AVIAN INFLUENZA: A CONTINUING THREAT

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Avian influenza commonly known as bird flu is a viral disease caused by *Influenza* Aviruses of *Orthomyxoviridae* family(Lamb and Krug, 2001).It is one of the most important diseases of poultry that negatively impacts poultry health and international trade of poultry and poultry products.Many species of birds are susceptible to *Influenza*A virus: aquatic birds being the major reservoir of the virus.*Influenza* A viruses infecting poultry are further classified as highly pathogenic avian influenza (HPAI) or low pathogenic avian influenza (LPAI) depending on their ability to cause disease in chickens. The HPAI causes mortality as high as 100% in poultry species. The disease course varies as seen in HPAI as sudden death with sometimes no clinical signs to a more characteristic one with variable clinical presentations depending on the species, age of the host and viral strain involved. In LPAI infection the disease may be seen as drop in egg production to mild respiratory symptoms. Viruses of H5 and H7 subtypes have been shown to cause HPAI in susceptible species, however not all H5 and H7 viruses are highly pathogenic (Peiris*et al.,* 2007).The influenza virus is continually evolving and under immune pressure; it may either evolve through small gradual changes in the virus (antigenic drift) or through abrupt major changes in the virus (antigenic shift) most frequently by genetic reassortments (CDC, 2010). Such changes can result in the emergence of new influenza viruses that can cause pandemics (CDC, 2010).Extensive research on avian influenza has revealed many facts about the disease and its causal agent and its thorough understanding would help in a pandemic preparedness. The present review briefly describes the disease and its status in India.

Historical background

Highly pathogenic avian influenza in chicken was first described by Perrocinto in 1878, though it is believed that prior outbreaks due to HPAI virus might have occurred in Italy and European countries. The disease was termed ‘‘fowl plague’’ and wasconfused with the acute septicemic form of fowl cholera (Perroncinto, *et al.,* 1878).The causative agent was identified as virus in the year 1901 and as type A influenza virus in 1955 (Centanni and Savunozzi, 1901; Schafer, 1955). Major outbreak of HPAI occurs during 1901 to 1930s with a human pandemic in 1918 (Taubenberger and Morens, 1918) but the first confirmed outbreak of HPAI was reported in Scotland in 1959 (Becker and Uys, 1967). Later, through intensive surveillance it has been recognised that wild birds harbour all identified subtypes of influenza virus (Dasen and Laver, 1970; Easterday*et al.,* 1968; Zakstel’skaja*et al.,* 1972). This has led to a change in the classification of viruses. Earlier, classification of influenza viruses were based on the species of origin and the antigenic properties ofthe nucleoprotein for typing and haemagglutinin (HA) and neuraminidase (NA) proteins for subtyping which has been modified to a unified system of classification regardless of species of origin (WHO, 1971; 1980). In the year 1981 there was the first international symposium on avian influenza where the name highly pathogenic avian influenza was proposed to substitute fowl plague. Since 2003 many Asian countries have reported outbreaks of HPAI H5N1 virus and it became endemic in Asia (Lupiani and Reddy, 2009).

Etiological agent

The *orthomyxoviridae* family contains five genera classified by variations in nucleoprotein

