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A STUDY OF SEASONAL INFLUENZA AND HEALTH GIRLS, AWARENESS ABOUT ITS VACCINE IN AL-RIFAI DISTRICT

Sarah Aziz Askar Gouda
Sumer University College of Science , Department of Pathological Analysis
kk8956094@gmail.com

Zahraa Jasim Hammoodi Majeed
University of Karbala College of Applied Medical Sciences Department of Pathological analyses
zahraa.jas2001@gmail.com

Marwa Shaalan Ghali Abbas
Dhi Qar University / College of Science Department of Pathological Analysis
marwashalaan4@gmail.com

Qayed Darwish ḵhasara Malhak
Samarra University, College of Applied Sciences Department Pathological analyses
qaydalkhzrjy295@gmail.com

Zahraa Ahmed Mohsen Muhammad Ali
University of Kufa / College of Science / Department of Pathological Analysis
annnner8@gmail.com

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Abstract: The influenza virus (IV) is still of great importance as it poses an imminent threat to humans and animals. Among the three IV-types (A, B, and C) influenza A viruses are clinically the most important being responsible for severe epidemics in humans and domestic animals. Aerosol droplets transmit the virus that causes a respiratory disease in humans that can lead to severe pneumonia and ultimately death. The high mutation rate combined with the high replication rate allows the virus to rapidly adapt to changes in the environment. Thereby, IV escape the existing immunity and become resistant to drugs targeting the virus. This causes annual epidemics and demands for new compositions of the yearly vaccines. Furthermore, due to the nature of their segmented genome, IV can recombine segments. This can eventually lead to the generation of a virus with the ability to replicate in humans and with novel antigenic properties that can be the cause of a pandemic outbreak. For its propagation the virus binds to the target cells and enters the cell to replicate its genome. Newly produced viral proteins and genomes are packaged at the cell membrane where progeny virions are released. As all viruses IV depends on cellular functions and factors for their own propagation, and therefore intensively interact with the cells. This dependency opens new possibilities for anti-viral strategies

Keywords: -.

Introduction

Influenza, commonly referred to as the flu, is an infectious disease caused by RNA viruses of the family Orthomyxoviridae (the influenza viruses), that affects birds and mammals. The most common symptoms of the disease are chills, fever, sore throat, muscle pains, severe headache, coughing, weakness/fatigue and general discomfort. Although it is often confused with other influenza-like illnesses, especially the common cold, influenza is a more severe disease than the common cold and is caused by a different type of virus. Influenza may produce nausea and vomiting, particularly in children ,but these symptoms are more common in the unrelated gastroenteritis, which is sometimes called "stomach flu" or "24- hour flu". Typically, influenza is transmitted through the air by coughs or sneezes, creating aerosols containing the virus. Influenza can also be transmitted by direct contact with bird droppings or nasal secretions, or through contact with contaminated surfaces. Airborne aerosols have been thought to cause most infections, Influenza viruses can be inactivated by sunlight, disinfectants and detergents. As the virus can be inactivated by soap, frequent hand washing reduces the risk of infection .

Influenza spreads around the world in seasonal epidemics, resulting in the deaths of between 250,000 and 500,000 people every year, up to millions in some pandemic years. Three influenza pandemics occurred in the 20th century and killed tens of millions of people, with each of these pandemics being caused by the appearance of a new strain of the virus in humans. Often, these new strains appear when an existing flu virus spreads to humans from other animal species, or when an existing human strain picks up new genes from a virus that usually infects birds or pigs.

An avian strain named H5N1 raised the concern of anew influenza pandemic, Vaccinations against influenza ,The most common human vaccine is the trivalent influenza vaccine (TIV) that contain purified and inactivated antigens against three strains.

Typically, this vaccine includes material from two influenza A virus subtypes and one influenza B virus strain. Antiviral drugs can be used to treat influenza, with neuraminidase inhibitors being particularly effect.

1.2 Amis of study

Study types of seasonal influenza viruses ,genetic changes and vaccines

2.2 Influenza

Influenza, commonly known as the 'flu, is an acute febrile viral illness that affects the respiratory tract of birds and mammals. It usually occurs between autumn to spring and appears in epidemic form in humans which spreads in a specific community and sometimes in pandemic form causing (high levels of mortality and economic losses (Timbury, 1997).

In humans, it has been documented that epidemic 'flu is responsible for 3–5 million typical infections and 250,000 to 500,000 fatal cases each year (WHO, 2011). New influenza viral strains may be generated over time causing sudden pandemic outbreaks which spread easily among humans especially infants, elderly, and immunocompromised persons (Timbury, 1997, Smith et al., 2001).

