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Avian/Bird Flu Virus: Poultry Pathogen Having Zoonotic and Pandemic Threats: A Review

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Avian/Bird flu, caused by Avian Influenza Virus (AIV) belonging to Orthomyxoviridae family, is the most fearful viral disease of birds. H5N1 subtype of AIV is of major concern for poultry as well as for humans due to its high economical impacts and zoonotic concerns. During the past ten years, the Highly Pathogenic Avian Influenza (HPAI) H5N1 subtype alone has affected more than 60 countries of the world. Domestic poultry is mostly affected by the disease episodes and outbreaks. Wild and migratory birds are the AIV reservoirs wherein H5N1 is found to be lethal. Major antigenic changes in Haemagglutinin (HA) or Neuraminidase (NA) result in periodic pandemics. Pigs can act as mixing vessel. The bird flu virus if gets the capability of transmitting from human to human can trigger a pandemic claiming millions of lives. A wide variety of serological tests and molecular tools have greatly aided in the diagnosis of avian flu. Disease management for the prevention of bird flu outbreaks including mass awareness and pandemic preparedness following World Health Organization (WHO) guidelines is of utmost importance. Interesting approaches of HPAI control are development of universal influenza virus vaccines and universal antibodies-based flu therapies. Vaccination using inactivated and recombinant vaccines is the common strategy adopted in different parts of the globe. Development of new generation vaccines is quiet noteworthy. Tamiflu is the drug of choice. Herbal therapy is gaining much attention to control disease in humans. All these aspects of the bird flu virus have been discussed vividly in the present review.

Key words: Bird flu, avian flu, influenza virus, HPAI, H5N1, poultry, zoonosis, pandemic, biosecurity, prevention, diagnosis, control, treatment

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INTRODUCTION

Bird flu, also known as avian influenza/fowl plague/fowl pest/avian flu/chicken ebola, is the most fearful viral disease of birds and particularly affects domesticated birds with very high flock mortality (upto 100%). The disease is of great economical significance along with having zoonotic potentials and probable pandemic threats. Its etiological agent Avian Influenza Virus (AIV) causes infections from subclinical to highly virulent disease in poultry birds. The virus was first detected in 1878 in poultry flocks in Italy and thereafter disease outbreaks in poultry have been reported worldwide (Sims *et al*., 2005). Important reservoirs of AIV are free flying migratory/wild birds, ducks and geese, where infection is mostly inapparent/subclinical and clinical disease is generally not observed (Singh *et al*., 2003). On the basis of pathogenicity the disease is categorized into Highly Pathogenic Avian Influenza (HPAI) and Low Pathogenicity Avian Influenza (LPAI). HPAI is an extremely contagious, multi-organ systemic disease and is a listed disease of World Organization for Animal Health (OIE) and there is risk of spread of the disease beyond the national boundaries. Control is difficult due to extreme genetic alteration. Earlier the AIV H5N1 subtype was restricted to poultry but now involves migratory birds also and has emerged in mammals and among human population too. Recent reports have indicated H5N1 subtype to be lethal in reservoir birds (migratory/wild) too (Dhama *et al*., 2005; Munster *et al*., 2007; Reperant *et al*., 2011; Cappelle *et al*., 2012). Thus, the virus is continuously evolving and becoming more and more lethal. During the past ten years, many countries have confirmed the AIV H5N1 subtype outbreaks in poultry populations resulting in tremendous monitory losses (Millions of chickens and other birds have died or been slaughtered to contain the infection). These recent waves of HPAI have highlighted the global impact of this transboundary animal disease (Swayne and Suarez, 2000; Chen *et al*., 2005; Dhama *et al*., 2005; Thomas and Noppenberger, 2007; Tiwari and Dhama, 2012).

The AI virus has yet to acquire the ability of rapid spread from human to human, as has been observed recently for the swine flu virus (H1N1 subtype) (Dhama *et al*., 2005, 2012a; Pawaiya *et al*., 2009). This kind of human to human transmission of bird flu virus can trigger a human pandemic claiming millions of lives like that happened during the 'spanish flu' of 1918 in which human influenza virus of H1N1 subtype got evolved causing more than 40 million deaths globally (Liu *et al*., 2009). If bird flu pandemic happens then this deadly avian pathogen could cause serious socio-economic and public health consequences (Dhama *et al*., 2013a). In preventing disease in fowls, judicious and strict biosecurity and disease surveillance measures along with proper culling and vaccination strategies are of utmost importance; thereby further helping to limit epidemic and human pandemic threats. Vaccine for human influenza does not prevent avian influenza infection in people. To protect both birds and humans against H5N1 effective vaccination is required (Mathew *et al*., 2006; Tiwari and Dhama, 2012).

