Reflections on historical pandemics and Mahidol University's research on the treatment and prevention of influenza outbreaks

Short title: Historical pandemics and Mahidol research

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Abstract

Epidemics of infectious diseases have threatened humans throughout our history. Devastating plagues are described in records from classical Greece and Rome and across the ancient world. Many epidemic diseases that now affect humans began from infections that originated in animals. In the 21st century, we live in a world in which there have been dynamic changes to the global ecology and expanded international travel, which has resulted in increased contact between humans, and between humans and animals. The result is that the emergence and spread of new contagious infections from animal reservoirs and between human populations has become both more probable and, in some aspects, also more difficult to contain. Pandemics in the 21st century are anticipated to have chiefly viral causes, such as influenza, corona and arboviruses. Unlike bacteria, virus proteins are readily altered through antigenic drift and shift. In the case of the latter, a new viral infection may enter an entirely susceptible human population who have never been exposed before and have no effective host immunity. Moreover, existing treatments may prove ineffective, and new vaccines will need to be developed. Another reason why animal-to-human, or "zoonotic" viruses are of great concern is that these can often be efficiently transmitted through droplets, from animal to person, and also from human to human. Unlike most viruses, the influenza virus exists as a seasonal epidemic and when a new influenza virus strain appears there is the potential for it to spread rapidly and widely across international borders and around the world, that is, to become a pandemic. In this short review we reflect on a several key aspects of past pandemics, with a focus on the influenza infections. We then present an overview of recent studies conducted by the Faculty of Tropical Medicine, Mahidol University to improve the treatment and prevention of influenza.

Keywords: Epidemics, Influenza, Pandemics, Mahidol, Siam,

Introduction

An epidemic is the increase of a disease above its normal level in a population, and is commonly termed an outbreak when the increase is sudden and the disease is caused by an infectious agent with a common mode of transmission. A major factor determining the importance of an outbreak is the rate and ease with which the disease can spread. There is greatest concern when a newly observed infectious disease has a high potential for rapid spread through air-borne human-to-

human transmission, where the disease is severe, and when existing drugs or vaccines are ineffective. A pandemic is a global epidemic or outbreak that spreads across international boundaries and thus contains the potential to affect a very large number of people.

Historical epidemics and pandemics

Plague

There is debate as to which specific pathogens caused the worst early epidemics and pandemics, two candidates the bubonic plague (*Yersinia pestis* – a bacteria) and smallpox (Variola – a virus) (Figure 1). A severe epidemic of the bubonic plague, which is also known as "Black Death", was recorded in the First Century AD and was later identified as a bacterial infection by *Y. pestis* (RWJF, 2013; Cochrane J, 1996). Wild rodents are the main reservoir for the fleas which carry *Y. pestis* bacteria, and bites from infected fleas can then transmit the infection to humans. The plague is believed to have spread along trading routes from Asia to Rome and then throughout Europe. During 1346-1353, the bubonic plague is estimated to have caused more than 75 million deaths, which was approximately half of the population of Europe and devastated panicking societies in Europe, Asia and North Africa (Figure 2).