Several pandemic outbreaks have been recorded and the most significant is the so–called ―Spanish flu‖, which occurred in 1918, killing more than 30 million people around the world. Other significant outbreaks were in 1957 (Asian flu), and in 1968 (Hong Kong flu); but they were less

severe than the 1918 pandemic avian influenza (Horimoto and Kawaoka, 2001, Horimoto and Kawaoka, 2005).

2.3 Avian influenza

The first description of avian influenza (AI) was in Italy in 1878 when researchers differentiated a disease of poultry from other diseases with high mortality rates (Alexander and Brown, 2009). The disease is highly contagious for poultry and associated with high mortality. It was named ―fowl plague‖ and it was initially confused with fowl cholera (avian pasteurellosis).

In 1880, according to the clinical and pathological properties, the disease was differentiated from fowl cholera and called Typhus exudations gallinarum. In 1901, scientists determined that a virus causes the disease, and in 1955, the classical fowl plague virus was confirmed to be a type A influenza virus based on the presence of type– specific ribonucleoprotein (Lupiani and Reddy, 2009).

The first isolation of influenza A virus from free–living wild ducks was in 1972 and at that time, numerous surveillance studies showed that wild birds including free– flying and shore birds are the natural hosts for all influenza A subtypes (Slemons et al., 1974).)

2.4. Influenza viruses

Influenza viruses belong to the "Orthomyxoviridae" family and are classified into five different genera: influenza A, influenza B, influenza C, Thogotovirus, and Isavirus (Cheung and Poon, 2007). They were initially isolated from pigs in 1931 and later from humans in 1933 (Shope, 1931, Smith W., 1995, Juozapaitis and Antoniukas, 2007). The most serious types that cause dangerous outbreaks with high morbidity and mortality are influenza A viruses because they mutate more rapidly and have a wider range of hosts (Khanna et al., 2008). Influenza A viruses infect animals, including birds, pigs, horses, whales, seals, and also humans (Ito and Kawaoka, 2000, Reperant et al., 2009).

Type B and C are generally found in humans, in addition to some mammals like seals, with less severity than influenza A. The infection is usually associated with a common cold–like illness, particularly in children (Greenbaum et al., 1998, Osterhaus et al., 2000). Wild aquatic birds of the order of Anseriformes (ducks, geese and swans) and Charadriformes (gulls, terns, surfbird and sandpiper) are considered to be the natural reservoir of all types of influenza A viruses. In these hosts, viral replication occurs mainly in the gastrointestinal tract, and to a lesser extent in the respiratory tract.

The infected birds generally have no apparent signs of illness, but with some exceptions after infection with highly pathogenic avian influenza viruses (Munster et al., 2007).

The main differences between the three main types of influenza viruses (A, B and C) are outlined in table 2.1 .

	Influenza A	Influenza B	Influenza C
Number of gene segments	8	8	7

Surface glycoproteins	HA and NA	HA and NA	HEF Haemaggluti) –nin–Esterase (Fusion
Host range	Wide range of ,hosts (humans ,pigs, horses whales, seals (and birds	Humans and seals	Mainly humans also found in) (swine

Table 2.1 Comparison of major properties of influenza viruses, adapted from Cheung and Poon (2007).

2.5. Structure and molecular biology of influenza A virus

Influenza viruses are roughly spherical with a size of around 100 nm or filamentous in shape, often in excess 300 nm in length (Bouvier and Palese, 2008). Morphological structure is known to be affected by several viral proteins (HA, NA, M1, and M2), in addition to the nature of the host cells (Cheung and Poon, 2007).

Influenza viruses are enveloped with surface glycoprotein spikes and a segmented RNA genome of negative sense (complementary to mRNA). RNA of influenza A virus is organized into 8 segments, in total around 13600 nucleotides long (Hoffmann et al., 2001). These are the polymerase basic (PB1 and PB2), the polymerase acidic (PA), haemagglutinin (HA), nucleoprotein (NP), neuraminidase (NA), matrix (M), and non– structural (NS) genes (Samji, 2009).

Each viral segment contains non–coding regions at both 5′ and 3′ ends. The 5′ terminus of each influenza A viral RNA segment has 13 conserved nucleotides, and the 3′ terminus has 12. The extreme ends are conserved among all segments, and this is followed by a segment–specific noncoding region (Hoffmann et al., 2001). These unique noncoding regions (U12 and U13) contain the promoter components, which are important for the initiation of transcription and replication as they are recognized by the polymerase complex consisting of PB1, PB2, and PA proteins (Hsu et al., 1987, Coloma et al., 2009). In between these highly conserved noncoding regions and the long central coding region of each gene there are additional untranslated regions (UTRs) at both 5′ and 3′ ends. Specific nucleotides and the UTRs and terminal coding regions act as the viral packaging signal (Hutchinson et al., 2010). Figure 2.1 shows the typical structure of influenza viral RNA.