ETIOLOGY

Avian/bird flu is caused by Avian Influenza Virus (AIV), a Type A influenza virus [Genus: *Influenzavirus* A, Family Orthomyxoviridae]. AIV is a negative sense single stranded (ss) RNA virus, 80-120 nm diameter. It is an enveloped virus with 10-12 nm long "spikes" or "projections" [rod shaped Hemagglutinin (HA) and mushroom shaped Neuraminidase (NA) glycoproteins]. Influenza viruses are classified into subtypes based on haemagglutinin (H1-17) and neuraminidase (N1-10) antigens. All the 16 H and 9 N in all possible combinations have been isolated from birds. Recently, H10 and N10 have been also discovered (Dhama *et al*., 2012b). Highly fatal infections are associated with the H5 and H7 subtypes which posses multiple basic amino acids at the cleave site of H protein (Dhama *et al*., 2005; OIE, 2012). HA glycoprotein is responsible for hemagglutinating activity and virus attachment to host cells and producing protective antibodies in host after infection (Kumari *et al*., 2007). NA glycoprotein aids in the release and spread of new virus from the infected cell. The virus is unstable in the environment and heat, high or low pH and dryness can inactivate the virus and is susceptible to a variety of detergents and disinfectants. Long term storage of viruses should be done at -70°C or following lyophilization. Avian influenza virus is killed at a temperature of 56°C for 3 h, 60°C for 30 min, 70°C for 3 min and 80°C for 1 min (Dhama *et al*., 2005; Hampson and Mackenzie, 2006).

Genomic/antigenic variation: Influenza viruses undergo genetic/antigenic variations by antigenic drift (point mutation) and genetic reassortment (antigenic shift). Evolution of new strains (belonging to same subtype) occurs due to accumulation of point mutation. These minor antigenic changes in HA or NA, results in frequent epidemics (Dhiman *et al*., 2010). A completely new subtype or a novel strain is evolved by genetic reassortment. Viral genome has eight segments and during mixed infection these are capable of re-assorting

with segments from other influenza viruses (human, avian, swine). In a mixed infection with two strains there is a possibility of generation of 256 (2^8) genetically different progeny viruses. Periodic pandemics are the outcomes of mutation in HA or NA. Genetic reassortment occurs as pigs can act as mixing vessels as well as intermediate host for evolution of influenza viruses having pandemic potential (Olsen, 2004). 17 H subtypes (H1-H16) and 10 N subtypes (N1-N9) can rise to 170 possible combinations (designated as H1N1, H1N2, H5N1, H7N2, H7N3, H7N7, H17N10 etc.) (Dhama *et al*., 2005, 2012a; Forrest and Webster, 2012; Gamblin and Skehel, 2010). Interest regarding the origin and evolution of influenza viruses has been raised by the H17N10 virus genome even though the H17 HA shares considerable amino acid sequence identity with the other 16 HA subtypes. The N10 genes of this virus (from little yellow-shouldered bats) code for the NA-like (NAL) protein and shows extensive divergence from known influenza NAs (Tong *et al*., 2012). H5N1 is of particular concern having the ability to mutate rapidly. Presently, the circulating H5N1 subtypes have gained the ability to cross/jump the species barrier and to affect animals (pigs and carnivores such as tigers, cats and leopards) as well as human beings besides causing high mortality in birds (Kuiken *et al*., 2011). Gene swapping between influenza viruses can occur in pigs between avian, swine and human viruses. AIV can escape infection as well as vaccination induced immunity, thus helping more pathogenic biovars to evolve thereby causing more outbreaks and epidemics (WHO/OIE/FAO H5N1 Evolution Working Group, 2012). Even though only H5 and H7 subtypes cause HPAI, most of these subtypes are of low virulence. But mutation can help converting viruses having low pathogenecity to those having high pathogenecity after short duration circulation (Webster *et al*., 1992; Dhama *et al*., 2005; Caron *et al*., 2008).

EPIDEMIOLOGY

Globally, AIV has caused chaos in poultry industry leading to enormous economic losses worldwide (Alexander, 2000, 2007; Dhama *et al*., 2005; Kataria *et al*., 2005a; Adams and Sandrock, 2010). Particularly, the H5N1 subtype affected more than 60 countries with losses of more than 300 million birds and 368 human lives, indicating the flu virus is becoming more and more dangerous. Following countries have reported most number of AIV H5N1 disease outbreaks in poultry (from end of 2003 to 12th March, 2013): Vietnam (2681), Thailand (1141), Egypt (1084), Bangladesh (546), Romania (273), Indonesia (261), Turkey (219), Russia (149), Myanmar (115), Korea (Rep. of) (112), China (People's Rep. of) (105), India (93), Nigeria (65), Pakistan (51). In the year 2013 (till 12th March, 2013), countries reporting bird flu are Bangladesh, Bhutan, Cambodia, Hong Kong (SAR-PRC), India and Nepal (Adams and Sandrock, 2010; WHO, 2011; WHO/OIE/FAO H5N1 Evolution Working Group, 2012).

Host range, transmission and spread: Virus can naturally infect a wide variety of avian species and is primarily a disease of domesticated poultry such as fowl and turkeys. Turkeys followed by chickens are the most susceptible species. Quails, guinea fowls, pheasants, partridges, geese and ducks (Mallards and Muscovy) are also susceptible. Ostriches, passerine birds and pheasants can also be infected (Alexander, 2000; Dhama *et al*., 2005, 2008a). Virus is highly contagious and can spread very fast and easily could cross the continents. AIV is usually introduced into one country through wild fauna and subsequently spreads to the domestic flocks directly via wild and domestic birds or indirectly through contaminated fomites (Munster *et al*., 2007; Feare, 2007). Virus is transmitted by direct contact between infected and susceptible birds or indirect contact through air borne droplets or exposure to contaminated feed and fomites, secretions, aerosols, faeces, water, equipments and clothings. Faeco-oral is the major route of transmission. One gram of infected bird feces is capable of infecting one million (10 Lakh) susceptible birds. Wild/migratory/free flying birds, ducks and geese, are important reservoirs of influenza viruses, these birds constantly shed virus through their respiratory secretions and droppings which helps virus to persist in environment (Brown and Stallknecht, 2008; Dhama *et al*., 2008a). AIV H5N1, H5N2, H5N3, H5N9, H7N1, H7N2, H7N3, H7N4, H7N7, H9N2, H10N7 and also H1N2, H2N2, H6N1 and H3 subtypes have been reported in poultry (Dhama *et al*., 2005, Alexander, 2007; Weber and Stilianakis, 2007; Pawaiya *et al*., 2009; Tiwari and Dhama, 2012).