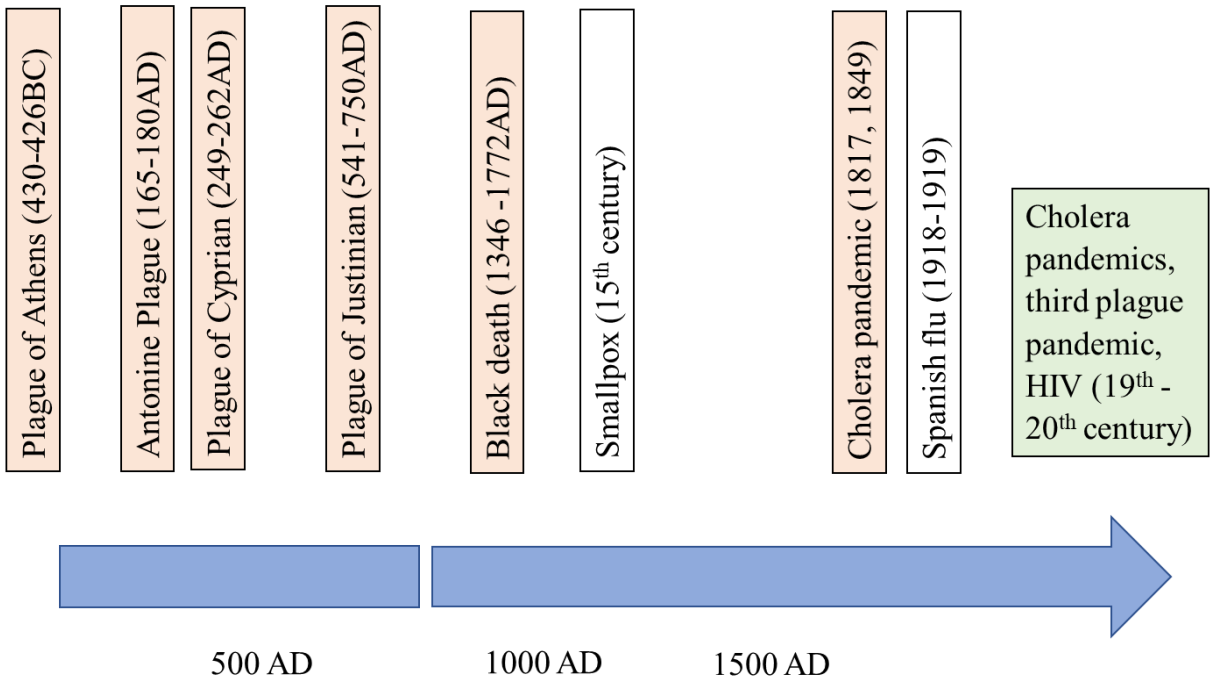

Figure 1. Notable historical epidemics and pandemics. The pathogenic causes are bacterial (pink) and viral (white) columns.

Figure 2. Special protective clothing to safeguard doctors against infection when treating plague, first advocated by Charles Delorme (1584-1678), a personal physician to King Louis XIII. (Cochrane J, 1996)

The first record of possible plague deaths in Thailand was in 1350, when the disease was taken to Ayutthaya via a merchant ship from China. During the reign of King Rama V, a Chinese ship was quarantined outside Bangkok for 9 days until it could be proven free from infection. The first official report of the Black Death in Siam is by Dr. Hugh Campbell Highet. (Bangkok's Principal Medical Officer) in the year 1904 (Figure 3) in his report on the plague outbreak among Indian people living in Thonburi (Hfocus, 2014).

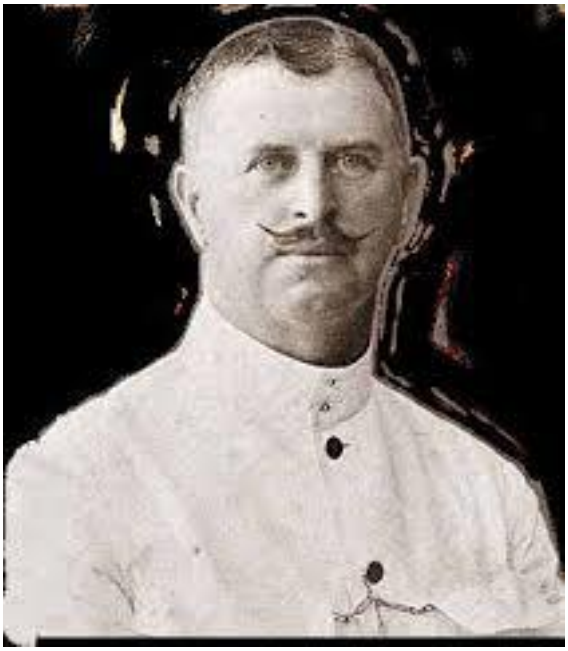

Figure 3. Hugh Campbell Highet, worked in Siam during 1898-1925 and was appointed as the first Thai Principal Medical Officer of Health in 1904 (source: Anupongphongphat & Chokevivat, 2013)

Smallpox

Smallpox is an acute communicable disease caused by the Variola virus and is spread through direct contact with an infected person, contaminated body fluids or objects. Smallpox is thought to have originated in India or Egypt at least 3,000 years ago. In the 1500s, the epidemic spread from trade routes in Asia to other continents. In the past, the virus killed about 30 percent of people infected and left pitted scars in survivors. According to Thai history, smallpox caused the deaths of two Ayutthaya kings, Borommarachathirat IV in 1553, and Naresuan the Great in 1605. An official account of the smallpox outbreak in the early history of Siam was written by Dr. Dan Beach Bradley who was present in Bangkok during reigns of King Rama the 3rd and the 4th. Smallpox was one of the first diseases controlled effectively by vaccination, using a vaccine invented by Edward Jenner in 1796. Bradley first introduced the smallpox vaccine to Siam in 1836, Figure 4 (Anupongphongphat & Chokevivat, 2013; Hfocus, 2014). There were repeated smallpox outbreaks in the early 19th and 20th centuries which are estimated to have killed many millions of people. In 1967 the World Health Organization (WHO) started a vaccination program to rid the world of smallpox and in 1980 the disease was declared eradicated.