A large open reading frame (green box) which represents the coding region is flanked by short untranslated region (UTRs) (black lines) containing terminal promoter sequences (blue boxes) that form the polymerase binding site. Those sequences are comparable between all genome segments and all virus subtypes. Specific nucleotide segments (red wedges), which overlap the UTRs and the terminal coding regions act as viral packaging signal. Figure adapted from Hutchinson et al. (2010).

Influenza A viral gene segments are known to encode at least ten proteins which are the RNA polymerase complex proteins (PA, PB1, and PB2), surface glycoproteins (HA, and NA), nucleoprotein (NP), matrix proteins (M1 and M2), and nonstructural proteins (NS1,NS2) (Samji,

2009, Wang and Taubenberger, 2010). In addition, PB1–F2 and a new viral protein (N40) which is translated from segment 2 have been recently identified in some influenza A virus isolates (Chen et al., 2001, Wise et al., 2009). Moreover, two more proteins, PA-X and M42 which are translated from segment 3 and 7, respectively, have been recently found (Jagger et al., 2012, Wise et al., 2012) .

Each viral RNA segment is surrounded by nucleoprotein (NP) forming ribonucleoprotein (RNP) and encapsidated by one copy of trimeric polymerase (PB1–PB2– PA complex) which is essential for viral replication (Digard et al., 1999). The lengths of the rod–like RNPs are varied (30–100 nm) and correlate with the length of each viral segment (Noda and Kawaoka, 2010). By longitudinally and transversally sectioning budding virions of different virus strains, a study has shown that the eight RNPs are highly organized in a distinct pattern; a central segment is surrounded by seven segments of different lengths (Noda et al., 2006). Such an organization is also observed in the isolated virion (Calder et al., 2010). The structural organization of viral ribonucleoprotein can be seen in figure 2–2.

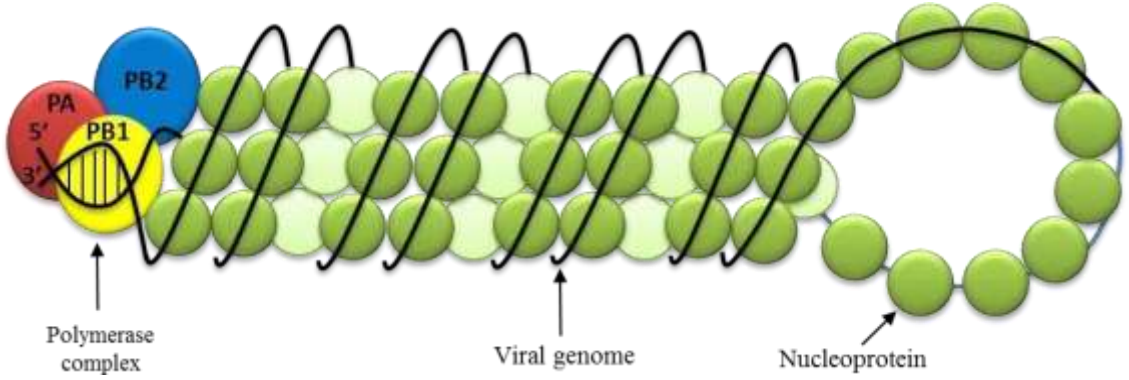

Figure 2–2 Structure of influenza virus ribonucleoprotein (vRNP).

Green spheres represent NP monomers, and the black line shows the associated single–stranded vRNA molecule. Influenza RNP folds into a double–helical hairpin structure. A short duplex formed between the 5′ and the 3′ ends provides the binding site for the heterotrimeric RNA–dependent RNA polymerase. Figure adapted from Portela and Digard (2002).

Four virus proteins (PB2, PB1, PA, and NP) are responsible for virus transcription and replication of the viral genome in the nuclei of infected cells. PB1–F2 protein plays a role in pro–apoptotic activity, while N40 protein, which is encoded by the same gene (PB1), interacts with the polymerase complex in the cellular environment but does not contribute to transcription function (Wise et al., 2009). PA-X protein has been shown to modulate host response and viral virulence (Jagger et al., 2012).

Haemagglutinin (HA or H) plays a role in virus attachment to the host cell and subsequent fusion with cell membranes, while neuraminidase (NA or N) supports the release of viruses from the host cell surface by hydrolyzing sialic acid from glycoproteins which helps to release the progeny virus particles from host cells (McCauley and Mahy, 1983, Odagiri, 1992). Non–structural protein 1 (NS1) has a major role in inhibition of host immune response via limitation of interferon (IFN) production (Hale et al., 2008).)

