Original Research Paper

VOLUME - 4 | ISSUE - 2 | April - 2020 | ISSN (O) : 2663-2888 | ISSN (P) : 2663-287X

NEW RESEARCH AND DEVELOPMENT OF INFLUENZA VIRUS

Bimalesh Kumar Jha Virologist, National Public Health Laboratory,

Bijendra Shah Lecturer, Ayurveda Teaching Hospital, IOM, Tribhuvan University,

Krishnadas Manandhar HOD, Department of biotechnology, Tribhuvan University, Kathmandu Nepal.

ABSTRACT Influenza viruses are noteworthy human respiratory pathogens that cause equally seasonal, endemic infections and periodic, unpredictable pandemics. The worst pandemic on record, in 1918, killed approximately 50 million people worldwide. Human infections caused by H5N1 highly pathogenic avian influenza viruses have raised concern about the emergence of another pandemic. Timely and perfect identification of influenza virus is very critical for the disease diagnosis, outbreak management and outpatient management, to avoid incongruous use of antibiotics, virus surveillance and initiation of antiviral therapy in patients with high risk of complications. Swine origin influenza virus A (H1N1) recent pandemic and highly pathogenic avian influenza A virus (H5N1) circulation highlighted the need of rapid and accurate diagnosis of influenza virus and its sub types. Technical advances in the last period have made it possible to investigate influenza virus infection from the cellular and subcellular level to intact animals and humans. As a result, we have expanded a new understanding of the virus and disease. This review deliberates different approaches for measuring the amount of influenza virus particles in the air and assessing their infectiousness. Here we also discuss the data describing the association between the influenza virus subtypes and virus air transmission, and distribution of viral particles in aerosol drops of different sizes. COVID-19, also referred to as “CORONAVIRUS,” is a respiratory illness that was first detected in the Hubei province city of Wuhan, China, in late December 2019(WHO).

KEYWORDS

Schwannomas, Encapsulated

***Corresponding Author** Bimalesh Kumar Jha

Virologist, National Public Health Laboratory, dr.anusofi@gmail.com

Introduction

Influenza virus are the major respiratory pathogen and are responsible for the high degree of morbidity and mortality in humans. The disease severity is higher in children, elderly and patients with underlying condition than in adults. It causes seasonal outbreak, endemic or pandemics too. The pandemic threat is even more due to the recent cross species transmission of avian influenza into the human. Pandemic and seasonal influenza have significant impact on health and economy. Influenza A and B viruses are the most common causes of influenza-like illness (ILI), pathogens other than influenza like Syncytial virus, Rhinovirus, Adenovirus, Parainfluenza viruses, Human Coronaviruses, also causes ILI.[1] Influenza occurs throughout the year in tropical areas whereas in the northern hemisphere, influenza season starts in early fall, reaches its peak in mid-February and ends in late spring of the following year. The severity and duration of influenza depends on the subtype of virus involved. Annual influenza epidemics results in about 3-5 million cases of severe illness and about 250,000 to 500,000 deaths according to the world health organization (WHO 2005, WHO 2010, CDC).The WHO Global Influenza Surveillance and Response System (GISRS) laboratories tested more than 65649 specimens in 2015/2016 from National Influenza Centres (NICs) and other national influenza laboratories in 91 countries out of which 10502 were positive for influenza viruses. Among these 80.8% were typed as influenza A and 19.2% as influenza B. Influenza A/H1N1 pdm09 (swine flu) was the most predominated strain (WHO 2016). In Asia in 2009,most H1N1 activities was reported from India, whereas low level of H1N1 activity were recorded in southeast Asian countries like China, Indonesia, Thailand, Myanmar, Bangladesh etc. However; Singapore reported high levels of seasonal A (H3N2) activity (WHO GISRS). India had Influenza outbreak in 2015 which had surpassed the previous numbers of reported cases and deaths. The total number of laboratory confirmed cases crossed 33000 mark with death of more than 2000 people (Hindustan Times 2015, BBC News 2015). Nepal has started Laboratory diagnosis of Pandemic influenza from mid-June 2009. The predominant strain circulating in Nepal was found to be Pandemic influenza A/H1N1. The case fatality ratio for Pandemic influenza A/H1N1 was 1.74%. According to the WHO Global Influenza Surveillance and Response System 2014, prevalence of Influenza in case of Nepal is 38 %. Timely and accurate identification of influenza virus is very crucial for the disease diagnosis, outbreak management, and outpatient management, to avoid inappropriate use of antibiotics, virus surveillance and initiation of antiviral therapy in patients with high risk of complications. Swine origin influenza virus A (H1N1) recent pandemic and highly pathogenic avian influenza A virus (H5N1) circulation highlighted the need of rapid and accurate diagnosis of influenza virus and its sub types. Influenza virus can be diagnosed by using rapid test (Immuno-chromatographic test) or RT-PCR or virus culture. RT-PCR and virus culture are the gold standard methods for the diagnosis of influenza virus. Rapid influenza diagnostic tests (RIDTs) are immunoassays that can identify the presence of influenza A and B viral nucleoprotein antigens in respiratory specimens, and display the result in a qualitative way (positive vs. negative). RIDTs can yield results in a clinically relevant time frame, i.e., less than 30 minutes, are less expensive than molecular diagnosis of viral nucleic acid, and laboratory staffs can easily be trained for this. However, RIDTs can't distinguish between influenza virus and its subtypes. Sensitivity of the RIDTs are found to be very low in comparison with PCR or Virus culture. The sensitivity and specificity of RIDTs vary with individual test, specimen collection site, and age of the participants. RIDTs have suboptimal sensitivity resulting in false negative results especially during high influenza virus activity.[2] Likewise, the positive predicted values (PPVs) and negative predicted values depend on the prevalence of influenza in the population tested. The limitations of RIDTs have been described by many literatures. Their sensitivity varies from 10 to 80% (most commonly from 40 to 70%); their specificity is better and ranges from 85 to 100%. In spite of these limitations RIDTs may be useful during the Seasonal influenza outbreak , endemic or even pandemic to identify the influenza so that patients with underlying conditions, children can be treated in time.[3] Reverse transcriptase-PCR (RT-PCR) techniques detect specific viral genomic nucleic acid sequences. It is a gold standard method for the diagnosis of Influenza virus and has high sensitivity and specificity. It is also called as quantitative PCR and used by many research laboratories. It can detect and measure minute amounts of nucleic acids in a wide range of samples. Most RT-PCR techniques are performed either by one-step or two-step RT-PCR. One step RT-PCR combines the reverse transcription and PCR together using one of the PCR primers, oligo-(dT)-primers or random primers for reverse transcription, while two-step PCR is