Role of migratory birds in the spread of bird flu: It is indicative that H5N1 viruses are now being transmitted between migratory birds (Dhama *et al*., 2005; Reperant *et al*., 2011; Cappelle *et al*., 2012; Tiwari and Dhama, 2012) and may pose a threat to humans (Sakoda *et al*., 2012). Spread of pandemic human strains of bird flu virus through wild birds however remains debated (Reid and Taubenberger, 2003). The most recent pandemic strain (H1N1) contains several segments that most likely originate in migratory birds. Intimate linkage is thus there among wild birds (migratory) and mammals

(including human) that governs dynamics of influenza infections (Vandegrift *et al*., 2010). More than one factor increases the risk of introduction or spread as well as maintenance of AIV via wild birds. These include: the species of susceptible animal; number and age of target individuals; characteristics of the geographical area of origin and destination; local (seasonal) abundance of that species and the gregariousness of the species during the breeding and migration and non-breeding seasons (Artois *et al*., 2009). In a specific geographical area, probability of spread of infection through contact with domestic avian species increases due to congregations of migratory wild bird population which are natural as well as principal reservoirs for most of the AIV subtypes (H1-H16 and N1-N9) (Dhama *et al*., 2005; Sengupta *et al*., 2007; Lee *et al*., 2008, Musa *et al*., 2009). Avian flu H5N1 virus outbreak in migratory waterfowl such as bar-headed geese, brown-headed gulls and great black-headed gulls in western China, killed nearly 1,500 migratory birds in 2005. In the United States, shore birds and great black-headed gulls are found to be reservoirs of subtypes H1-H13 and N1-N9 (Olsen *et al*., 2006; Krauss *et al*., 2007). However, majority of the AIVs that are circulating in migratory birds belong to Low Pathogenicity Avian Influenza (LPAI) viruses (Gonzalez-Reiche *et al*., 2012; Henaux *et al*., 2012).

DISEASE IN BIRDS (AVIAN/BIRD FLU)

The disease syndrome in birds ranges from asymptomatic/subclinical to mild upper respiratory illness and loss of egg production to a highly contagious and rapidly fatal systemic disease resulting in severe epidemics, with very high morbidity and mortality in the affected poultry flocks. Incubation period of the virus is few hours to 3 days (Alexander, 2000; Dhama *et al*., 2005; Capua and Alexander, 2007, 2009).

Highly pathogenic avian influenza (HPAI): HPAI is an acute, generalized, highly infectious and dynamically evolving disease of birds causing huge morbidity and mortality leading to very high economical losses. Infections with HPAI H5 and H7 subtypes can occur in poultry including other types of birds and result into the clinical picture of bird flu. These subtypes can replicate throughout the bird's body (pantropism), resulting in disease with a very high mortality rate. HPAI have multiple basic amino acids at the cleavage site, are cleavable by ubiquitous proteases and causes extensive damage to all systems and vital organs (Dhama *et al*., 2005; Eagles *et al*., 2009).

Clinical signs: In domestic poultry (chickens and turkeys primarily), there is sudden onset of severe illness, rapid death, high mortality and morbidity which may reach to 100% within few days. It is a fatal systemic disease affecting most organ systems including the nervous and cardiovascular systems. In peracute cases, birds are seen dead prior to observance of any clinical signs. Signs include severe respiratory distress/sounds, depression, coughing, sneezing, watery eyes and sinuses with excessive eye discharges, cyanosis of head, combs, wattles and shanks, edema/swelling of head, face and sinuses, ruffled feathers and diarrhoea (initially bright green, later white) and nervous signs. Drops in egg production occur in breeders and layers with typical declines including total cessation of egg production within six days (Alexander, 2000; Dhama *et al*., 2005; Gowthaman *et al*., 2009).

Lesions: Petechial hemorrhages of whole viscera and peritoneum with generalized congestion and periorbital edema are the most commonly observed lesions. In visceral organs, there are serosal or mucosal surface hemorrhages and necrotic foci within parenchyma. Especially prominent are the petechial hemorrhages on the epicardial fat, in pectoral muscles and in mucosa and around the ducts of proventricular glands. Death is usually by mutiple visceral organ failure (Alexander, 2000; Dhama *et al*., 2005; Mathew *et al*., 2006; Korteweg and Gu, 2008).