NS2 (also called nuclear export protein or NEP) plays a role in the export of RNPs from the nucleus to the cytoplasm during viral replication, in addition, it also regulates virus transcription and replication processes (Robb et al., 2009). Matrix protein 1 (M1), the major structural protein, is the dominant protein in determining virus morphology and also plays an important role in virus assembly and budding (Rossman and Lamb, 2011).

Matrix protein 2 (M2) is the ion channel that regulates the pH, and is responsible for virus uncoating, the step that follows virus entry into the host cell (Holsinger et al., 1994). In addition, this protein also plays an important role in membrane scission in the last stage of virus life cycle (Roberts et al., 2013). Matrix protein 42 (M42) can functionally replace M2 and support efficient replication in null M2 influenza viruses (Wise et al., 2012). The viral envelope is made of a lipid bilayer which is derived from the host cell‘s plasma membrane. Three surface viral antigens are embedded in the lipid bilayer: the HA spike, which has a rod like–shape, represents approximately 80% of the total surface proteins; the NA spike, which is almost mushroom–shaped, represents 17%; with minor components of M2 represented by few molecules (only 16 to 20 molecule per virion) (Schroeder et al., 2005, Nayak et al., 2009).

Underneath the lipid bilayer, the M1 protein forms a layer that separates the viral segments from the virus membrane. Inside the virion, 8 segments of different length are associated with the nucleocapsid protein (NP) and three large proteins (PB1, PB2, and PA). NEP is also associated with the virus but in low amounts (Cheung and Poon, 2007).Figure 2–3 illustrates the typical structure of influenza A virus.

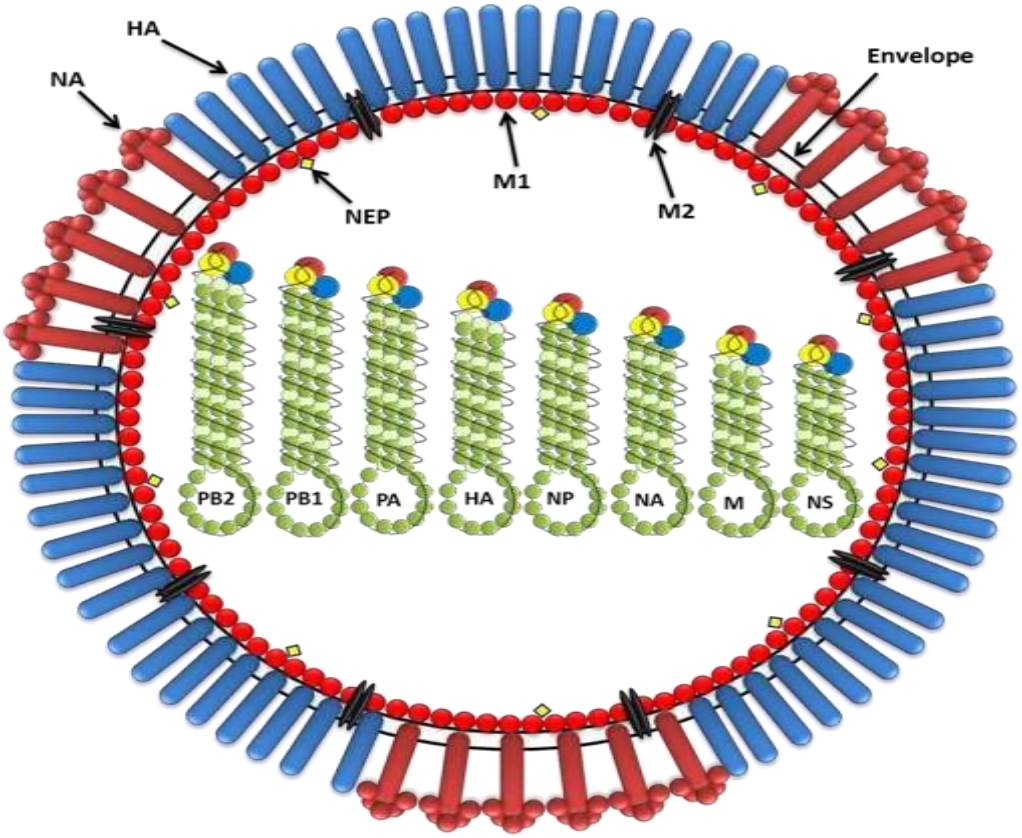

Figure 2–3 Schematic diagram of influenza virus A particle

The RNA is segmented and each segment encodes one or more proteins. The segments are not identical in length (ranging from 2341 to 890 nucleotides). The longest segment encodes PB2 protein and the shortest encodes NS protein. The RNA segments are coated with nucleoprotein forming ribonucleoprotein (RNP), and a small amount of transcriptase (polymerase complex) represented by PB1, PB2, and PA is also associated with it. The haemagglutinin (HA), neuraminidase (NA), and M2 proteins are inserted into the host–derived lipid envelope. The matrix (M1) protein underlies the lipid envelope. A nuclear export protein (NEP) is also associated with the virus. .