VOLUME - 4 | ISSUE - 2 | April - 2020 | ISSN (O) : 2663-2888 | ISSN (P) : 2663-287X

carried out by performing reverse transcription first followed by PCR. Nevertheless, RT-PCR is the expensive methods, needed highly equipped laboratory and very well trained laboratory staffs. [4] So its use is circumscribed in central laboratory only and all the sentinel sites in Nepal are unable to detect influenza virus.

Influenza Pathology

Influenza virus replicates in the epithelial cells throughout the respiratory tree, with virus being recoverable from both the upper and lower respiratory tract of people naturally or experimentally infected.[5] As histologic changes are nonspecific, histologic analysis alone is insufficient to make a specific diagnosis; diagnosis typically requires supporting diagnostic tests such as viral isolation, rapid diagnostic tests (including RT-PCR), serologic studies, or a biopsy or autopsy tissue section confirmed by in situ hybridization or immunohistochemically techniques. Non-fatal influenza viral infections predominantly involve the upper respiratory tract and trachea, but fatal cases of influenza usually show evidence of pneumonia. This review concentrates on the pathology of the lower respiratory tract.[6]

Materials and Methods:

The details of the concerned information were retrieved from various sources such as literatures of both classical as well as modern, web based database searches and published papers.

Influenza virus

Influenza A, B, and C viruses belongs to the family Orthomyxoviridae. It represents three of the five genera of the family; are characterized by segmented, negative-strand RNA genomes. Influenza A viruses are further characterized by the subtype of their surface glycoproteins, the hemagglutinin (HA) and the neuraminidase (NA). 16 HA and 9 NA – have been found in circulating influenza A viruses, only three HA (H1, H2, and H3) and two NA (N1 and N2) subtypes have caused human epidemics as defined by sustained, widespread, person-to-person transmission. The typical structure of influenza virus genome and function of its viral proteins are responsible antigenic drift and antigenic shift. These processes helps the viruses to evade the long-term adaptive immune responses in many hosts.[7]

Influenza A virus (influenza virus net 2016): It is the only one species under the genus influenza A virus. Among the three Influenza virus, type A virus is the most virulent pathogens and causes most severe disease. Though the natural host of the large variety of Influenza A virus is wild aquatic birds, sometimes the viruses are transmitted to the other species and may then cause the devastating outbreaks in domestic poultry or give rise to human influenza pandemic. H1N1, H1N2, and H3N2 are the only known Influenza A virus subtypes currently circulating among humans. Based on the antibody response to these viruses, Influenza A virus can be subdivided into different serotypes. The serotypes that have been confirmed in humans are as follows;

