease such as Aspergillosis

Highly pathogenic Avian Influenza must be differentiated from other causes of high mortality such as

* Velogenic Newcastle Disease
* Per acute septicemic fowl cholera
* Heat exhaustion
* Severe water deprivation

**2.8. Prevention**

**2.8.1. Prevention of Avian Influenza in birds:**

Poultry can be infected by contact with newly introduced birds or fomites, as well as by contact with wild birds, particularly waterfowl. The risk of infection can be decreased by all-in/all-out flock management, and by preventing any contact with wild birds or their water sources. Birds should not be returned to the farm from live bird markets or other slaughter channels. In addition, strict hygiene and bio-security measures are necessary to prevent virus transmission on fomites.

Outbreaks can be controlled by rapid depopulation of infected and exposed flocks, proper disposal of carcasses and contaminated materials, and strict bio-security measures. Farms should be quarantined, and movement controls and surveillance should be established. Infected premises must be thoroughly cleaned and disinfected. Insects and mice on the premises should be eliminated, then the flock depopulated and the carcasses destroyed by burying, composting or rendering. Once the birds have been killed, the manure and feed should be removed down to a bare concrete floor. If the floor is earthen, one inch or more of soil should be removed. The manure can be buried at least five feet deep. It may also be composted for 90 days or longer, depending on the environmental conditions. The compost should be covered tightly with black polyethylene sheets to prevent the entry of birds,

There are many steps that can be taken to prevent AI in birds:

1. Keep your distance
   * Do not allow visitors and animals to have access to your birds.
   * People that work with your birds should not own or be around other birds.
   * Provide visitors with disposable boots, or have them clean their shoes before and after their visit.
2. Keep it clean
   * Keep a pair of shoes and a set of clothes to wear only around your birds, or clean and disinfect your shoes and launder your clothes before you check on or work around your birds.
   * Scrubbing your shoes with a long-handled scrub brush and disinfectant will remove droppings, mud, or debris.
   * Wash your hands thoroughly with soap, water, and disinfectant before entering your bird area.
3. Don't haul disease home
   * If you travel to a location where birds or present or where bird owners visit (i.e. feed store), clean and disinfect your vehicle, and other items that have travelled with you.
   * Scrubbing your shoes with a long-handled scrub brush and disinfectant will remove droppings, mud, or debris.
   * When returning from a fair or exhibition, keep birds that attended separate from the rest of the flock for at least two weeks.
   * New birds should be kept separate from the flock for at least 30 days.
4. Don't borrow disease from your neighbors
   * Don't share equipment, birds, or items with neighbors or other bird owners.
   * Scrubbing your shoes with a long-handled scrub brush and disinfectant will remove droppings, mud, or debris.
5. Know the warning signs of infectious bird diseases
6. Report sick birds
   * Report signs of unusual illness to your veterinarian, or the Washington State Department of Agriculture (WSDA) Avian Health Program at 1-800-606-3056.

**Cleaning and disinfection**

Influenza viruses are very sensitive to most detergents and disinfectants. They are readily inactivated by heating and drying. However, flu viruses are well-protected from inactivation by organic material and infectious virus can be recovered from manure for up to 105 days. Complete removal of all organic material is part of any effective disinfection procedure.

Contaminated houses are heated for several days to inactivate virus. Organic material is removed followed by complete cleaning and disinfection of all surfaces. Contaminated litter and manure is problematic and should be composted or buried to ensure that it does not spread infectious virus.