Both NA and HA genes encode surface glycoproteins and influenza A virus can be classified into several subtypes according to the antigenic diversity of those surface antigens. There are 18 HA and 11 NA subtypes described as H1–H18 and N1–N11 with amino acid sequences differing by 30% or more between subtypes (Hampson and Mackenzie, 2006). Of those subtypes, 16 HA (HA1–HA16) and 9 NA (NA1–NA9) circulate in waterfowl and two of HA and NA (HA17–HA18 and NA10–NA11) have been isolated from bats (Tong et al., 2012, Tong et al., 2013). The most frequently circulating subtypes of influenza A viruses in the human population are H1N2, H3N2 and H1N1 (Nelson and Holmes, 2007).

In addition, many different subtypes have been generated over time because of the genetic reassortment (antigenic shift). In the last few years, humans have been infected with swine and bird flu in different parts around the word, raising concerns for public health for humans as well as for pork and poultry production worldwide (Metzgar et al., 2010, Van-Tam and Sellwood, 2010).

2.6 Replication of influenza A viruses

2.6.1 Virus attachment and entry

The first step of viral replication is virus attachment to its host cell through N–acetyl neuraminic (sialic) acid, a nine–carbon acidic monosaccharide (Couceiro et al., 1993). The most common linkages of sialic acids are α 2,3 and α 2,6 linkage with which influenza viruses have the affinity to bind. The different sialic acid linkages can be one factor in host specificity. Both types of receptors are wide spread in many organs in chickens, ducks, cats, and pigs (Kuchipudi et al., 2009, Nelli et al., 2010, Trebbien et al., 2011, Wang et al., 2013); with a dominant expression of SA α 2,6Gal in the respirator tissues of humans including epithelial cells in the nasal mucosa, paranasal sinuses, pharynx, trachea, bronchi and bronchioles; while SA α 2,3Gal is occasionally detected in the nasal mucosa and on the non–ciliated cuboidal bronchiolar cells at the junction between the respiratory ronchiole and alveolus (Shinya et al., 2006).

Once a host cell is infected with influenza virus, the HA glycoprotein is produced as a precursor, HA0, which is cleaved into two subunits (HA1 and HA2) by host serine proteases before virus particles become infectious (Klenk and Garten, 1994). The H1 portion contains the antigenic sites (the receptor binding domain), while the H2 portion mediates fusion of the virus envelope and cell membranes (Steinhauer, 1999). Virulent and a virulent avian influenza A viruses can be differentiated by the sequence of a few basic amino acids at the point where the HA0 is cleaved (cleavage site); the so–called cleavage sequence (Zambon, 1999).

The virus enters the host cell via receptor (clathrin) mediated endocytosis at the inside face of the plasma membrane forming an endosome (Rust et al., 2004). Although other endocytic routes (non–clathrin–dependent) may provide additional entry pathways, the endocytic pathway seems to be the most common (Sieczkarski and Whittaker, 2002).

The endosome has a low pH of around 5 to 6, which induces a conformational change in HA0, displaying the HA2 fusion peptide. This fusion peptide inserts itself into the endosomal membrane and mediates the fusion of the viral envelope with the endosomal membrane, reviewed in Stegmann (2000). This mechanism is not only important for inducing the conformation change in HA0, but also opens up the M2 ion channel during fusion of viral and endosomal membranes, allowing the virion interior to become acidic which releases the vRNP from M1. This permits the vRNP to enter the host cell‘s cytoplasm, reviewed in Pinto and Lamb (2006).

2.6.2 Transcription, replication and protein synthesis

Transcription and replication occur inside the nucleus. Because of the negative sense of the viral genome, the viral RNA is copied into positive sense mRNA by the polymerase complex to act as a template for the production of the viral RNAs. The polymerase complex responsible for viral transcription and replication is formed by PB1, PB2, and PA. The viral RNA transcription is catalyzed by the RNA dependent

RNA polymerase. The mRNA acquires a 5′ capped primer in a process known as ―cap snatching‖. The PB2 protein has a role to capture this primer from host mRNA. The cap is cleaved by PA endonuclease into short sequence which is polymerized by RNA dependent RNA polymerase PB1.The resultant positive sense viral mRNA is exported to the cytoplasm through nuclear pores to start viral translation by ribosomes (Figure 1.3–6). Positive sense viral mRNA also serves as a template to produce the negative sense RNA that is packaged into new virions (Bouloy et al., 1978, Swayne, 2008).