Influenza A/H1N1 Virus: It was the most common cause of human influenza (flu) in 2009. Some strains of H1N1 are endemic in humans and causes illness in human. Other strains of H1N1 are endemic in pigs (Swine Influenza) and in birds (Avian Influenza).The World Health Organization declared the new strain of swine-origin H1N1 as a pandemic in June 2009. This strain is also called as swine flu. This novel virus spread worldwide and had caused about 17,000 deaths by the start of 2010. Spanish Flu (1918-1920), Fort Dix outbreak (1976), Russian flu (1977–1978), Pandemic H1N1 (2009), all was caused by strains of Influenza H1N1 virus. The 2009 flu pandemic was found to be made up of North American swine influenza, North American avian influenza, human influenza, and swine influenza virus typically found in Asia and Europe.

H1N2 Influenza virus: H1N2, sometimes called bird flu, is a subtype of the species Influenza A virus. Currently in both human and pig populations, it is pandemic. The virus cause milder illness than other influenza viruses.

H2N2 influenza virus: It's a subtype of the type influenza virus A. Mutation in H2N2 has evolved several strains like H3N2, the Asian flu strain which is extinct in the wild now, and various strains found in birds. It is suspected for causing a pandemic in human in 1889.

H3N2 influenza virus: H3N2 is a subtype of Influenza virus A, which causes influenza (flu). Birds and mammals can be infected by H3N2 virus. Many mutated strains of H3N2 virus are found in birds, human, and pigs. H3N2 kills an estimated 36,000 people in United States yearly, and is increasingly abundant in seasonal influenza.

H5N1 influenza virus: H5N1virus is a influenza A virus subtype. It is also called as "bird flu. Illness in humans and many other animal species can be caused by H5N1.The HPAI A strain of subtype H5N1 is a bird-adapted highly pathogenic avian influenza and is causative agent of H5N1 flu, also known as avian influenza" or "bird flu". The HPAI A (H5N1), although has limited human-to-human transmission, it is considered as avian disease. The virus transmission from infected birds to human is inefficient although handling of infected poultry is a risk factor for contracting the virus. Still, mortality rate for individual infected with current Asian strain of HPAI A that is Highly Pathogenic Influenza A virus A(H5N1) is around 60%. H5N1 in human may mutate or reassert into a strain having capability of human-to-human transmission. The current endemic of H5N1 is considered world's largest pandemic of today because of its highly virulent circulating strain HPIA A(H5N1), high lethality, its increasingly large host reservoir, and its significant ongoing mutations. There is a possibility of new influenza pandemic of H5N1 due to the expectation of continuing mutation in birds.[8]

H7N2 influenza virus: H7N2 Influenza virus is a subtype of the species Influenza A virus. Sometime, it is called bird flu virus. Many times, H7N2 was confirmed in poultry farm.

H7N3 influenza virus: H7N3 Influenza virus is a subtype of the species Influenza A virus. Sometime, it is called bird flu virus. At several poultry farms its presence was confirmed. The H7N3 strain was first detected in turkeys in Britain in 1963. Both in 1963 and in 2006 it was found in UK.

H7N7 influenza virus: H7N7 Influenza virus is a subtype of Influenza virus A, which is responsible for influenza. Two pathogenic strains, the highly pathogenic strains (HPIA) and low pathogenic strains (LPAI) exist. Humans, birds, pigs, seals, and horses are the hosts in the wild; and mice were also found as host in laboratory setting. This wide host range makes it as potential threat for future pandemics. In the Netherlands an outbreak was spotted on several poultry farms in 2003 with 89 human cases of H7N7 influenza virus. Among the 500 people tested for antibodies for H7N7 influenza virus more than half found positive according to the final report by the Dutch government .Final analysis of Dutch avian influenza outbreaks disclosed much higher levels of transmission to humans than previously thought."

H9N2 influenza virus: H9N2 influenza virus is a subtype of the species Influenza A virus (bird flu virus). In the domestic poultry of Asia, the H9N2 influenza virus of domestic ducks has been established. In Hong Kong several H9N2 influenza virus cases found in children aged nine months to 5 years last occurred in December 2009.[9]

Influenza B virus (influenza virus net 2016): This genus has one species, influenza B virus. Influenza B almost exclusively infects humans and is less common than influenza A. The only other animals known to be susceptible to influenza B infection are the seal and the ferret. This type of influenza mutates at a rate 2–3 times slower than type A and consequently is less genetically diverse, with only one influenza B serotype. As a result of this lack of antigenic diversity, a degree of immunity to influenza B is usually acquired at an early age. However, influenza B mutates enough that lasting immunity is not possible. This reduced rate of antigenic change, combined with its limited host range (inhibiting cross species antigenic shift), ensures that pandemics of influenza B do not occur.