PUBLIC HEALTH SIGNIFICANCE

Influenza A viruses infects a variety of animal species including humans, pigs, horses, marine mammals and birds. The virus has expanded its host range and has infected dogs and other mammals. Only HPAI viruses have been reported to have zoonotic importance. Zoonotic potential of AIV was first observed in 1997, wherein six persons died out of eighteen affected following infection with HPAI (H5N1) in Hong Kong. Zoonotic alarm of bird flu virus mounted again in 2003 with human deaths in Vietnam (3) and China (2) with subsequently increasing number of human casualties till date, as reported in Thailand, Azerbaijan, Cambodia, China, Indonesia, Cambodia, Egypt, Iraq, Turkey, Nigeria, Pakistan, Laos and Bangladesh (Peiris *et al*., 2004; Dhama *et al*., 2005; Tiwari and Dhama, 2012). Till date (March 15, 2013), the bird flu virus has caused 368 human deaths out of a total of 622 confirmed human cases (nearly 60% Mortality Rate). Highest number of human casualties has been reported from Indonesia (160), Vietnam (61),

Egypt (61), China (29), Combodia (25) and Thailand (17) (WHO, 2008). The close proximity of birds to humans increases the risk of transmission to humans. Strains jumping to humans are limited to 4 HA types: H5, H7, H9 and H10 (AIV subtypes H7N2, H7N3, H7N7, H9N2 and H10N7). The exact conditions for human infection are not clear, but it would appear that these mostly occur in situations of high exposure to virus and oro-nasal route, affects predominantly the respiratory tract (breathing passages) and is acquired by virus inhalation (Beigel *et al*., 2005; Perdue and Swayne, 2005). Infected birds or egg/meat when handled possess more risk of transmitting bird flu than edible poultry products but well cooked food hardly transmits the disease (Beato *et al*., 2009; Taubenberger and Morens, 2010; Tiwari and Dhama, 2012).

PANDEMIC THREAT

Bird flu virus still has not attained the capability to undergo human to human transmission in a rapid/pandemic manner as during 1918 'spanish flu' (H1N1 killed 40-50 million persons globally in 2 years). A completely new subtype could be generated when avian and human influenza viruses exchange genes during a co-infection of a person with both viruses. Newly evolving virus if contains sufficient human genes, spread directly from one person to another can occur, in a rapid, easy and vicious manner and human flu could acquire the deadly virulence of the avian flu-the start of a new influenza pandemic could begin therein. A change in viral receptor specificity from avian type (α-2, 3 sialic acid receptor) to human type (α-2, 6 sialic receptor) could make a probable deadly pandemic. The disease spreads explosively due to the world population being 'immunologically naive' (Dhama *et al*., 2005; Sellwood *et al*., 2007; Iwami *et al*., 2008; Taubenberger and Kash, 2010; Tiwari and Dhama, 2012).

Vaccination of persons at high risk of exposure to infected poultry, using existing vaccines effective against currently circulating human influenza strains, could reduce the likelihood of co-infection of humans with avian and human influenza strains. Influenza epicenter is the region where birds, other animals and humans live closely together. Ducks/chickens with pigs sharing ponds into which they discharge household wastewater, including human and pig excreta and the bird's faeces, the significant carriers of viruses, can very well contribute to the development of a reassortant virus (World Health Organization Global Influenza Program Surveillance Network, 2005). In generating reassortants of novel flu virus, role of birds become evident due to past pandemics; and in last 40 years the first global pandemic is recorded caused by H1N1 triple human/avian/swine reassortant virus (of human) resulting in substantial illness, hospitalizations of millions of peoples and thousands of deaths throughout the world (Beveridge, 1991; Dhama *et al*., 2005, 2008a; Vijaykrishna *et al*., 2008; Pawaiya *et al*., 2009; Centers for Disease Control and Prevention, 2010; Tiwari and Dhama, 2012).

CLINICAL FEATURES OF BIRD FLU IN HUMANS

The incubation period for AIV in humans is probably between 3-7 days. A rapid onset of severe viral pneumonia with a high fatality rate is seen in humans affected with bird flu. Typical influenza-like symptoms are observed: Fever, sore throat, cough, breathing problems (acute respiratory distress), chest pain, acute respiratory distress, muscle aches, malaise, fatigue and lethargy. Eye infections (conjunctivitis), severe bilateral pneumonia, myocarditis (inflammation of the heart muscle) and other severe and life-threatening complications can occur (Dhama *et al*., 2005; Malik Peiris, 2009; Riquelme *et al*., 2009; Tiwari and Dhama, 2012).

DIAGNOSIS

Tentatively diagnosis is based on the clinical signs and very high flock mortality. Confirmatory diagnosis requires isolation, identification and characterization of the virus from suspected samples (tracheal/cloacal swabs, feces, tissue samples including trachea, lungs, etc.). Definitive diagnosis requires direct detection of AI viral antigen/nucleic acid in affected tissues, swabs, clinical samples and inoculated cell cultures or embryonating chicken eggs (Babiuk *et al*., 2003; Dhama *et al*., 2005; Kataria *et al*., 2005a, b; Schmitt and Henderson, 2005; Brown, 2006; Suarez *et al*., 2007). Hemagglutination (HA) test done with the infected chicken embryo allantoic fluid during virus isolation indicates towards the hemagglutinating nature of the virus. Hemagglutination Inhibition (HI) assays using reference AIV antiserum, confirms the virus infection. For demonstration of viral antigen in the suspected clinical samples, the techniques employed are Agar Gel Immunodiffusion (AGID), Immunofluorescence Test (IFT), Immunoperoxidase Test (IPT) and Enzyme-linked Immunosorbent Assays (ELISA). Detection of antibodies to AI virus by AGID and HI tests are of significant value. Virus Neutralization Test (VNT), IFT, IPT and ELISA are important diagnostic tools (He *et al*., 2007; Alexander, 2008; Dhama and Mahendran, 2008). Subtyping of AIVs can be done by

using mono-specific antisera prepared against antigens of each of the 17 HA and 10 NA can be used in AGID. Haemagglutination and neuraminidase inhibition (HI/NI) tests against a battery of polyclonal antisera are also useful in this direction. By Reverse Transcription-polymerase Chain Reaction (RT-PCR), real time -PCR, PCR-ELISA and other molecular tools also subtyping can be achieved (Alexander, 2008; Cattoli and Terregino, 2008; Dahlhausen, 2010; Tiwari and Dhama, 2012).