2.6.3Virus packaging, budding and release

Influenza progeny virions are not infectious unless they have all eight genome segments (Bancroft and Parslow, 2002). Formation of new vRNP complexes from the newly synthesized PB1, PB2, PA, NP, and NS2 proteins occurs in the nucleus of the infected host cell. M1 proteins catalyze the transport of vRNP to the cytoplasm after forming M1– vRNP complexes. Nuclear export of vRNA complexes is directed by NEP protein and the nuclear export signal (NES) carried by NP proteins and inhibited by the M1 proteins. Consequently, newly synthesized vRNA accompanied by M1 proteins are unable to penetrate into the nucleus .gain (Portela and Digard, 2002).

Two models for the packaging of the segmented influenza A virus genome have been identified: random and segment specific packaging (Hutchinson et al., 2010). In the random model, the segments of the viral genome are distinguished from cellular RNA and also non–genomic viral RNAs and then integrated into a new virion; however, this mechanism does not distinguish between different segments. In this case, the possibility of the formation of fully infectious virus might be through chance by acquiring 8 different segments, or by packaging with more segments than the minimum. Conversely, in a mechanism of the specific packaging model, one copy of each viral segment is specifically selected producing fully infectious virus, (cited by Bancroft and Parslow (2002).

The final step of viral replication is budding and release. Budding occurs at the apical plasma membrane of the host cell, possibly initiated by the accumulation of M1 protein at the cytoplasmic side of the lipid bilayer. The protein complexes represented by M1 interact with the cytoplasmic tail of envelope proteins (M2, HA, and NA proteins). This interaction leads to the formation of a bud and assembly site in the cellular membrane (Bouvier and Palese,(2008). .

2.8. Mode of transmission

All influenza A subtypes can be transmitted in two main ways: inhalation of contaminated aerosols and by direct contact. Many studies have shown that inhalation of aerosol and infectious respiratory droplets play an essential role in the spread of the disease (Tellier, 2009, Tellier, 2006). Transmission by contact may occur directly from the infected persons or animals or indirectly by touching contaminated tissues and surfaces (Collier and Oxford, 2006) .

Persons who are in contact with infected birds may be infected with the highly .)2010) pathogenic strains (Khanna et al., 2008). Such transmission could happen in wet markets where live birds are sold, leading to direct close contact with infected poultry, via feather plucking and preparation of poultry for consumption, as well as poultry slaughtering facilities, commercial poultry farms, and eating of raw or poorly cooked animal parts (Paul Tambyah and Leung, 2006, Ma et al).

Transmission between birds usually occurs by the faeco–oral route which is the predominant means of spread in wild bird reservoirs (University Jawa State, 2010). The stability of avian influenza viruses in water supplies may spread the infection to other birds such as shore birds and also to aquatic mammals such as seals and whales (Stallknecht et al., 1990). Mallard ducks are of great interest because they are widely distributed and can travel large distances carrying the viruses from one region to another (Achenbach and Bowen, 2011). Transmission also occurs through inhalation of respiratory secretions contaminated with influenza virus particles (Zambon, 1999).

2.9 Clinical signs and symptoms in humans

Generally signs and symptoms appear directly after the incubation period which is 24 to 48 hr after being exposed to infection, but sometimes they may take four days to appear (Van-Tam and Sellwood, 2010). The severity of symptoms varies with virus subtype. A person who is infected with the disease starts to spread the viruses to other people one day prior to the beginning of symptoms and remains infectious for five to seven days (Collier and Oxford, 2006, Van-Tam and Sellwood, 2010). The typical symptoms of influenza A in people include fever (38˚C or more), rhinitis, runny nose, cough, headache, myalgia (muscle pain), body aches especially joints and throat, nasal congestion, chills, tiredness, watering eyes, loss of appetite, weakness and general discomfort, diarrhea or vomiting (especially in children) (Collier and Oxford, 2006, Van-Tam and Sellwood, 2010). The common symptoms (fever, headache and fatigue) are caused by the secretions of large amounts of cytokines, including interferons and interleukins which are produced from influenza infected cells (Hampson and Mackenzie, 2006).