Influenza C virus (influenza virus net 2016): This genus has one species, influenza C virus, which infects humans, dogs and pigs, sometimes causing both severe illness and local epidemics. However, influenza C is less common than the other types and usually only causes mild disease in children.

Antigenic drift: is a gradual and relatively continuous process which yields change in the viral HA and NA proteins. It occur due to the accumulation of point mutations in the HA and NA genes of Influenza A and B virus during viral replication and responsible for the emergence of new viral strain. Thus, it necessitates the frequent updating of influenza vaccine virus strains as antibodies to previous influenza infections may not provide full protection against the new strains resulting from antigenic drift, individuals can have many influenza infections over a lifetime (WHO).

Antigenic shift: Influenza A virus can undergo antigenic shift which it is a more dramatic and abrupt type of change. Antigenic shift occurs infrequently and unpredictably. Antigenic shift can occur in a virus bearing new HA and NA proteins. It can arise through the genetic re-assortment of non-human and human influenza viruses; or an influenza virus from other animals (e.g. birds or pigs) can infect a human directly without undergoing genetic re-assortment; or a non-human virus may be passed from one type of animal (e.g. birds) through an intermediate animal host (such as a pig) to humans. Since

antigenic shift results in the emergence of a new influenza virus and if it is capable of causing illness in humans and sustained chains of human to-human transmission, it can lead to community-wide outbreaks and pandemic as well (WHO).

Flu: Influenza (flu) is a contagious respiratory illness caused by Influenza virus Illness can be mild to severe .serious cases of flu infection can results in hospitalization or death. Peoples at high risk of developing serious complications are older people, young children, and people with certain health conditions. Getting vaccinated in each year is the best way to prevent the flu .Influenza virus type A and B are the main two types of flu virus. The influenza A and B viruses that routinely spread in people (human influenza viruses) are responsible for seasonal flu epidemics each year. Influenza A viruses can cause pandemic (CDC).

Influenza symptoms: The flu is different from a cold. The flu usually comes on suddenly. People who have the flu often feel some or all of these symptoms: Fever* or feeling feverish/chills, Cough, Sore throat, Runny or stuffy nose, Muscle or body aches, Headaches, Fatigue (tiredness), Some people may have vomiting and diarrhea, though this is more common in children than adults. * It's important to note that not everyone with flu will have a fever (CDC).

Flu complications: Influenza will recover in a few days to less than two weeks in most of the cases. Nevertheless, there is a chance of having complications (such as pneumonia) as a result of the flu, some of which can be life-threatening and result in death. Some examples of flu complications are Pneumonia, bronchitis, sinus and ear infections. The flu can exacerbate chronic health problems. For example, people with asthma may experience asthma attacks while they have the flu, and people with chronic congestive heart failure may experience exacerbation of this condition that is triggered by the flu (CDC).

People at high risk from flu: There is a chance of serious flu related complications in some people if they get sick. This includes people 65 years and older, people of any age with certain chronic medical conditions (such as asthma, diabetes, or heart disease), pregnant women, and young children (CDC).

Transmission of Influenza Virus: Influenza is a contagious disease. People with flu can spread it to others up to about 6 feet away. When people with flu cough, sneeze or talk flu viruses are spread mainly by droplets and people who are nearby may inhaled the droplets into the lungs. A person might also get flu by touching a surface or object that has flu virus on it and then touching their own mouth or nose but it is very less often mode of transmission. Most healthy adults can infect other people 1 day before symptoms develop and up to 5 to 7 days after becoming sick. Children may shed the virus for longer than 7 days. Symptoms start 1 to 4 days after the virus enters the body (CDC).

Influenza like Illness (ILI): Influenza like illness is an acute respiratory infection with fever measured ≥ 38 C°, cough and/or sore throat (in absence of known cause other than Influenza) with onset within the last 10 days (CDC).