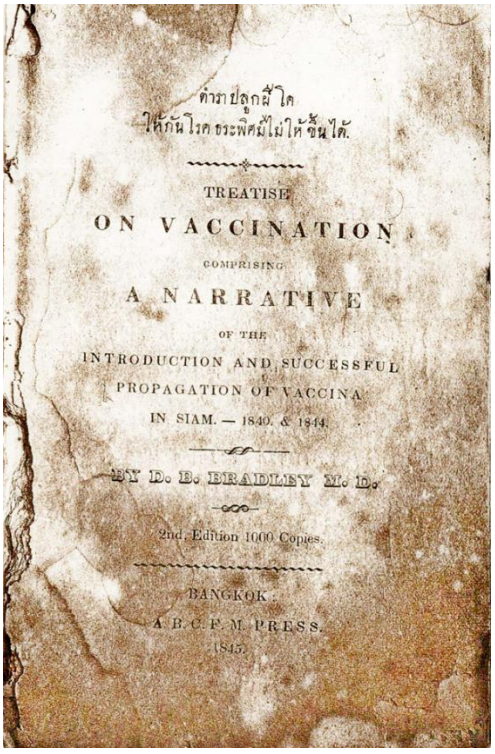

Figure 4. The Thai text book on smallpox vaccination by DB Bradley in 1845 (source: Anupongphongphat & Chokevivat, 2013)

Cholera

Cholera

Another important pandemic disease is cholera. In 1817 the disease spread from India to other countries, both inside and outside Asia, killing millions. Cholera is an acute diarrhea caused by consuming food or water contaminated with the bacterium Vibrio cholerae. In 1849, John Snow, a British physician, undertook a novel public health investigation into a cholera outbreak in London. His systematic work to identify the source of the outbreak is considered a landmark event in the history of epidemiology. Through detailed interviews and carefully plotted maps he traced the source of the epidemic to the water supply, namely, a public water pump from which he then removed the handle. Despite advances in the management of cholera and the existence of vaccines, the risk of cholera epidemics continues to the present day in environments such as

refugee camps where there is overcrowding and inadequate access to clean water and sanitation (Figure. 5). In 2016, WHO recorded 132,121 cholera cases and 2420 deaths.

Siam was severely affected in 1820 when cholera was recorded as the leading cause of death (Hfocus, 2014). In the 19th and early 20th centuries Siamese habitations consisted mainly of stilted or floating houses lining canals. Natural river water was the only source for drinking and daily utilities. Contamination of this source was the cause of cholera outbreaks during the dry seasons (Figure 5). Public supply of clean water in Siam was planned and initiated by King Rama V in 1907 after returning from his second visit to Europe. The clean water pipe system was successfully implemented in Bangkok in 1914. In the next 4 decades 1918-1958, there were 5 cholera outbreaks in Thailand after which only few isolated cases have been reported. Cholera has been removed from the national disease alert list in 1989.

Figure 5. Saen Saeb Canal is an excavated canal, depicted here during the reign of King Rama 3 in 1837 (source Khaosos online, 2016). In the 19th and early 20th centuries Siamese habitation consisted mainly of stilted or floating houses lining canals.

Spanish flu

In 1918, the Spanish flu, caused by the H1N1 influenza virus, became pandemic. The 1918-1919 Spanish flu was among the deadliest pandemics in history, with an estimated 500 million people

infected worldwide and 50 million deaths. The number of deaths from this pandemic is far greater than the combined number of deaths recorded during combat in World War One (1914-1918). Despite its name, the Spanish flu is no longer believed to have originated in Spain, but it was first observed in Europe before spreading to other parts of the world, including Asia. During this pandemic, public health policy was scientifically implemented for the first time as citizens were ordered to wear masks and schools, theaters, and businesses were shuttered. The return of the Royal Thai Armed Forces from France in 1918, sent by King Rama VI in support of the Allied Forces during World War I, led to an outbreak of Spanish flu in Thailand. In October 1918, influenza emerged in the harbor city in the south of Thailand where the troops disembarked. By November 1918, the virus had spread throughout the whole country and eventually subsided in March 1919. The number of victims from the Spanish flu was well documented in Thai public health records. In 1918-1919 when the population was estimated at 8.5 million, around 2.3 million individuals or 37% of the Thai population had been infected by the H1N1 influenza virus. There were 80,263 deaths during this outbreak approximating to a mortality rate of 1% of the population, or 3.5% of those infected. (Thongcharoen P, 2017).