(NP and M) antigens: *Influenza virus A*, *Influenza virus B*, *Influenza virus C*, *Thogotovirus* and *Isavirus* (Swayne and Halvorson, 2003; Alexander, 2007) Classification of Influenza virus types are made on the basis of antigenic properties of nucleoprotein (NP) and matrix (M) proteins (Cox *et al.,* 2000; Lamb and Krug, 2001). Type A influenza viruses have a wide host range including birds and mammals. Type B viruses affect human beings only whereas type C viruses affect human beings and pigs. Isaviruses infect fish while Thogotoviruses are tick-born arboviruses infecting livestock and humans (Kawaoka*et al*., 2005). Out of the three influenza types only Influenza A viruses are known to infect birds (OIE, 2014). Influenza A viruses exhibit a variety of shapes and sizes, ranging from fairly spherical particles of 80-120 nm in diameter to elongated filamentous forms depending on the virus strain and passage history (Cox *et al.,* 2000). The virion is enveloped with two prominent surface glycoproteins: rod-shaped trimers of haemagglutinin (HA) and mushroom-shaped tetramers of neuraminidase (NA) (Padhi*et al.,* 2004). Both HA and NA are major determinant of a protective immune response. *Influenza A* viruses are further subtyped based on antigenic properties of HA and NA. There are a total of known 18 HA and 11 NA serologically distinct influenza virus subtypes and any combination of HA and NA is possible (Tong *et al.,* 2013).Of these 18 HA subtypes, two subtypes H3 and H7 infect equines; six subtypes H1, H2, H3, H5, H7 and H9 infect humans and five subtypes H1, H2, H3, H5 and H9 infect pigs. The latest introductions of H17-18 have been identified in bats. Of the 11 NA subtypes, N1, N2 and N7 subtypes, including a unique H1N2 reassortant, had been isolated from pigs in nature and four NA subtypes N1, N2, N3 and N7 were isolated from humans (Tong *et al.,* 2013.,Brown *et al.,* 1997, Fouchier*et al.,* 2004)The viral genome is a single stranded segmented negative sense RNA molecule contained within the viral envelope. Each of the eight segments of the gene codes for 10 different proteins viz. PB2, PB1, PA, HA, NP, NA, M1, M2, NS1 and NEP (Krug *et al.,* 2003). A recent addition of protein is PB1-F2 encoded by the +1 open reading frame (ORF) of PB1 (Bouvier and Palese, 2008). The genome is in tight association with the NP and three polymerase proteins (PB1, PB2 and PA) forming ribonucleoprotein (RNP) complex. The RNP complex is the basic functional unit of replication and is responsible for transcribing messenger RNAs (mRNAs), synthesizing complementary RNAs (cRNAs) which are further transcribed into viral RNAs and finally assembled into progeny viruses. The matrix protein (M1), which lies inside the lipid envelope, is associated with both the RNP and the viral envelope. The M1 protein plays a fundamental role in virus assembly. The NS2 protein, also known as the nuclear export protein (NEP), wasearlier thought to be a non-structural protein, but is present in small amounts in the virions in association with the RNP through interaction with theM1 protein. The NS2 functions to mediate the export of newly synthesized RNPsfrom the nucleus (Lee *et al.,* 2008).Among the viral proteins encoded, only NS1 is truly non structural protein with numerous functions (Krug *et al.,* 2003). The NS1 is a regulator of both mRNA splicing and translation, and also plays a critical role in competing against the cell's antiviral defence and is thus directly related to the pathogenicity of the influenza strain(Palese, 2006). The schematic representation of the virus is presented.

Pathogenicity

Avian influenza has been currently defined by OIE Terrestrial Animal Health Code as an infection in poultry by any influenza virus either highly pathogenic Influenza A virus (HPAI) and low pathogenic H5 and H7 subtypes (H5/H7 LPAI). Till date, naturally occurring highly pathogenic avian influenza have been associated with H5 and H7 subtypes. Though most viruses of the H5 and H7 subtype have been of low pathogenicity for poultry, there is always a risk of becoming highly pathogenic

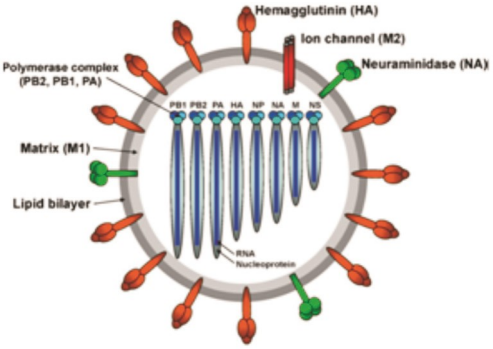

Fig 1. A schematic diagram of the structure of the influenza Avirus. Lee C-W, Saif YM, Avian influenza virus, Comp Immunol Microbiol Infect Dis. 2009 Jul;32(4):301-10.

by mutation (OIE, 2014). The disease course of avian influenza in poultry ranges from asymptomatic to fatal cases (Swayne and Halvorson, 2003). Incubation period is generally short varying from few hours to weeks in poultry (Beard 1989). Typical signs in HPAI infection include ruffled feathers, respiratory signs, rales, excessive lacrimation, sinusitis, cyanosis of unfeathered skin, wattles and combs, edema of head and face, swelling of infraorbital sinuses, decreased egg production andin-coordination. Primary difference between low pathogenic (LPAI) and highly pathogenic avian influenza virus is in the cleavage site of HA protein. Post translational cleavage of HA molecules into HA1 and HA2 subunits is a major determinant in the spread of infection, tissue tropism and pathogenicity (Webster and Rott, 1987). HA of LPAI viruses are cleaved externally where appropriate extracellular proteases are present leading to a localized and less severe infections. HA of HPAI gets cleaved intracellularly in the Golgi apparatus by ubiquitous subtilisin like proteases. Thus HPAI viruses can replicate in a wider range of host tissues leading to a fatal systemic disease in poultry (Horimoto*et al.,* 1994).