Epidemiology:

Global Scenario: Every year influenza virus epidemic occur seasonally throughout the world. Occasionally pandemics is caused by novel subtypes of the virus. Annual influenza epidemics occur during the winter months in temperate region, in both the Northern Hemisphere (November through March) and Southern hemisphere (April through September). In tropical regions, there is year-round activity of influenza virus with larger epidemics in between those found in the Northern and Southern Hemispheres. The WHO GISRS laboratories tested more than 65649 specimens in 2015/2016 from National Influenza Centers (NICs) and other national influenza laboratories in 91 countries. 10502 were positive for influenza viruses, of which 80.8% were typed as influenza A and 19.2% as influenza B. influenza A(H1N1)pdm09 was the most predominated strain. Influenza A viruses are responsible for causing recurrent epidemics and global pandemics. Pandemics occurs typically when a new HA subtype are introduce into human population .Re-assortment and inter-species transmission are the two mechanism result in the introduction of viruses with new HA subtypes into human populations (Neumann et al 2009) .World has witness five major pandemics.[10] They are as follows:

Spanish flu (1918-1919): The flu occurred in 1918, also known as the Spanish flu. It had unprecedented severity. Some people felt fine in the morning but died by nightfall. People who caught the Spanish Flu but did not die from it often died from complications caused by bacteria, such as pneumonia. Approximately 20% to 40% of the worldwide population affected with Spanish flu and an estimated 50 million people died. [11] Nearly 675,000 people died in the United States. Unlike earlier pandemics and seasonal flu outbreaks, the 1918 pandemic flu saw high mortality rates among healthy adults. In fact, the illness and mortality rates were highest among adults 20 to 50 years old. The reasons for this remain unknown (Neumann et al 2009).[12]

Asian influenza (H2N2): The 'Asian influenza' originated in Southern China in February 1957. From there, it spread to Singapore (March 1957), Hong Kong (April 1957), Japan (May 1957), and the United States and the United Kingdom (October 1957). A second wave was detected in January 1958. In the United States, excess mortality was estimated to be 70,000. The pandemic was caused by a human/avian re-assortment that introduced avian virus H2 HA and N2 NA genes into human populations. In addition, the 'Asian influenza' virus also possessed a PB1 gene of avian virus origin. (Flu.gov 2016)

Hong Kong influenza (H3N2): In 1968, viruses of the H2N2 subtype were replaced by another human/avian re-assortment that possessed an H3 HA gene of avian virus origin. Again, the PB1 gene of the pandemic virus was derived from an avian virus. The virus was first isolated in Hong Kong in July 1968 and caused a pandemic in the winter of 1968-1969 and 1969-1970. In the United States, an estimated 33,800 people died from the 'Hong Kong influenza'. (Flu.gov 2016)

Russian influenza (H1N1): In May 1977, an influenza virus outbreak was reported in China that affected young adults in the northern hemisphere in the winter of 1977/1978. The outbreak was caused by influenza viruses of the H1N1 subtype that closely resembled viruses that had circulated in the early 1950s suggesting accidental release of this virus. The re-emerging H1N1 virus did not replace the H3N2 viruses circulating at the time and both subtypes are co-circulating in humans to this day. Re-assortment between viruses of these subtypes resulted in the emergence of H1N2 viruses in human populations in 2001. However, these H1N2 viruses have since disappeared.[13]

H1N1 (swine flu): A new flu virus spread quickly across the United States and the world in the spring of 2009. On April 15, 2009, the first U.S. case of H1N1 (swine flu) was diagnosed. H1N1 was declared as public health emergency by U.S government on April 26, 2009. By June, 18,000 cases of H1N1 had been reported in the United States. A total of 74 countries were affected by the pandemic. 80 million people were vaccinated against H1N1, which minimized the impact of the illness. The CDC estimates that 43 million to 89 million people had H1N1 between April 2009 and April 2010. They estimate between 8,870 and 18,300 H1N1 related deaths. On August 10, 2010 the World Health Organization (WHO) declared an end to the global H1N1 flu pandemic. []

Influenza in Nepal: Nepal has started the laboratory diagnosis of influenza virus from 2009 only. No data are available regarding the mortality and morbidity associated with the influenza virus before 2009. The most circulated strain in 2009 influenza surveillance was found to be Pandemic influenza A/H1N1 and seasonal influenza A .Most of the pandemic cases (53%) were found among young people with ≤ 20 years.1.74% was the case fatality ratio for pandemic influenza A/H1N1.(13) Nepal had several threat due to the avian influenza outbreaks. Though world health organization confirmed the presence of highly pathogenic avian influenza virus H5N1 in Nepal, but till date no evidence of human infections associated with H5N1 (UN 2009).The influenza activities is high during winter season and subsequently fall down during summer season. Severity of the disease is high in children but mortality associated data are limited. In 2015, higher in 10-13 weeks and gradually fall down from 13 to 17 weeks and influenza activity is very low from 18 weeks to 53 weeks. Most circulated strain was pandemic A/H1N1 (swine flu) followed by seasonal influenza A and B (WHO GISRS).[14]