Molecular tools for AIV detection: The virus can be identified by employing RT-PCR using a set of primers specific to the Nucleoprotein (NP) gene or matrix gene. RT-PCR has been used for identification and HA-subtyping which can be further confirmed by sequence analysis (Dhama *et al*., 2005, 2008b; Alexander, 2008). Subtypes H5 and H7 can be detected by H5 and H7 primers covering the cleavage site of the HA gene, by the presence or absence of multiple basic amino acids determined by sequencing of PCR product (Sidoti *et al*., 2010). *In situ* hybridization assay is also available for detection of AIV. An H9-based RT-PCR-ELISA has been found highly sensitive when compared to virus isolation method in detecting H9N2 AIV. RT-PCR Heteroduplex Mobility Assay (HMA), M gene RT-PCR, can be used to detect and partially characterize influenza A viruses from different species (human, avian and swine influenza A viruses) and offers a rapid and sensitive means for screening for novel or unusual influenza viruses. Multiplex-PCR (m-PCR) including two or more primer pairs specific for target sequences of different viral pathogens has been developed for detecting H5 and H7 AIVs (Rashid *et al*., 2009). Nucleic acid sequence-based amplification (NASBA) s a rapid and sensitive method of detection as well as identification of pathogenic influenza viruses, can detect a portion of the HA gene of AIV subtypes H5 and H7 (Cavanagh, 2001; Kataria *et al*., 2005b). NASBA/ECL (electrochemiluminescent detection) is an isothermal technique especially suitable for amplifying RNA, from a diverse range of sources.

RRT-PCR (Real time-RT-PCR), a real-time detection has eliminated need for post-PCR screening by electrophoresis allowing definitive confirmation of a virus within minutes and is highly useful for AIV surveillance and monitoring programs. LUX (Light Upon eXtension) real-time RT-PCR utilizes lux (light upon extension) fluorogenic primer for rapid detection of AIVs in real-time PCR assays. Sequencing and phylogenetic analysis helps in tracing the origin of virus and gemomic relatedness/lineages (Kim *et al*., 2008; Munster *et al*., 2008; Takekawa *et al*., 2010). Loop-mediated Isothermal Amplification (LAMP) and Real-time RT-LAMP assay for AIV H5 and H7 strains holds some promise for routine veterinary diagnostic purposes (Capua and Alexander, 2004; Postel *et al*., 2010). Sensitivity and specificity odf real time RT-PCR assays are more and at the same time it is cost effective and can rapidly detect and screen H5 and H7 isolates (alternative to isolation of virus) during outbreaks due to influenza A virus. To understand in a better manner the involvement of wild birds in HPAI H5N1 transmission and to tally the results of surveillance portable real-time RT-PCR (with lyophilized reagents) is helpful. DNA micro-arrays are also being developed (Peeters, 2008). Recently, a rapid H5N1 bird flu test kit (real-time RT-PCR assay based), detecting all known strains of H5N1 virus with a single test and with almost 100% accuracy, has been reported to be developed for diagnosing bird flu within a few hours in humans (Tiwari and Dhama, 2012).

Proper collection and dispatch procedures need to be followed for clinical samples of bird flu, also care should be taken to prevent leakage and the spoilage during transport. Samples must be sent at earliest and appropriately preferably by special messenger to the referral laboratory. Diagnosis of avian flu requires referral laboratories equipped with trained scientific manpower and minimum level 3 biosecurity (BSL-3) measures. Suitable samples are required to be sent worldwide in designated referral laboratories in order to diagnose the disease timely and precisely (Dhama *et al*., 2005; Tiwari and Dhama, 2012).

DIFFERENTIAL DIAGNOSIS

Differential diagnosis of avian influenza should be done from Newcastle disease virus, avian pneumovirus and other parmayxoviruses, infectious laryngotracheitis, infectious bronchitis, *Chlamydia*, *Mycoplasma* and fowl cholera (Dhama *et al*., 2005; Kataria *et al*., 2005b; Centers for Disease Control and Prevention, 2007; George, 2012).

TREATMENT

No specific drug therapy is practiced in birds. To reduce the secondary bacterial infections, antibiotics and supportive therapy have been recommended. Anti-Flu drugs (Amantadine, Rimantadine, Zanamivir/Relenza and Oseltamivir/Tamiflu) may be used both to prevent people from catching bird flu and to treat those who have it. They work best if given within 2 days of becoming ill, but may be given later if illness is severe or for those at a

high risk for complications. They can turn the illness into a milder form and help in preventing serious complications. These must strictly be prescribed by a medical doctor only. Antipyretic medications and suitable anti-inflammatory drugs are also prescribed. Relenza and Tamiflu are neuraminidase inhibitors which check the flu viruses from multiplying within the host cell and are found to be most effective. Tamiflu is the drug of choice (Dhama *et al*., 2005; Hsu *et al*., 2012; Jarhult, 2012). Ayurveda plays a substantial role in promoting immunity of the host. Various herbs for boosting immunity include: basil (*Ocimum basilicum*); Ginger (*Zingiber officinalis*); garlic (*Allium sativum*); Gooseberry (*Embelica officinalis*); Aloevera (*Aloe barbadensis*); Camphor (*Cinnamomum camphora*) and Eucalyptus oil; Ginkgo (*Ginkgo biloba*) leaf extract; Red Sea grass (*Thallasodendron ciliatum*); flavanoids of various plants and acidic polysaccharides from green algae (*Coccomyxa gloeobotrydiformi*) (Parmar *et al*., 2011; Costa *et al*., 2012; Haruyama and Nagata, 2012; Ibrahim *et al*., 2012; Komatsu *et al*., 2013).