**2.8.2. Prevent Avian Influenza in humans**

**People travelling to areas affected by avian influenza** are likely to have only a very small risk of infection. This risk can be reduced further by:

* avoiding places where there may be contact with infected birds, particularly live bird markets
* washing hands thoroughly after any contact with birds, their faeces or body fluids
* Ensuring all uncooked poultry and eggs are handled hygienically during food preparation, with careful attention to hand washing after handling.
* ensuring all poultry and eggs are cooked thoroughly before eating

**People caring for patients with avian influenza should always:**

* use appropriate personal protective equipment (PPE) - including P2 masks, goggles, gloves and protective outer clothing;

**2.9. Occurrence in the world:**

A pathogenic and mildly pathogenic influenza A viruses occur worldwide. Highly pathogenic avian influenza A (HPAI) viruses of the H5 and H7 HA subtypes have been isolated occasionally from free-living birds in Europe and elsewhere. Outbreaks due to HPAI were recorded in the Pennsylvania area, USA, in the years 1983-84. More recently outbreaks have occurred in Australia, Pakistan and Mexico. There is evidence that H5 viruses of low pathogenicity may mutate and become highly pathogenic. HPAI infections are very rarely seen, and should not be confused with viruses of low pathogenicity, which may also be of H5 or H7 subtypes.

**HPAI outbreak in Bangladesh**

Highly pathogenic Avian Influenza (HPAI) of pathotype H5NI was first reported February 5, 2007 in Bangladesh. Since the epidemic wave in poultry, the country has attempted to control the diseases through a range of control measures. To date, HPAI H5N1 has been isolated from more than 350 poultry farms in different areas of the country (FAO Technical unit for Avian Influenza, Bangladesh). The H5N1 virus struck 47 of Bangladesh's 64 districts, according to a recent Area flu profile (AFP) report. To date a 16 month old baby in Bangladesh has an H5NI at infection in January but has since recovered. But Bangladeshis are at great risk as 4 million people are directly or indirectly associated with poultry farming. They are often forgetting to wash the egg before handling, disinfecting the slaughtering places or to dispose feces of the infected birds loaded with the H5N1 properly. These pose a great threat for the spread of the virus along with the migratory routes of wild waterfowl. Diagnostic tools and surveillance systems should be improved immediately for early warnings and to detect the maximum human cases.

**Table-1: According to Department of Livestock Services (DLS) aerial attack rates (infected upazillas) of the infected districts based of Avian influenza outbreaks in Bangladesh in 2007-2008 (25/64 districts).**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **District** | **No. of AIAFS** | | **No. of**  **commercial farm** | **No. of**  **SH farms** | **Attack rate (95%-CI)** | |
| **Commercial** | **SH** | **Commercial** | **SH** |
| Naryangong | 9 | 0 | 925 | - | 0.009 | - |
| Nilphamari | 0 | 5 | 119 | 277,984 | - | 0.00001 |
| Noakhali |  | 0 | 97 | - | 0.010 | - |
| Pabna | 1 | 0 | 313 | 71,380 | 0.003 | - |
| Rajbari | 1 | 2 | 395 | 311,279 | 0.002 | 0.0006 |
| Rajshahi | 1 | 0 | 81 | 65,752 | 0.012 | - |
| Rongpur | 0 | 2 | 95 | 1473,263 | - | 0.0001 |
| Tangail | 1 | 0 | 227 | 91,650 | 0.004 | - |
| Thakurgaon | 0 | 1 | 1137 | 73391 | - | 0.00001 |
| Barguna | 1 | 0 | 101 | 50,765 | 0.10 | 0.0003 |
| Barisal | 0 | 2 | 68 | 68,718 | 0.004 | 0.00008 |
| Bogra | 16 | 0 | 255 | 72,275 | 0.004 | - |
| Dhaka | 3 | 1 | 744 | 11,485 | 0.021 | 0.00008 |
| Dinajpur | 0 | 1 | 219 | 271967 | 0.013 | 0.00007 |
| Feni | 2 | 1 | 108 | 55573 | - | 0.0002 |
| Gaibandha | 3 | 2 | 311 | 232,550 | 0.006 | 0.00008 |
| Gazipur | 5 | 0 | 1956 | - | 0.001 | - |
| Jamalpur | 3 | 0 | 144 | 58,962 | 0.034 | - |
| Jessore | 2 | 3 | 298 | 331,295 | 0.010 | 0.0003 |
| Joypurhat | 0 | 1 | 382 | 229,702 | 0.005 | 0.000004 |
| Kurigram | 0 | 1 | 81 | 58,950 | - | 0.0002 |
| Lalmonirhat | 0 | 3 | 145 | 120,417 | - | 0.0001 |
| Magura | 0 | 1 | 82 | 83,607 | - | 0.0001 |
| Moulavibazar | 1 | 0 | 86 | 76,520 | 0.011 | - |
| Naogaon | 0 | 2 | 109 | 80,752 | - | 0.0002 |
| Total | 51 | 28 | 8,478 | 26,72270 | 0.0006 | 0.0001 |