Coronavirus disease (COVID-19) Pandemic: COVID-19, also referred to

VOLUME - 4 | ISSUE - 2 | April - 2020 | ISSN (O) : 2663-2888 | ISSN (P) : 2663-287X

as "CORONAVIRUS," is a respiratory illness that was first detected in the Hubei province city of Wuhan, China, in late December 2019(WHO). Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). Coronavirus disease (COVID-19) is a new strain that was discovered in 2019 and has not been previously identified in humans. Coronaviruses are zoonotic, meaning they are transmitted between animals and people. Detailed investigations found that SARS-CoV was transmitted from civet cats to humans and MERS-CoV from dromedary camels to humans. Several known coronaviruses are circulating in animals that have not yet infected humans. Common signs of infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death. Standard recommendations to prevent infection spread include regular hand washing, covering mouth and nose when coughing and sneezing, thoroughly cooking meat and eggs. Avoid close contact with anyone showing symptoms of respiratory illness such as coughing and sneezing. (WHO 31ST December 2019) [15]

Real time PCR: For avian influenza virus (AIV) detection; Real-time PCR has become the most useful tool for routine surveillance, outbreak and research. Real-time PCR and real-time RT-PCR, they have high sensitivity, good reproducibility and wide dynamic quantification range. TaqMan®, Molecular Beacons, Scorpions® and SYBR® Green are four different techniques that are currently in use for real-time PCR. All of these allow detection of PCR products via the generation of a fluorescent signal (WHO, Charlton et al 2009). Mostly, TaqMan approach are being used in influenza A virus surveillance and diagnosis .In TaqMan approach, a probe is designed to hybridize an internal region of the PCR product so that the highest sensitivity and specificity can be achieved during the PCR amplification. Specific matrix gene primers and probes have often been designed for influenza virus typing (i.e., distinguishing type A and type B influenza virus) in human samples because of the conserved nature of the matrix gene segment among different type A influenza viruses . (Aguero et al 2007).

Shortly after the emergence of the outbreak, the CDC developed a real-time PCR protocol for 2009 H1N1 influenza A virus detection. It is a one-step RRT-PCR approach and targets the matrix gene of the novel influenza A/H1N1. It detect novel H1N1 in clinical specimens and do not cross-react with seasonal influenza A, subtypes H1N1 and H3N2 viruses and swine influenza A (H1N1) (Wang and Taubenberger 2010, Carr 2009). Conventional RT -PCR reactions typically require a pair of oligonucleotides (known as primers), four deoxyribonucleoside triphosphates (dNTPs), template RNA, reverse transcriptase and Taq DNA polymerase. Following reverse transcription of the RNA target to cDNA, the cDNA is subjected to repeated thermal cycling. In the case of conventional RT -PCR this causes template denaturation (at 95 °C), primer annealing (at 45–60 °C) and product extension (at 72 °C). For some assays and for real-time RT -PCR, product extension at 72 °C is not necessary. Taq DNA polymerase is DNA-dependent and thermostable, and is therefore not inactivated during the denaturation steps and does not need to be replaced at every round of the amplification cycle. Because the products of one round of amplification serve as templates for the next, each successive cycle essentially doubles the amount of the desired DNA product. The recent availability of improved "one-step" RT -PCR strategies have decreased the number of pipetting steps required, making the process technically easier and less susceptible to contamination. (WHO 2011)

Cycle threshold value: In a real time PCR assay a positive reaction is detected by accumulation of a fluorescent signal. The Ct (cycle threshold) is defined as the number of cycles required for the fluorescent signal to cross the threshold (ie exceeds background level). Ct levels are inversely proportional to the amount of target nucleic acid in the sample (ie the lower the Ct level the greater the amount of target nucleic acid in the sample). Cts <29 are strong positive reactions indicative of abundant target nucleic acid in the sample Cts of 30-37 are positive reactions indicative of moderate amounts of target nucleic acid Cts of 38-40 are weak reactions indicative of minimal amounts of target nucleic acid which could represent an infection state or environmental contamination.

Quality control: Quality-control procedures are intended to monitor reagent and assay performance. Test all positive controls prior to running diagnostic samples with each new reagent kit lot to ensure all reagents and components are working properly. Good laboratory practice recommends including a positive RNA extraction control (EC) in each nucleic acid isolation batch for each run. The EC provides a secondary negative control that validates the nucleic extraction procedure and reagent integrity. Each sample RNA extract is tested by separate primer sets. Negative template controls (NTCs) and positive template controls (PTCs) for all primer/probe sets should be included in each run. The human RNase P gene (RNP) primer and probe set serves as an internal positive control for human RNA. Reaction assay mixtures are made as master mix cocktails and dispensed into a PCR reaction plate or strip tube that meets the manufacturer's requirements and specifications for the PCR platform being used. Extracted nucleic acid. (WHO 2011)

QuickVue A+B Influenza Test: Quickvue A+ B influenza diagnostic test are commercially available rapid diagnostic tests. It is a screening tests for influenza A and B virus infections which provide results within 10 minutes. RIDTs are immunoassays and detect influenza viral antigen. QuickVue detect and distinguish between influenza A and influenza B infections. (WHO 2005).