Host range and transmission

Avian influenza viruses are distributed globally without any geographical boundaries and the infection with AI viruses has been detected in seventeen taxonomic orders of birds across all the seven continents (Abubakar*et al.,* 2009). Although it is basically a disease of birds (Alexander, 2000; Perkins and Swayne, 2002) viruses have been shown to infect humans, pigs, horses, mink, marine mammals (Kawakoa*et al.,*1990; Webster *et al.,* 1992) tiger, dogs, eagles (Krawford*et al.,*2005), cats (Kuiken*et al.,*2004) ferrets, mice, hamster and macaques (Thiry*et al.,* 2007). It infects a variety of birds including domestic poultry, free-living and captive birds. Influenza virus infectivity is primarily influenced by the type of linkage to galactose on the host cell surface (Skeheland Wiley2000). All AI viruses, with the exception of some viruses of the H5N1 and H9N2 subtype (Yamada *et al.,* 2006), possess HAs with high affinity for sialic acids attached to galactose sugars in the α2,3-linkage. In contrast, human influenza viruses have HAs which preferentially bind to sialic acid attached to sugars in the α 2,6-linkage. Thus, the adaptive mutations in their HAs, which result in preferential binding from the α 2,3- to the α 2,6- linkage, is crucial for enabling the switch from birds to human infection (Matrosovich*et al.,* 2000).There are reports of influenza virus isolation from 105 wild bird species of 26 families (Olsen *et al.,* 2006). The virus can survive longer in cold weather but fortunately there is no evidence of virus survivability in well cooked meat. In soil, water or contaminated equipments, AI virus can remain for weeks to months depending on temperature and humidity (Horimoto and Kawaoka 2001). Transmission of influenza virus between pigs and humans and chicken and humans have been demonstrated but not between wild birds and humans.There are reports on transmission of H5N1 to tigers, leopards and cats when fed with uncooked infected domestic fowl (Knobler*et al.,* 2005). The mechanism by which bird to bird transmission occurs depends on various factors like strain of virus, species of bird environment and is extremely complex. HPAI tend to show poorer transmission than LPAI owing to little virus excretion due to extremely rapid deaths in infected birds (Alexander, 2007). Faecal materials from infected birds directly contaminate soil, water leading to faecal/oral route of transmission infecting many aquatic birds (Horimoto and Kawaoka 2001).

Pandemic potential of HPAI virus

Influenza viruses of birds rarely infect human beings because of the difference in cell receptor binding requirements of the HA molecule which is determined by differing conformations of *N*-acetyl neuraminic acid residues on cell surfaces in different species (Naeve*et al.,* 1984). But a significant change in receptor preference can occur even with a single amino acid mutation at the HA receptor site (Harvey *etal*., 2004).Rapid point mutation of the virus, gene recombination between different virus subtypes and genetic reassortment diversified the virus subtypes and it has been reported that HPAI virus circulating in South East Asia have the ability to cross the species barrier from birds to humans causing severe disease with very high case fatality rate (Stohr and Esveld, 2004). Pigs act as a natural host of avian influenzavirus and the causative viruses for swine influenza are H1N1, H1N2 and H3N2 subtypes (Brown, 2013) which are antigenically