PREVENTION AND CONTROL

Pre-requisites for effective control programs aimed at eradication of AI virus infection in poultry include disease awareness, early detection, culling and stamping out, proper disposal of affected birds, timely notification, strict biosecurity measures, isolation, zoning and quarantine, control of live bird market and judicious vaccination strategy. Continuous global surveillance of influenza is a key factor (Dhama *et al*., 2005; Graham *et al*., 2008; Tiwari and Dhama, 2012). The best way to check HPAI from spreading is to prevent exposure of flocks and rapid elimination/culling of the virus infected birds which are essential for preventing a major outbreak (Chen *et al*., 2006). Adapt the key principles of biosecurity i.e., isolation, traffic control and sanitation. Follow strict cleanliness, good sanitation and hygienic practices, along with suitable decontamination and disinfection procedures on the farm. Virus spreads via movement of birds, crates or vehicles/trucks to other farms and/or market, therefore necessary precautions need to be adapted. Vehicles coming from other poultry farms or poultry market should be sanitized before and after arrival.

Prevention of the exposure of poultry flocks is the best measure to eliminate the virus infected birds. Human traffic need to be checked and visitors are to be avoided. Employees and crews should wear clean clothing supplied at the farm each day. Disinfectant boot dips should be placed to reduce the probability of introducing and spreading the infection. Contact of poultry with migratory/wild/free flying birds and waterfowls should be avoided (Dhama *et al*., 2005, 2008a; Munster *et al*., 2007; Krauss and Webster, 2010). The accumulation of standing and stagnant water should be prevented as it is a great source of attraction to migrating waterfowl and shorebirds. Employees of the poultry farm house need to be educated about the dangers of live birds markets which are potent source of AIV infection. Sick or dying and dead birds should be appropriately and immediately submitted to recognized laboratories for a timely diagnosis. All the infected or exposed poultry flocks should be culled and slaughtered (stamping out) following the prescribed procedures appropriately and timely. Dead birds should be properly disposed off following burial or incineration methods. Cleanliness and sanitation/hygienic measures should be upgraded at the farm level with follow up of washing of hands and feet frequently with soap and water and suitable disinfectants after handling affected birds or contaminated materials. Surveillance and monitoring of Avian influenza virus should be followed regularly to know the disease status (Kataria *et al*., 2005b; Tiwari and Dhama, 2012). Epidemiological investigations with strict biosecurity measures are followed to prevent further spread. Stamping out of all domestic poultry is applied in an approximately 3-km-radius zone around the outbreak, with an exhaustive supervision and monitoring campaign in a 10 km radius zone. Prohibition needs to be imposed on sale and transportation of poultry products and closure of poultry markets in the infected zone. Disinfection of premises after culling of birds is an important aspect. Restocking is advised in accordance with a specified protocol and period (Kataria *et al*., 2005a; Bunn *et al*., 2011).

Disease management for preventing the 'bird flu' outbreaks: Bird flu being a 'Notifiable' disease therefore, any suspected disease condition or an outbreak should be immediately reported to the regulatory authorities and officials and leave handling of the poultry to experienced personnels (veterinarians, cullers, clean-up personnel etc.). Only trained veterinarians and professionals wearing protective gloves, masks etc., as biosafety measures should handle suspected birds. Affected poultry birds should not be necropsied in the field. In case, bird flu is detected in any country all the movements of birds from area where disease has appeared should be strictly restricted. AIV infected or exposed poultry flocks should be culled and slaughtered (stamping out). Field veterinarians should be trained for collection and dispatch of appropriate samples and the suspected/clinical samples should be immediately processed for timely diagnosis (Dhama *et al*., 2005;

Kalthoff *et al*., 2010). While handling dead or sick poultry follow appropriate safety measures such as wearing protective clothing, gloves, face masks (nano masks), goggles, gown, rubber boots etc (MacMahon *et al*., 2008). Confine live birds being submitted to laboratory in boxes that will not return to farm. Dead bird should be put in a leak proof plastic bag, double packed, sealed and transported under chilled conditions immediately to the investigation laboratory. Assistance from local animal husbandry authorities should be sought on how to bury dead birds safely and appropriately. Local authorities should keep under close monitoring persons having exposure to bird flu virus infected chickens and suspicious farms. Liaison with neighboring countries for international trade should be monitored to check the influx of avian influenza (Koh *et al*., 2008). Cross border trades with affected countries should be strictly regulated or banned completely. Strict surveillance and vigilance for bird flu virus are required at international airports as well as in railways and land transport (Gowthaman *et al*., 2010; Dhama *et al*., 2013b). Mass media should take part in providing public awareness widely in order to prevent and control spread of bird flu to humans. Along with it education and training programmes should be organized for veterinary para-professionals, farmers, marketers, poultry transport contractors, egg collectors and concerned professionals. Thorough cleanliness and heightened sanitation and hygiene measure should be adapted; specifically hands should be washed with a soap/detergent every time after handling any contaminated items (Kataria *et al*., 2005a; World Health Organization Global Influenza Program Surveillance Network, 2005; WHO, 2005; Bunn *et al*., 2011; Tiwari and Dhama, 2012).