Acceptable respiratory specimens for RIDTs: Nasal aspirates, nasal washes, sputa and nasopharyngeal swabs, especially those specimens containing cellular material, are preferable to nasal swabs and throat swabs. They should be collected as close to the onset of symptoms as possible and not after 4–5 days in adults as virus shedding typically diminishes. In young children, viral shedding may occur for longer periods, and the collection of specimens for testing after 5 days of illness may still be useful. Nevertheless; for the upper respiratory tract infection throat swab and nasal swab are the suitable specimens and can easily be collected. (WHO 2005, WHO 2011)

Clinical accuracy: The accuracy of an influenza diagnostic test is determined by the sensitivity and specificity of the test to detect an influenza virus infection compared with a "gold" standard (usually culture) and the prevalence of influenza in the community. (WHO 2005)

Sensitivity: It is the percentage of "true influenza cases" detected as positive by a test. In general, the sensitivity of rapid tests is variable (median 70–75%) and lower than that of cell culture and polymerase chain reaction. (WHO 2005)

Specificity: It is the percentage of "true non-influenza cases" detected as being negative by a test. Specificity of rapid influenza diagnostic kit test is high (median 90–95%). Because of the low sensitivity, false negative results are a major concern with these tests. (WHO 2005)

Positive predictive value (PPV): Positive predictive value of a test is the percentage of test positive cases that have influenza. (WHO 2005)

Negative predictive value (NPV): Negative predictive value of a test is the percentage of test negative cases that do not have influenza. (WHO 2005)

Discussion

Influenza viruses are common respiratory pathogens in humans. Influenza viruses can cause serious infections, leading to the development of pneumonia. Influenza A viruses, because of their host-range diversity, their genetic and antigenic diversity, and their ability to reassort genetically, are continual sources of novel influenza viruses that lead to the emergence of periodic pandemics. Pandemic influenza viruses cause much higher morbidity and mortality than annual, epidemic influenza virus outbreaks. Influenza virus infection includes both upper and lower respiratory tract involvement. Influenza virus pneumonia, either alone or with secondary bacterial pneumonias, can often be fatal. The worst influenza pandemic on record, the 1918 influenza, killed up to 50 million people globally. The pathologic spectrum of fatal influenza virus infections during the 1918 pandemic was not significantly different from that observed in other pandemics or even from fatal cases in seasonal influenza outbreak. Influenza infection is a serious health concern, especially for pregnant women and young children. While influenza vaccines are generally considered to be safe, vaccine uptake remains suboptimal. Vaccination of

VOLUME - 4 | ISSUE - 2 | April - 2020 | ISSN (O) : 2663-2888 | ISSN (P) : 2663-287X

pregnant women provides protection against influenza infection in both the expectant mother as well as the infant due to trans placental transfer of influenza-virus-specific IgG antibody. Additionally, breastfeeding provides antibodies (in particular IgA that is not passed through the placenta) and immunomodulatory factors to prevent and/or combat influenza infection. These factors are particularly important in the first six months of life, as active vaccination is not recommended for this age group. For infants born prematurely, complicating factors such as chronic lung disease increases the risk for developing severe illness after influenza infection. Despite having an immature immune system, vaccination is recommended on schedule in this population and has been shown to be protective. Furthermore, vaccination of these populations will help to guard against the development of ARDS, which is a major health concern following infection with influenza virus. Influenza viruses undergoes continuous antigenic drift, which leads to lower than ideal vaccine efficacy in some seasons. Furthermore, antigenic shifts resulting in pandemic outbreaks is not uncommon, with 4 pandemics in the 21st century. Next generation influenza vaccines targeted against highly conserved regions of the influenza virus are being developed that may provide more universal protection against even potentially pandemic influenza strains. However, even if conventional vaccine strains of influenza are not well matched to circulating strains, reduced viral shedding and a shorter duration and severity of illness are often observed in the vaccinated population. Such information must be effectively disseminated to members of the public in order to improve vaccine uptake, as misconceptions about influenza vaccine adverse effects and effectiveness remains major obstacles to improving worldwide influenza vaccination rates.