AIDS

The most deadly pandemic to emerge in the late 20th century was AIDS (acquired immune deficiency syndrome) caused by the human immunodeficiency virus (HIV), a blood borne and sexually transmitted infection. The virus originated from primates in West Africa and spread to humans early in the 20th century, with the earliest documented case from the Congo in 1959. AIDS was first clinically recognized in 1981 at the start of the epidemic in America and by 1983 scientists had established that a retrovirus was the cause of AIDS. Epidemics of HIV were reported in many countries in the 1980s and by 1999, the World Health Organization announced that AIDS was the fourth-leading cause of death globally and the leading cause of death in Africa. By the first decade of the 21st century approximately 33 million people were living with HIV and 14 million people had died from AIDS since the start of the epidemic in 1981 (WHO, 1999, World Health Report 2009). In 1997, highly active antiretroviral therapy (HAART) became the new standard treatment and this has approximately halved the number of deaths. Nevertheless, HIV/AIDS still places huge strains on the healthcare systems of a number of countries and in 2018 it was estimated that approximately 38 million people were knowingly or

unknowingly living with HIV. Currently there is no licensed HIV vaccine but the RV 144 vaccine has shown some promise, having prevented HIV infection in some individuals in Thailand (Rerks-Ngarm & Pitisuttithum et al, 2009).

Modern pandemics and emerging infectious diseases

Emerging contagious diseases are now recognized as a leading problem facing global health. The causes of these new emerging infections are multifactorial such as, a changing ecology (which may accelerate with global warming), increased urbanization, and other rapidly evolving social and environmental factors. These include frequent air travel, an aging society (as older people are often more susceptible to disease), immigration, civil and political unrest, wars, natural disasters, and the ease with which people can become infected and infect others when living in densely populated areas and where there is close contact with either wild or domestic animals. Since the 1980s, approximately 50 new infectious diseases have been discovered. This reflects both the improvements in modern medical technology and diagnostic techniques, but it is also likely that the rate of emerging infectious diseases is greater now than at any time in our past (Table 1).

Table 1. Notable emerging infectious diseases since 1980

Years	Diseases
1980	Human T-lymphotropic virus
1982	Escherichia coli O157:H7
1982	Lyme borreliosis (Lyme disease)
1982	Human T-lymphotropic virus type 2
1983	Human immunodeficiency virus (HIV): AIDS.
1988	HBE (epidemics of jaundice in hot climates)
1988	Human herpesvirus
1989	Hepatitis C (liver cancer, liver disease)
1991	Guanarito virus (Venezuelan haemorrhagic fever)
1992	Vibrio cholerae O139 (epidemic cholera)
1993	Hantavirus pulmonary syndrome (Sin Nombre virus)
1994	Sabia virus (Brazilian haemorrhagic fever)
1994	Hendra virus
1995	Human herpesvirus 8 (Kaposi's sarcoma virus)
1996	NV Creutzfelt-Jacob disease (CJD, a prion) Australian bat lyssavirus (a rabies-like virus)
1999	Nipah virus

2002	SARS coronavirus
2003	Avian influenza (H5N1)
2009	Swine flu (H1N1)
2010s	Influenza H2N2, H3N2, H5N1, H7N7, H1N2, H9N2, H7N2, H7N3, H10N7
2012	MERS-CoV (Middle East respiratory syndrome coronavirus)
2013	Ebola virus (causing Ebola haemorrhagic fever)
2015	Zika virus (a mosquito-borne flavivirus)
	Consider adding CoVID-19?

(WHO 1999, WHO 2016)

In the 21st century, emerging infectious diseases develop into pandemics every few years, and the rate that this is occurs may be increasing (Table 2). Recent pandemics and major epidemics have chiefly been viral in origin: including severe acute respiratory syndrome ("SARS", a corona virus), Avian influenza ("bird Flu", most commonly caused by the H5N1 influenza subtype), H1N1 influenza ("Swine Flu"), Middle East Respiratory Syndrome ("MERS", a corona virus), the Ebola virus epidemic in Africa, and mosquito-borne Chikungunya, and Zika viruses. Since the Millennium, the most deadly pandemics have been caused by influenza and coronaviruses, both of which are efficiently transmitted by air borne droplets.