Pandemic preparedness: The persons to be included for bird flu pandemic preparedness include: virologists and epidemiologists experts from human and animal health professionals; military and paramilitary forces; representatives of NGOs; press and media persons and administration (Dhama *et al*., 2005; Rebmann *et al*., 2012; Tiwari and Dhama, 2012). Extension works and strategies must be employed in order to fill the gaps in knowledge about the pandemic and vaccine particularly in underdeveloped nations where there is evidence of substantial disparities in education and media access (Kouassi *et al*., 2012; Cantey *et al*., 2013). There is a need for developing simple and easy to use tests for the characterization of emerging influenza strains as the bird flu virus is a very dynamically evolving and changing virus (Mak *et al*., 2012). Safe international trade in the least trade restrictive manner controls the international movement of birds and products which should be based on OIE recommendations. Measures may be modified in the light of specific risk assessments and agreements between trading partners. In order to develop disease control and prevention strategies Veterinary authorities must consider the cross-boundary leakages. There is need to implement measures to prevent wild bird populations from infecting domestic poultry (WHO, 2004; Dhama *et al*., 2005, 2008a). It is essential to thoroughly understand the factors that contribute to the willingness of health care professionals to work during an influenza outbreak and is critical in planning for pandemic preparedness (Devnani, 2012; Godderis and Rossiter, 2013). It is also important to have overall tight coordination along with communication as well as integration and alignment in any management structure (Fieldston *et al*., 2012). Any country faces an extra burden on resources to control the disease during a pandemic but this situation may help in understanding of the disease and effect of preventive measures in order to contribute to global knowledge. The research directions needed may include virus transmission; antigenic and molecular characterization of virus strain; antiviral drug resistance and vaccine efficacy along with socioeconomic impact of the pandemic (Dhama *et al*., 2005; Fouchier *et al*., 2012; Van Gageldonk-Lafeber *et al*., 2012). By virtue of the differences in the poultry sector infrastructures, restructuring the poultry sector may be an important strategy to guard against the damaging effects of HPAI and requires different approaches at different levels of poultry sector in different countries. The general principles to be undertaken in this regard include: well-defined socio-economic impact analysis; government commitment with stakeholders' full support as well as collaboration between private and public sectors and above all public awareness. For enforcing measures to control animal diseases and to support trade both within regions and globally, the need to strengthen regulatory policies is recognised by many countries that are directly affected or are at risk. Realigning the veterinary regulations and policies to meet world trade organization (WHO)/OIE standards is the need of hour for this reason. These mechanisms include: quality and evaluation of Veterinary Services along with animal quarantine and institutional reforms; introduction of OIE standards, guidelines and recommendations for international as well as domestic trades; certification for exports and designation of disease free zones and compartments. For creating necessary cordial environments, long-term national or regional HPAI control or prevention interventions should be supported strengthened (Dhama *et al*., 2005; Pawaiya *et al*., 2009).

Salient precautionary measures for general public: Appropriate sanitation, hygiene and safety measures need to be followed during bird flu outbreaks to avoid AIV infection. During a disease epidemic, avoid going to poultry farms and markets where birds are sold and make sure to keep children away from dead or sick poultry/birds. During laying, egg shells may be contaminated with bird's faeces that contains sufficient amount of virus, thus the live bird as well as poultry products including eggs, egg products, chicken and duck meat and objects contaminated with faeces from infected birds can carry disease spreading virus. Proper cooking procedures destroy the virus in poultry meat and eggs, therefore good kitchen hygiene practices and eating properly cooked eggs and poultry meat/products need to be encouraged (Dhama *et al*., 2005; Chmielewski and Swayne, 2011; Tiwari and Dhama, 2012). Thorough and frequent hand washing using suitable disinfectants need to be practiced especially by food handlers at home and in restaurants which should become a routine practice to help avoid infection. All persons exposed to AIV infected chickens or to farms under bird flu suspicion should be under close monitoring by local health authorities (Kataria *et al*., 2005a; MacMahon *et al*., 2008).