**\*B=Commercial breeder farm; Br=Commercial broiler farm; L=Commercial layer farm; S=small holding farm; N= 58(4B+ 2Br+ 27L+25S).**

**2.10. Economic impact:**

Outbreaks of highly pathogenic avian influenza can be catastrophic for single farmers and for the poultry industry of an affected region as a whole.

Economical losses are usually only partly due to direct deaths of poultry from HPAI infection. Measures put up to prevent further spread of the disease levy a heavy toll. Nutritional consequences can be equally devastating in developing countries where poultry is an important source of animal protein. Once outbreaks have become widespread, control is difficult to achieve and may take several years **(**[**WHO, 2004/01/22**](http://www.who.int/csr/don/2004_01_22/en/index.html)**).**

**2.11.Control Measures Against HPAI**

Due to its potentially devastating economic impact, HPAI is subject world-wide to vigilant supervision and strict legislation (***Pearson, 2003,*** [***OIE Terrestrial Animal Health Code, 2005***](http://www.oie.int/eng/normes/mcode/en_chapitre_2.7.12.htm)***).*** Measures to be taken against HPAI depend on the epidemiological situation of the region affected. For these purposes, control and surveillance zones are erected around the index case with diameters varying from nation to nation (3 and 10 kilometers, respectively, in the EU**).**. The quarantining of infected and contact farms, rapid culling of all infected or exposed birds, and proper disposal of carcasses, are standard control measures to prevent lateral spread to other farms([***OIE - Terrestrial Animal Health Code***](http://www.oie.int/eng/normes/mcode/en_chapitre_2.7.12.htm)***, 2005).***

Moreover, domestic ducks attract wild ducks and provide a significant link in the chain of transmission between wild birds and domestic ﬂocks (***WHO, 2005***). These circumstances may provide the grounds for HPAI viruses to gain an endemic status. Endemicity of HPAI in a certain re

**2.12. Vaccination.**

The ﬁrst aim, which is the protection from clinical disease induced by HPAIV, is achieved by most vaccines. The risk of infection of vaccines with and excretion of, virulent field virus is usually reduced but not fully prevented. This may cause a significant epidemiological problem in endemic areas where exhaustive vaccination is carried out. In the field of influenza vaccination, neither commercially available nor experimentally tested vaccines have been shown so far to fulfill all of these requirements **(Lee and Suarez, 2005).** . For practical use several requirements must be observed **(Lee and Suarez, 2005).** By detection of NA subtype-speciﬁc antibodies, vaccines and infected birds can be distinguished **(Cattoli, 2003).**

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**Chapter-III**

**MATERIALS AND METHODS**

The study was aimed to determine the prevalence of avian influenza infection in chicken at Chittagong district.

**3.1. Study area and study period**

A cross sectional study was conducted at 4 thana under Chittgong Metropoliton Area (Pahartoli, Chandgaon, Bakolia, and Potenga) and two upazilla (Hathazari and Potia) in Chittagong District for a period of 2 months from March,2012 to April,2012.

**3.2. Collection of Data:**

Before going to retrospective study, all of the data were collected from the record sheet kept by the government surveillance and monitoring team. In the presence of District Livestock Office, Directorate of Livestock Services, Chittagong. A preset questionnaire was developed to gather demographic information (population, type of bird, age location and months etc.) of the Avian Inflenza (AI) affected farms.

**3.3. Data Entry and Analysis**

After fill up the questionnaire, these are brought to the Computer Lab, Chittagong Veterinary and Animal Sciences University. All data were sorted out and entered into the MS-Excel-2003. Later these data were imported to the STATA -11.00 for the descriptive statistical analysis (Mean, SD, % etc.).