2.10 Pathogenicity of influenza A viruses

According to the pathogenicity and severity of the disease in chickens, avian influenza A viruses can be classified into two pathotype groups: highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI). The mortality rates of the poultry flocks infected with HPAI viruses may reach to 100%, while infection with LPAI cause only milder and primarily respiratory disease (Capua and Alexander, 2009). In HPAI viruses, the region that encodes the cleavage site of the surface glycoprotein (HA) molecule contains multiple basic amino acids (arginine and lysine) which allows cleavage of the HA molecule by cellular endogenous proteases widely distributed throughout the cells of the body (Wood et al., 1993, Senne et al., 1996). This molecular structure is important in determining the virulence of these strains because it allows the virus to replicate in a considerably broader tissue range, causing widespread damage in tissues and death of the bird, with a mortality rate approaching 100% (Kim et al., 2009, Adams and Sandrock, 2010). The most pathogenic subtypes of avian influenza are restricted to subtypes H5 and H7 (Hampson and Mackenzie, 2006). On the other hand, LPAI viruses have only one basic amino acid (arginine) in the cleavage site of the HA molecule. This limits the site for the viral cleavage by host proteases such as trypsin–like enzymes, and as a consequence, the replication process occurs in limited tissues and organs, particularly in respiratory and digestive tracts, causing only mild disease (Alexander, 2000). LPAI viruses which cause asymptomatic or low pathogenic infection may mutate and convert to HPAI viruses through an adaptation process after infection of poultry (Mundt et al., 2009). This also reflects the importance of the role of wild birds as a primary source of zoonotic introduction of influenza and spreading the pandemics (Causey and Edwards, 2008).

2.11 Genetic variations

During influenza viral replication, genetic variations occur frequently. This is due to the structure of the viral RNA (segmented) and the low fidelity of the RNA dependent

RNA polymerase which generates replication errors during virus life cycle (Zambon, 1999, Zambon, 2001). Consequently, influenza A viruses can undergo recurrent antigenic changes (Shors,

2009). The resultant change in structure allows the virus to evade neutralizing antibody, the main mechanism of protective immunity against influenza infection. Such changes may lead to the creation of a new virus strain distinctive from those previously circulating viruses (Zambon, 1999, Smith et al., 2001).

2.11.1. Antigenic shift

Antigenic shift is a result of reassortment and it occurs when two or more different influenza A viruses subtypes infect a single cell simultaneously. Because influenza A viruses are segmented, it is possible to produce new viruses with a variety of segment combinations by the acquisition of entirely new gene segments. The newly assembled progeny virions may have mixed genes from the two parent viruses (Holmes et al., 2005, Nelson et al., 2008). Genome segmentation therefore confers evolutionary advantages by allowing genetic reassortment. This may result in the emergence of new subtypes which may be more pathogenic than the original parent viruses and may be associated with pandemics (Neumann et al., 2009b, Van-Tam and Sellwood, 2010). Pigs are thought to have an important role in reassortment because of their ability to become infected with different types of influenza A viruses (avian and human viruses), and thus they act as an intermediate host, or ―mixing vessel‖

The new reassortant strain may cause a pandemic or panzootic because the hosts (humans or birds) Firas Al–Mubarak 2014 Chapter 1: Introduction and Aims 43 have little or no immunity against it (Smith et al., 2001, Van-Tam and Sellwood, 2010). Such a scenario happened recently in April 2009 where a swine origin H1N1 virus originated from a triple reassortant, composed of genes from avian, porcine and human virus origin (Michaelis et al., 2009).

2.11.2 Antigenic drift

Genetic change in influenza A virus also occurs by _antigenic drift‘. This is due to the accumulation of point mutations over time, which results from a lack of proofreading mechanism in the RNA polymerase, leading to incorrect ribonucleotide insertions during replication (Zambon, 1999, Adams and Sandrock, 2010). Such changes occur progressively over a period of time accompanied by a gradual change in surface glycoproteins (HA and/ or NA). The accumulation of basic amino acids in the HA gene product may result in the transition of low pathogenic viruses to high pathogenic forms (Adams and Sandrock, 2010). The newly created viruses can escape immunity acquired after infection or vaccination and cause seasonal epidemic influenza, in humans, which usually occurs in winter every year and can infect the same person multiple times . As a result of this, influenza vaccines must be updated each year with changes in the circulating influenza viruses to achieve the best match with the circulating strain possible (Chen and Deng, 2009). These changes can be confirmed by phylogenetic analysis of H and N gene sequences (Hampson and Mackenzie, 2006). Influenza viruses produced as a result of antigenic drift are usually not changed much in their virulence in comparison with those produced from antigenic shift (Timbury, 1997). However, such viral gene mutations may play a role in virus evolution and spread.

2.12 Treatment

Treatment of influenza infection using antiviral medication plays an important role in modulating disease severity and in prevention and management of the disease. There are two main antiviral classes for influenza: adamantine (M2 blockers), and neuraminidase inhibitors (Hurt et al.,2006).