Vaccination: Inactivated AIV vaccines are the commonly used vaccines throughout the world at present. Live conventional influenza vaccines against any subtype are not recommended in birds. Inactivated homologous (same field strain), heterologous (same H but different N-'marker vaccine') and oil emulsion vaccines have been developed for use in avian species. Inactivated monovalent and polyvalent viral vaccines, with adjuvants, are capable of inducing antibody (Dhama *et al*., 2005; Kataria *et al*., 2005b). Haemagglutinin (HA) based vaccines protect against a broad array of homologous HA subtype viruses, but provide poor protection against a heterologous HA virus. It is not practical to use preventive vaccination against all possible AIV subtypes. For stopping the spread of disease and for improving chances of eradication, vaccination combined with selective culling is effective. Widespread use of vaccine against HPAI is being used with increasing frequency in countries experiencing large disease outbreaks of H5N1. Routine use of AI vaccine in poultry production system is rare (Dhama *et al*., 2005; Kataria *et al*., 2005b). Virus infection can not be prevented completely by the use of commercial AIV vaccines but certainly multiple goals can be achieved if vaccines are used properly. Newer vaccines include recombinant vaccines, DNA vaccines, reverse genetics based vaccines, vector vaccines, or subunit vaccines and DIVA (differentiating infected from vaccinated animals) strategy (Dhama *et al*., 2005, 2008b; Peeters, 2008; Tiwari and Dhama, 2012; Yang *et al*., 2012). Gene-deleted mutants allow the use of live bird flu vaccines (Swayne, 2004). During the use of such vaccines it should be kept always in mind that there are inherent dangers for gene reassortment with field viruses in the generation of disease-causing strains. A different Neuraminidase (NA) is used in the vaccine to differentiate with the field virus infection by looking for specific antibodies against the NA of circulating field virus in the vaccinated birds in order to allow the differentiation of infected from vaccinated flocks (Capua *et al*., 2003). DIVA strategy using these kinds of marker vaccines with a heterologous strain differing in NA from the circulating field virus during the outbreaks of H7N1 is useful (Marangon *et al*., 2003). This strategy can help countries to escape from trade restrictions. 'Field' strain (similar H subtype but different N subtype) is incorporated in vaccines for DIVA strategy (Capua and Marangon, 2003; Bano *et al*., 2003; Capua *et al*., 2004).

In the recent past, there have been development of vectored bird flu vaccines using Fowl Pox Virus (FPV) and Infectious Laryngotracheitis Virus (ILTV), baculovirus; vaccinia virus and new castle disease virus (NDV) expressing H5, H7 of bird flu virus hemagglutinin gene insert (Swayne *et al*., 2000a; Veits *et al*., 2003; Dhama *et al*., 2005; Cornelissen *et al*., 2012). These vaccines however replicate poorly in birds that have had field exposure (Swayne *et al*., 2000b). A single dose of plasmids expressing H5 and H7 hemagglutinins can protect the birds from infection by either subtype (Kodihalli *et al*., 2000). For the development of candidate vaccine viruses against the HPAI viruses including H5N1 subtype virus the reverse genetics techniques have been exploited (Nicolson *et al*., 2005; Tian *et al*., 2005). The reassortant viruses generated using this technology, containing the same H5 and H7 hemagglutinin gene as the challenge virus, but a heterologous neuraminidase gene, can help in differentiating the infected and vaccinated birds (DIVA strategy) (Lee *et al*., 2004). To control the spread of avian influenza and ND alike recombinant NDV expressing HA of AIV/H5N1 generated through reverse genetics can be useful (Ge *et al*., 2007). India has not adapted vaccination for controlling AIV and followed culling and containing the virus in affected areas and preventing further spread (Meeusen *et al*., 2007; Murugkar *et al*., 2008; Tiwari and Dhama, 2012).

CONCLUSION

Disease outbreaks of bird flu, its public health impacts with probable potential of a deadly human pandemic have created an alarming situation worldwide. Continuous global efforts are on the way so as to better

understand about the basic knowledge of the virus focusing on its pathogenesis, genetic versatility, zoonosis, pandemic potential, therapy and control possibilities. AIV has caused havoc in poultry industry recently leading to enormous economic losses worldwide and in last ten years only the H5N1 subtype has affected more than 60 countries with losses of more than 300 million birds and 360 human lives. This is indicative of the fact that flu virus is becoming more and more dangerous, especially in south-eastern Asian countries. Prevention and control strategies focus on strict biosecurity, adequate disease surveillance, timely diagnosis, appropriate culling measures and judicious vaccination practices along with adequate public health and biosafety measures. Even though commercial antiviral drugs such as Zanamivir and Oseltamivir are available for treatment of the human flu, effective vaccination strategies together with careful and far-sighted disease prevention and control measures is the need of the hour. This may help to wipe down the bird flu virus before it re-emerges with a changed genetic make-up (reassortant or mutant virus) with much more lethal virulence or killing ability and acquire human to human transmission abilities that too in a rapid way to cause a devastating pandemic. Such kind of a probable pandemic if happens practically then the situation could be much dangerous for human life and the existence of mankind. The avian flu virus is posing a great challenge due to its high mutational abilities, various combinations and many subtypes, interspecies transmissions which are all the impeding factors for developing an effective prophylactic strategy. Being a global problem and a huge challenge, the solutions for bird flu requires international and coordinated efforts, collaborative projects and integrated approaches. Since the appearance of this disease is unpredictable, these responses must be prompt and timely, well planned and complete. For tackling bird flu, multidisciplinary approach, effective co-operation and networking among scientists, veterinary/medical and public health officials, wildlife specialists, avian disease experts, life science researchers and the regulatory authorities is required. These measures adapted on priority would help in preventing and eradicating the bird flu and restricting its zoonotic impact. Now-a-days, advancements in biotechnology and molecular biology have provided rapid and confirmatory diagnostics for this deadly disease. The recent advances in vaccinology and biotechnology are being exploited for developing effective and safer vaccines for protecting birds as well as the humans against the AIV H5N1 subtype. Formulation and adaptation of effective and sound strategies for preventing and controlling bird flu would alleviate the economic losses to the poultry industry as well as save the precious lives of millions of people living under a possible and deadly pandemic threat.

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