**Chapter-IV**

**RESULTS AND DISCUSSION**

**Table-1(a):** Morbidity and Mortality rate of Avian Influenzea affected farm in different Thana of Chittagong district

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Location** | **Total bird** | **Dead bird** | **Culled bird** | **Morbidity rate (%)** | **Mortality rate (%)** | **P-value** |
| Pahartoli | 2250 | 262 | 1988 | 100 | 11.6 | 0.22 |
| Chandgaon | 11740 | 1332 | 10408 | 100 | 11.3 |
| Bakolia | 11500 | 687 | 10813 | 100 | 5.9 |
| Hathajari | 4000 | 80 | 3920 | 100 | 2 |
| Potia | 9619 | 2283 | 7336 | 100 | 23.7 |
| Potenga | 1700 | 95 | 1605 | 100 | 5.6 |

The different rate of mortality and morbidity among different thanas are shown in the table-1. In table-1, the highest mortality rate was observed in Potia (23.7%) and lowest in Hathajari (2%) thana. However, there were no statistically significant differences were observed between places for mortality rate. These differences were observed due to lack of proper bio-security measure. On the other hand, the morbidity among different location was similar (100%) for all the places. Similar results were found by Sharmin for poultry farm in Chittagong region.

**Table: 1(b):** Average number of Total, Dead and Culled bird

|  |  |  |
| --- | --- | --- |
| **Variables** | **Mean ±SD** | **Min-Max** |
| Total bird | 6801.5± 4668.894 | 1700-11740 |
| Dead bird | 789.8333±871.703 | 80-2283 |
| Culled bird | 6011.667 ± 4101.66 | 1605 - 10813 |

Table 1(b) showed that average number of total dead and culled bird in different study areas. Around 6800 bird was recorded per thana that number ranged between 1700-11740. The mean dead bird was 789 and culled bird was 6011.

**Table-2:** Mortality and morbidity rate between layer and broiler bird

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Types of bird** | **Total bird** | **Dead bird** | **Culled bird** | **Morbidity rate (%)** | **Mortality rate (%)** | **P-value** |
| Layer | 34809 | 4671 | 30138 | 100 | 13.4 | 0.157 |
| Broiler | 6000 | 68 | 5932 | 100 | 1.13 |

In addition, Table 2 showed the morbidity and mortality rate of layer and broiler bird in different locations. The highest mortality rate (13.4%) was found in layer birds than broilers(1.13%). But there was no significant differences observed between the broilers and layers. This difference in mortality rate between layer and broiler bird could be due to the energy utilization for egg production and exposure for long period of time. . The result showed similarity with the study conducted by **Brug *et al.,* (1987).**

**Table-3:** Seasonal variation of Morbidity and mortality

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Month** | **Total bird** | **Dead bird** | **Culled bird** | **Morbidity rate (%)** | **Mortality rate (%)** | **P-value** |
| January | 7150 | 343 | 6807 | 100 | 4.8 | 0.000 |
| February | 7500 | 357 | 7143 | 100 | 4.8 |
| March | 2900 | 80 | 2820 | 100 | 2.8 |
| April | 7000 | 671 | 6329 | 100 | 9.6 |
| May | - | - | - | - | - |
| June | 1000 | 44 | 956 | 100 | 4.4 |
| July | - | - | - | - | - |
| August | - | - | - | - | - |
| September | 15259 | 3244 | 12015 | 100 | 21.3 |
| October | - | - | - | - | - |
| November | - | - | - | - | - |
| December | - | - | - | - | - |

Table 3, portrayed the variations in morbidity and mortality of AI infected birds according to the months. The highest mortality rate (21.3%) was found in September and lowest mortality rate was found in March. There was a highly significant difference (p>0.000) in mortality rate observed in birds among the different months of the year. The mortality rate was found in January, February, March, April, June and September was 4.8%, 4.8%, 2.8%, 9.6%, 4.4% and 21.3%, respectively. In the remaining months were no cases of Avian Influenzea out breaks.