related to human and avian viruses. Pigs have receptors in their respiratory tract that bind swine, human, and avian influenza viruses (Ito *et al.,* 1998). Thus pigs can act as 'mixing vessels' in which genetic reassortment between human and/or avian and/or swine influenza viruses can occur and lead to the generation of new viruses (Ma *et al.,* 2009). HPAI strains like H5N1 pose a severe threat to human beings because of high pathogenicity, with mortality rates in humans exceeding 60% (Bahlky, 2009). The spread of avian influenza viruses from one affected individual to another has been reported very rarely, and has been limited, inefficient and unsustained (CDC, 2010). Most of the human infection have resulted from direct or close contact with infected poultry or contaminated surfaces (Kallon*et al.,* 2013). Major concern about HPAI is the potential to possibly change into a form of the virus that is able to spread easily from person to person. Avian Influenza viruses with a hemagglutinin against which humans have little or no immunity that have reassorted with a human influenza virus are more likely to result in sustained human-to-human transmission and pose a major public health threat of pandemic influenza (Taubenberger and Morrens, 2009).Novel influenza subtypes including H7N2, H7N7, H7N9, H9N2 and H10N7 are also found to have the potential to infect humans (Watanabe *et al.,* 2013). Genetic changes in avian influenza virus hamper treatment, prevention and control of the disease (Gavin and Thomson, 2004). It is currently impossible to predict which influenza virus will cause the next epidemic or pandemic, the pathogenic potential of these viruses can be anticipated more precisely with continued research and development in surveillance, diagnostics and genomic studies of the virus and its key hosts (Salomon and Webster, 2009).

Indian scenario

Diagnosis and surveillance on HPAI started in the year 2001 at ICAR-National Institute of High Security Animal Diseases, Bhopal. In India, only low pathogenic H9N2 could be isolated till the year 2006. First H9N2 was isolated from Haryana and Punjab in the year 2003 (Nagarajan*et al.,* 2009). HPAI H5N1 strain in poultry was first isolated fromNavapur sub-district of Nandurbar district of Maharashtra in the year 2006 (Pattnaik*et al.,* 2006). It was identified by phylogenetic analysis that the viruses isolated were grouped with H5N1 viruses from China. The virusmight have spread through migratory waterfowlsthat survived the HPAI H5N1 infection. These viruseswere able to replicate in cultured cells of avianand mammalian hosts and posses lysine at position627 of the PB2 protein, indicating that they might beable to cross the host barrier to infect mammals. In the subsequent years many outbreaks due to H5N1 have been reported from different states. This includes Manipur in 2007, West Bengal and Tripura in 2008, Assam, Sikkim and West Bengal 2008-2009, (Tosh *et al.,* 2007, Murugkar*et al.,*2008 and Nagarajan*et al.,* 2009, Dubey *et al.,* 2009). The outbreaks were seen almost every year thereafter too; in 2010 from West Bengal in 2011 from Tripura, Assam and again West Bengal, in 2012 from Odisha, Meghalaya and Tripura and Karnataka, in 2013 from Bihar and Chattisgarh and very recently from Kerala in November 2014 (http: // www.oie.int /). H5N1 has been isolated from many species in India and it was isolated from crow first time in 2008. (Nagarajan*et al.,* 2010) and subsequently it was also isolated in 2011 and 2012. (Das *et al.,* 2014). Since crow is a universal scavenger, it poses a threat of potential source for transmission if the virus gets adapted to this species. The H5N1 has also been isolated from other species like goose, turkey and ducks. (Tosh *et al.,* 2014). Phylogenetic analysis of the virus isolates revealed that two clades, clade 2.2 and clade 2.3.21 have circulated in Indian subcontinent. Clade 2.2 was isolated in India till the last out break from West Bengal in 2010 and thereafter in subsequent outbreaks only viruses belonging to clade 2.3.2.1 were isolated and characterised (Nagarajan*et al.,* 2012, Dubey *et al.,* 2009). The survivability of Indian H5N1 HPAI virus in dry and wet poultry faeces has been identified to be for 5 days at 24 °C and 8 weeks at 4 °C in dry and wet faeces (Kurmi*et al.,* 2013). First time a trial of various crude extracts of indigenous medicinal plants for their antiviral activity against HPAI H5N1 was conducted *in vitro* as well as *in ovo*. Cold and hot aquoueus extract of bark and hot aqueous extract of leaves of *Eugenia jambolana* showed 100% virucidal activity and antiviral potential of hot aqueous extract of bark of *E. jambolana* was demonstrated by a high selective index of the extract.(Sood *et al.,*