Conclusion

Influenza viruses remain to be a major health threat in both endemic and pandemic forms. The rapid, continuous, and unpredictable nature of influenza viral evolution makes vaccine strategies and pandemic planning difficult. It is crucial that future pathology studies be performed on autopsies of victims with fatal influenza infections, whether caused by endemic strains, seasonal strains, or zoonotic strains such as the recent H5N1 viruses. Careful analysis of the histopathological changes of infection coupled with molecular genetics, virologic, and immunologic analyses will contribute to our understanding of the variable pathogenesis of influenza viruses. COVID-19, in early 2020, after a December 2019 outbreak in China, the World Health Organization (WHO) identified a new type, 2019 novel coronavirus (2019-nCoV), which is fatal. The organization named the virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and named the disease it causes COVID-19. The outbreak quickly moved from China around the world. Symptoms of COVID-19 include fever, cough, and shortness of breath. It is rapidly killing millions of people in worldwide till now. (WHO)

Conflict of interest: Author declares that there is no conflict of interest.
Source of support: None

References:

1. Luytjes, W.; Krystal, M.; Enami, M.; Parvin, J.D.; Palese, P. Ampli cation, expression and packaging of a foreign gene by in uenza virus. Cell 1989, 59, 1107–1113.
2. Neumann, G.; Watanabe, T.; Ito, H.; Watanabe, S.; Goto, H.; Gao, P.; Hughes, M.; Perez, D.R.; Donis, R.; Hoffmann, E.; et al. Generation of in uenza A viruses entirely from cloned cDNAs. Proc. Natl. Acad. Sci. USA 1999, 96, 9345–9350.
3. Taubenberger, J.K.; Reid, A.H.; Krafft, A.E.; Bijwaard, K.E.; Fanning, T.G. Initial genetic characterization of the 1918 "Spanish" in uenza virus. Science 1997, 275, 1793–1796.
4. Nair, H.; Brooks, W.A.; Katz, M.; Roca, A.; Berkley, J.A.; Madhi, S.A.; Simmerman, J.M.; Gordon, A.; Sato, M.; Howie, S.; et al. Global burden of respiratory infections due to seasonal in uenza in young children: A systematic review and meta-analysis. Lancet 2011, 378, 1917–1930.
5. Wright, PF.; Neumann, G.; Kawaoka, Y. Orthomyxoviruses. In: Knipe, DM. Howley, PM., editors. Fields Virology. 5th. Phladelphia: Lippincott Williams & Wilkins; 2007. p. 1691-740.
6. Taubenberger JK, Layne SP. Diagnosis of influenza virus: coming to grips with the molecular era. Mol. Diagn 2001;6:291–305.
7. Kash, J.C.; Tumpey, T.M.; Proll, S.C.; Carter, V.; Perwitasari, O.; Thomas, M.J.; Basler, C.F.; Palese, P.; Taubenberger, J.K.; Garcia-Sastre, A.; et al. Genomic analysis of increased host immune and cell death responses induced by 1918 in uenza virus. Nature 2006, 443, 578–581.
8. Peiris JS, De Jong MD, Guan Y. Avian Influenza Virus (H5N1): A Threat to Human Health. Clinical Microbiology Reviews. 2007; 20: 243-267.
9. Peiris, M.; Yuen, K.Y.; Leung, C.W.; Chan, K.H.; Ip, P.L.; Lai, R.W.; Orr, W.K.; Shortridge, K.F. Human infection with in uenza H9N2. Lancet 1999, 354, 916–917.
10. Harfoot, R.; Webby, R.J. H5 in uenza, a global update. J.Microbiol. 2017, 55, 196–203.
11. Taubenberger, J.K.; Reid, A.H.; Janczewski, T.A.; Fanning, T.G. Integrating historical, clinical and molecular genetic data in order to explain the origin and virulence of the 1918 Spanish in uenza virus. Philos. Trans. R. Soc. Lond. B Biol. Sci. 2001, 356, 1829–1839.
12. Morens DM, Taubenberger JK, Fauci AS. The Persistent Legacy of the 1918 Influenza Virus. New England Journal of Medicine. 2009; 361: 225-229.
13. Wrammert, J.; Koutsonanos, D.; Li, G.M.; Edupuganti, S.; Sui, J.; Morrissey, M.; McCausland, M.; Skountzou, I.; Hornig, M.; Lipkin, W.I.; et al. Broadly cross-reactive antibodies dominate the human B cell response against 2009 pandemic H1N1 in uenza virus infection. J. Exp. Med. 2011, 208, 181–193.
14. Jha BK, Mahato RK, Upadhya BP and Manandhar KD. Influenza Virus and Its Preparedness in Nepalese Scenario. SM Virol. 2018; 3(1): 1015.
15. https://www.who.int/health-topics/coronavirus#tab=tab_1