The variation of mortality and morbidity among different seasons are significant

**Table-4:** Morbidity and mortality among different age groups

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Age of birds (wks) | Total bird | Dead bird | Culled bird | Morbidity rate | Mortality rate (%) | p-value |
| 0 to 12 | 13769 | 2443 | 11326 | 100 | 17.7 | 0.199 |
| >12 to <16 | 3900 | 245 | 3655 | 100 | 6.3 |
| >16 | 23140 | 2051 | 21089 | 100 | 8.9 |

Table-4 showed that the morbidity and mortality rate among the different age groups of bird. The highest proportion of mortality (17.7%) rate was found in between 0-12 weeks of age followed by >16 weeks of age (8.9%) and >12-16> (6.3%). There were no significant differences between different age groups of bird.

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**DISCUSSION**

Fig 1: No. of AI affected chicken in different thanas of Chittagong district

Figure 1 shows number of AI affected birds in different thanas of Chittagong District. The highest number of bird was found in Chandgoan and lowest number of bird was found in Potenga. The number of highest AI infected was recorded in Chandgaon due to the proper monitoring and higher number of birds. Farm utensils used on the AIAFs (Avian influenza affected farms) were either disinfected or destroyed by burning. For disinfection, Di-potassium per-oxisulphide (Vikon-S®) was used on 82% Avian Influenza affected farms (AIAFs) and CFs (contagious Farms). The disinfectant required for the AIAFs and CFs were supplied free of cost to the farmers from the DLS, Live birds to be culled were killed by cervical dislocation, The carcasses were then packed up in plastic bags before dumping them into the newly excavated burial pits. The depth of the pits varied from 3 to 8 feet, inner and outer surfaces, along with the contaminated surrounding areas of the pits were also disinfected chickens on each AIAFs, and all domestic and commercial birds around 1km radius of the AIAF (called infected zone) were culled. In presence of the DLS officials (100%). local District commissioner (DC) / Upazilla Nirbahi, (Executive) officer/ Upazilla magistrate (59.5%) and the combined forces (Police, Rapid action battalion [RAB] and members of the Army), the culling operations were conducted predominantly by hired labors (65.8%). The safety of the labors was ensured by providing personal protection equipment (PPE) kits supplied from the DLS. However, in all cases these personnel had been administered an anti influenza drug. Oseltamivir (Aviflu;® Oseflu®) before the operation begun.

**Chapter-V**

**CONCLUSION**

It has already met all prerequisites for the outbreak of a pandemic. The rising outbreak of bird flu both in birds and humans has renewed fears of a pandemic that could kill millions of people. Highly pathogenic H5NI strain of bird flu virus is gradually increasing its ability to spread efficiently and substantially among humans. The World Health Organization (WHO) states this condition as "Pandemic Alert Period". High officials of WHO recently urged governments to act swiftly to control the spread of bird flu warning that the world is in grave danger of a deadly pandemic triggered by the virus since its first detection in 2008. There were 40809 birds reported to have culled due to highly pathogenic Avian Influenza (HPAI) respectively, (in Chittagong) according to DLS. Experts have warned the H5NI virus could become far deadlier if it mutates into a form that can be easily transmitted among humans. Experts identified one of the biggest challenges in controlling avian flu is in altering traditional farming practices in Bangladesh where animals live in close, often unsanitary quarters with people.

**RECOMMENDATION**

Taking extra precaution like wearing protective devices like gloves, glasses, hand washing with simple soap and hot water after handling chickens and birds, eating properly cooked meat and egg (above 70°C) can reduce the transmission to a significant level. Diagnostic tools and surveillance systems should be improved immediately for early warnings and to detect the maximum human cases. Vaccination and early treatment has a great role in slowing the process of spread and preventing a pandemic, according to the experts. The H5N1 virus has spread in more than 25 districts and caused at least 8,478 poultry commercial farms outbreaks since (2007-2008). This is why coordinated movement is badly needed to work on a pandemic preparedness plan so that even in an emergency we can provide.

**Chapter-VI**

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