2012) In another study the cold aqueous extract of bark of *Acacia arabica*var. *indica* revealed 100% virucidal activity, hydro-methanolic extract of leaves of Azadirachtaindica showed 92% inhibition and hot aqueous extract of leaves of *Ocimumtenuiflorum*showed 58% inhibition of HPAI H5N1 in MDCK cells. (Sood *et al.,* 2013). As per the directives of Government of India immediate biosafety and biosecurity measures are enforced immediately in the areas of outbreak and culling in the perimeter of 1km is followed. In long run culling cannot be economically viable option as it affects the poultry industry and ultimately economy of the country. Vaccination is the second line defence for avian influenza and in a bid to keep the preparedness ready and an eventuality of a major outbreak work has been carried out at NIHSAD, Bhopal where antigenic cartography has been demonstrated for identification of the vaccine seed virus among the circulating viruses (Bhatia *et al.,* 2013). Phylogenetic analysis revealed that all the viruses isolated so far have 20-amino acid deletion in the NA stalk region, which is characteristic of adaptation of the virus to terrestrial poultry. Emergence of drug resistance has also been identified in Indian isolates and has been characterised genotypically as well as in cell culture (Tosh *et al.,*2011, 2014; Sood *et al.,* 2014). Oseltamivir and Zanamivir are the two currently approved drugs against influenza. In India drug resistance has been identified against both these drugs in viruses belonging to clade 2.2. Genotypic characterisation revealed two mutations; one at amino acid position 294 where Asparagine was replaced with Serine, and the other at position 119 where Glutamic acid was replaced by Alanine conferring more than 100-500 time decreased sensitivity to the drugs (Sood *et al.,* 2014).

A continuous surveillance of the susceptibility of the circulating influenza viruses is a must as it addresses an important human animal interface to keep a vigil not only in preparedness to pandemic outbreak but also in safeguarding the public health workers at the time of culling operations and wet market operations carried out during Avian Influenza outbreaks in the country**.** Control measures adopted during HPAI H5N1 outbreaks have been described in form of Action plan for preparedness, control and containment of avian influenza (Department of Animal Husbandry Dairying and Fisheries 2015). Further information of the disease can be obtained from the following web sites; http://dahd.nic.in/birdflue.htm, http://www.cdc.gov/flu, http://www.offlu.net/.

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A COMPARATIVE EVALUATION OF FAECAL B-GLUCURONIDASE ACTIVITY OF ADULT ALBINO MICE FED WITH PROBIOTIC (*BIFIDOBACTERIUM ANIMALIS* SUBSP. *LACTIS* B420), PREBIOTIC (INULIN) AND THEIR COMBINATION (SYNBIOTIC) *

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Abstract

A study was conducted to assess the effect of Bifidobacterium animalis subsp. lactis B420, inulin or their combinations on the activity of β- glucuronidase, a procarcinogenic enzyme, in the faeces of adult albino mice. Compared to control group, a statistically significant reduction (P<0.05) in the faecal β-glucuronidase activity was observed in all the three treatment groups after 10 days of administration of feed supplements and this effect was persistent throughout the treatment period. In view of their ability to reduce the faecal β-glucuronidase activity, it is suggested that administration of Bifidobacterium animalis subsp. lactis B420, inulin or their combinations can be potential options in reducing colon cancer risk.

Keywords: *Probiotics, prebiotics, synbiotics, β-glucuronidase activity.*

The gut microflora and their metabolism have a strong influence on the etiology of colorectal cancer -one of the major health problems in the world. As scientific evidences are found to link the occurrence of colon cancer to the imbalance of intestinal microbiota, dietary options like probiotics, prebiotics or synbiotics which modulate intestinal microbiota can be effective options, reducing the risk of colon cancer. Studies show that bacterial enzymes like β -glucuronidase, β-glucosidase, azoreductase and nitroreductase play an important role in cancer development as they hydrolyse pre-carcinogenic compounds to carcinogens. Of these β -glucuronidase, a commonly considered marker for procarcinogenic activity (Gadelle et al., 1985) is important in initiating colon cancer due to its wide substrate specificity and ability to hydrolyse different glucuronides. The present study was carried out to evaluate the effect of probiotic organism (*Bifidobacterium animalis subsp. lactis B420*), prebiotic substrate (inulin) and a synbiotic combination (i.e. inulin in combination with *Bifidobacterium animalis subsp. lactis B420*) on the faecal β -glucuronidase activity in adult albino mice.

Materials and Methods

Prebiotic and Probiotic

The prebiotic inulin was procured from Orafti, Belgium. The probiotic culture, *Bifidobacterium animalis* subsp. lactis B420 (B-420, Danisco, Germany) was maintained in

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