Many of these contagious viral infections have been zoonotic which subsequently adapted to air borne transmission between people. New corona viruses have caused repeated epidemics in recent years. Meanwhile, seasonal influenza spreads across the world each year causing many tens to hundreds of thousands of deaths.

Table 2. Pandemics and major epidemics of the early 21st century (WHO, 2003; 2019, 2020; Hickok K, 2020)

Years	Disease outbreaks	Total Cases	Mortality rate	R0
2002-2003	SARS coronavirus	8,096	11%	3-4
2003-2004	Avian influenza (H5N1, H7N9)	861	53%	-
2009-2010	Swine flu (H1N1)	700 million - 1.4 billion	0.01 - 0.08%	1.2-1.6

2010s	Influenza H2N2, H3N2, H5N1, H7N7, H1N2, H9N2, H7N2, H7N3, H10N7	500,000/year	<0.5%	1-2
2012	MERS-CoV	2,494	34.4%	0.6-0.7
2013-2016	Ebola virus	28,600	70%	1.5-2.5
2015-2016	Zika virus*	1.5 million	53 cases	-

R0 = average number of people that can be infected by one single patient. * Infection of Zika virus (a mosquito-borne flavivirus) during pregnancy may be associated with fetus microcephaly from congenital Zika syndrome.

Pandemic influenza

In 2009, the World Health Organization classified influenza epidemics into six phases. Phases 1-3 describe the intensifying force of transmission of animal infections and risk of infection to humans but not efficient transmission between humans. Phase 4 marks the development of sustained human-to-human transmission, while Phases 5-6 denote the increasing intensity of transmission leading up to widespread human infection. The six-phased framework is currently under revision with a special focus on the geographical spread of the virus, the disease severity and its impact on society and disease control. The outbreak alert form issued by the World Health Organization for diseases that pose a threat to the public includes the following information: the potential for the spread of disease, the ability to cause serious illness or death, the international surveillance requirements, the availability of prevention and control measures, the availability of reliable and meaningful case definitions and tests (WHO 2012).

Influenza is a contagious respiratory virus, spread mainly through respiratory droplets expelled by an infected person. Influenza viruses aresimple pathogens consisting of a capsule or ball-like structure with RNA strands enclosed by protein capsule with numerous antigenic spikes at the surface. Each individual influenza virus has a very short life of only minutes but is capable of rapid multiplication by hijacking host genomic multiplication. An infected host can eliminate the virus only after the development of one specific immunity, an acquired process that take several

days to weeks to develop. Being a simple pathogen, it mutates readily causing antigenic drift (small, gradual changes) and antigenic shift (rapid, major changes), resulting in new but usually similar virus that can escape previous host immunity. Seasonal influenza is a yearly epidemic that requires an updated vaccine during each flu season, however, influenza has the potential to develop into a pandemic every few years. Many pandemic influenzas are zoonotic and originate from circulating animal influenzas.

There are four types of influenza viruses: A, B, C and D. Seasonal influenza in human is usually caused by Type A (~75%) and Type B (~25%). Influenza A viruses are the only viruses known to cause flu pandemics. Influenza type C infections generally cause mild illness, and Influenza D viruses primarily affect cattle. A pandemic can occur when a new and very different influenza A virus emerges that has the ability to both infect and to spread efficiently between people. Unlike other bacteria and viruses (measles, smallpox and poliomyelitis), influenza viruses are usually undergoing constant antigenic changes and continue to exist within animal reservoirs making them harder to eradicate.

The influenza A virus is an 80-120 nm capsule composing 8 single-stranded RNA segments with numerous 'spikes' at the outer layer. There are 2 spike-shaped proteins on the surface of the virus, Hemagglutinin (H) and Neuraminidase (N), and more than 16 types of H and N antigens. There are more than 10 important influenza virus subtypes e.g. influenza A (H1N1, H3N2), avian influenza (H5N1), and the 2009 pandemic influenza A (H1N1). (Table 3).