2.13 Antivirals

Influenza antivirals are essential components in countries clinical management of influenza since they play an important part in treating and reducing disease severity in people infected with influenza. Antivirals complement vaccination in both seasonal influenza programs and pandemic preparedness planning, and thus, serve as an additional tool for countries to protect vulnerable risk groups and health systems.

2.14 Vaccine types

Seasonal influenza vaccines are available and have been used for more than 60 years, although they remain largely underused in many LMICs. They target the three or four influenza virus strains that are likely to be the most circulating for the season (Trivalent and quadrivalent vaccines). WHO convenes the Vaccine Composition Meeting (VCM) twice a year to review the latest influenza surveillance data, predict which strains of the virus are most likely to be circulating during the next influenza season, and recommend the composition of both trivalent and quadrivalent vaccines (Bhan at el.,2019).

2.14.1 Trivalent and quadrivalent vaccines

- Trivalent influenza vaccines (TIVs) protect against two influenza A viruses and one influenza B virus. WHO notes that there are three main types of TIV: whole virus vaccines, split virus vaccines, and subunit vaccines.
- Quadrivalent influenza vaccines (QIVs) target two influenza A viruses and both influenza B viruses. Originally licensed in the United States of America (USA) in

2012, QIVs have since been increasingly incorporated into other countries‘ national immunization programmers. They provide wider protection against influenza B disease. (Tisa V, Barberis I, Faccio V, et al., 2016) .

2.14.1.1 Inactivated versus live attenuated Influenza vaccines

Both TIVs and QIVs can be made using a weakened form of the virus (live attenuated vaccines) or using a protein or other small piece of the dead virus

(inactivated vaccines). Inactivated vaccines are recommended for pregnant women, the elderly, health workers, and immunocompromised people. Older adults may require the use of more antigen, multiple doses, or adjuvants to achieve the efficacy comparable to younger adults. (Grohskopf LA,at el., 2019)

2.14.1.2. Live attenuated influenza vaccines

(LAIVs) can be delivered through a nasal spray and can induce a more protective and longer-lasting immune response in naive populations (i.e. children) because they are more similar to the real virus. Studies have shown that LAIVs are particularly effective in children above two years of age. However, for seasonal influenza vaccines, LAIV have lower efficacy in adolescents and adults. Because they contain a small amount of live virus, they are not recommended for people with weakened immune systems or long-term health problems. Most influenza vaccines (both TIVs and QIVs) that are licensed for use are inactivated vaccines; although some LAIVs are available. The efficacy and effectiveness of both LAIV and inactivated vaccines vary considerably with season and age group. For example, LAIV is recommended for children in Germany and the United Kingdom. (Sridhar S at el., 2015)

2.14.1.3 Universal vaccines

Research is underway to develop vaccines that do not need to be changed every year to match the circulating strains. Numerous clinical trials are ongoing but no vaccine has yet been approved that achieves this goal.

Two things in particular differentiate seasonal influenza vaccines from most other vaccines:

1- Annual re-vaccination. Unlike most other vaccines, seasonal influenza vaccines must be re-formulated and re-administered every year to account for the constant changes in circulating strains and rapid evolution of the virus. Vaccine formulations can also differ between northern and southern hemispheres, which may have different circulating strains.

2- Variable effectiveness. Because influenza viruses change so quickly, the effectiveness of seasonal vaccines depends, in part, on the match between the circulating viral strains and the strains included in the vaccine. If the match is poor, then the vaccine is less effective. Individual immune response to influenza vaccination also influences effectiveness of the vaccine and differs by target group. Past exposure to influenza may also influence individuals‘ immune response to the vaccine and so impact vaccine effectiveness. (Grohskopf LA,at el., 2019)

2.15 Vaccine composition

Circulating influenza virus strains change and evolve rapidly so seasonal influenza vaccines must be regularly reformulated to ensure they cover the strains with the highest probability of circulation . Twice a year, the VCM reviews the latest surveillance data so that WHO can be confident in its recommendation for which virus strains to include in the seasonal influenza northern and southern hemisphere vaccines. These data are generated by the GISRS network of WHO Collaborating Centers ,

National Influenza Centers, WHO Essential Regulatory Laboratories and WHO H

Reference Laboratories. They include information on: • the antigenic and genetic characteristics of circulating viruses; and • vaccine effectiveness and antiviral resistant strains. At the VCM, experts use the data to forecast the strains likely to circulate and make recommendations on how to optimize the next vaccine. The recommendations inform the development of candidate vaccine viruses, for use in manufacturing the vaccine.(Falkenhorst G at el., 2013.

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