Influenza is transmitted primarily by infected droplets being inhaled, whereupon the virus attaches to host cells of the upper respiratory tract. Viral attachment is the key process of viral invasion and subsequent replication, and is initiated via a binding of the H spike with a receptor on the host cell, sialic acid. This is followed by cell invasion via the mechanism of endocytosis and the release of viral genetic material (RNA) into the cell cytoplasm. The free viral RNA then enters the cell nucleus, and hijacks the host's transcription machinery. This results in the production of viral proteins, not those of the host cell. The newly replicated viral particles then migrate to the outside of the infected cells. The emerging viruses adhere to the sialic acid molecules via the H spikes. The viral N (Neuraminidase) enzymatically cleaves these bonds, releasing the young viruses to infect other cells. This process results in the death of the host cell, and the complete infection cycle is completed in as little as 30 minutes.

A virus is an intracellular pathogen with no cell wall and therefore does not respond to antibiotics. Recovery from a viral infection is therefore mainly driven by acquired host immunity. Unlike most viral infections, influenza virus is susceptible to neuraminidase inhibitors and the seasonal influenza vaccine is a partially effective prophylaxis. Neuraminidase inhibitors (NIs) have similar structures to sialic acid, and so compete with the viral N antigen. This unattached new progeny of viruses remains stuck to the outside of the cell and are eventually destroyed by the host immune system. The most widely available NIs are oral oseltamivir (Tamiflu ®, Roche) and inhaled Zanamivir (Ralenza ®, GSK) and intravenous zanamivir. Recently, an alternative new medication that inhibits viral replication by a selective inhibitor of influenza cap-dependent endonuclease, Baloxavir marboxil (Xofluza ®, Roche), has been approved for the treatment of influenza.

Influenza research at Mahidol University

The Faculty of Tropical Medicine, Mahidol University has investigated and published the detailed pharmacokinetic properties of both NI drugs (Oseltamivir and Zanamivir), focusing on both the treatment and prevention of influenza infection (Chotivanich & Pukrittayakamee, 2014). Six different studies in healthy volunteers using the anti influenza drugs, oseltamivir and zanamivir, the most widely used drugs, were conducted with a special emphasis on the pharmacokinetics and drug safety (Table 3). The results of these studies are a valuable contribution towards the effective treatment and prevention of epidemic and pandemic influenza. (Figure 6).

Table 3. Clinical/laboratory research on anti-influenza drugs conducted by the Faculty of Tropical Medicine, Mahidol University.

Year	Main results	Reference
2009	Oseltamivir is well tolerated at up to 675 mg or 9 times of the usual 75 mg dose	Wattanagoon et al, 2009
2011	Intravenous zanamivir would be ideal for treatment of severe influenza. Zanamivir is safe when given alone or in combination with oral oseltamivir	Pukrittayakamee et al, 2011
2013	A large-scale study of safety of daily 4-month chemoprophylaxis in health personals. Either 75 mg oseltamivir or zanamivir inhaler are both safe without adverse health effects. This is a very important	Anekthananon & Pukritayakamee et al, 2013

	finding, as both drugs appear to be good candidates for the long term prophylaxis of influenza infection	
2013	A simple dry blood spot is feasible for measuring the drug level from dry blood spot samples. It is an ideal sample for storage and transportation.	Instiaty et al, 2013
2013	A use of three oral anti-influenza drugs – amantadine, oseltamivir and ribavirin was well tolerated with no significant pharmacokinetic drug-drug interaction.	Seo et al, 2013
2014	No significant pharmacokinetic differences between obese and non-obese patients using oseltamivir. The normal oseltamivir dosage could be safely given to obese patients.	Jittamala et al, 2014

Figure 6. Anti-influenza drugs studied in trials conducted by Clinical Therapeutic Unit, Faculty of Tropical Medicine, Mahidol University. From left to right are intravenous Zanamivir, Oral Oseltamivir and inhaler peramivir.

Summary

Humans have suffered from epidemic and pandemic diseases throughout history, and will continue to face new emerging pathogens in the 21st Century. Influenza infections remain the cause of much disease in humans and animals and are deserving of being the focus of research. Fortunately, the influenza virus generally responds to anti-viral medications and the infection may be partially mediated as a result of previous flu vaccines. Seasonal influenza is a yearly occurrence and new influenza epidemics and pandemics will continue to occur in the future, while eradication of the disease may be a long way away. As part of the global scientific community, The Faculty of Tropical Medicine, Mahidol University will continue working to develop effective medicines for the fight against our deadly old enemy.

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