An unchanging reservoir continually providing new variants: an updated review of the case of influenza virus

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Abstract. *Influenza virus infection usually provokes a strain-specific immunity that prevents the same virus strain from causing epidemics in two successive years without at least minor antigenic variation. Influenza in waterfowl avoids this rule and constitutes an unchanging reservoir because of the annual production of large numbers of immunologically-naive offspring that may be contaminated by virus preserved from the previous year in frozen lakes. Humans are usually infected when a variant of the preceding year's strain spreads world-wide. This process of gradual antigenic change, called drift, is cumulative and results in considerable antigenic change over a period of years until a new strain appears. New strains derive from the waterfowl reservoir, probably after reassortment in swine which are susceptible to both avian and human influenza viruses. This change, termed shift, results in highly virulent pandemics. The change in animal husbandry brought about by modern farming techniques may also allow swine to play an important role as a reservoir in addition to their recognized role as a mixing vessel. This review presents the process of variation and modification of viruses from virulent epidemic to benign nuisance drawing examples from influenza virus infections of man, of animals, and of birds.*

Influenza virus is a well-known human pathogen having originated world-wide epidemics, or pandemics, one of which killed about 2% of the world's human population (20,000,000 people) in 1918-19. Many serious epidemics of lethal influenza virus have occurred in the 20th century in animals and birds in addition to 4 pandemics in humans. A variant lethal to chickens is currently threatening the poultry industry in Mexico and has already infected 26,000,000 chickens, reminiscent of a 1983 outbreak in the United States that killed 17 million chickens and cost $63 million to control (Wuethrich, 1995). In 1980, an influenza outbreak killed 30% of the seal population on Cape Cod. New influenza variants suddenly appeared in swine in Europe in 1979 and in horses in China in 1989. Not only influenza but a number of other viruses have suddenly appeared in recent years: a hantavirus called Sin nombre in the US southwest; two Ebola epidemics in monkeys and one in humans; the human immunodeficiency virus suddenly appeared less than 20 years ago. We might ask where do these lethal variants come from.

The structure of viruses and the host's immune response to infection help us understand how and why viruses suddenly appear.

I. Virus structure

All viruses are composed of genetic material (RNA or DNA) packaged with nucleoprotein in a capsid. Many viruses, including influenza, are further surrounded by an envelope. After infection, viruses employ host enzymes together with a few viral genes to synthesize progeny virus.

1) Surface. Polypeptides on the viral surface are normally responsible for virus attachment to cellular receptors and the initial stages of infection. They are normally the primary antigenic target for neutrali-

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zing antibodies. Two influenza polypeptides are present on the viral surface: hemagglutinin and neuraminidase (see Figure 1). The hemagglutinin polypeptide is considered to be the most important protein in determining the epidemiology of influenza. It is responsible for virus binding to cellular receptors and entry into the cell. It is the primary target on the virus for neutralizing antibodies and therefore the most likely to change through the pressure of antibody selection. Fourteen different subtypes of hemagglutinin have been identified of which three are found in human influenza (H1, H2 and H3). Within each subtype, various lineages have evolved that infect different hosts. For example, within H3 influenza, variants infecting humans are different from those infecting swine, horses and fowl. Still greater differences however distinguish subtypes such as H3 from H1. For instance human H3 influenza is more similar to avian H3 influenza than it is to human H1 influenza.

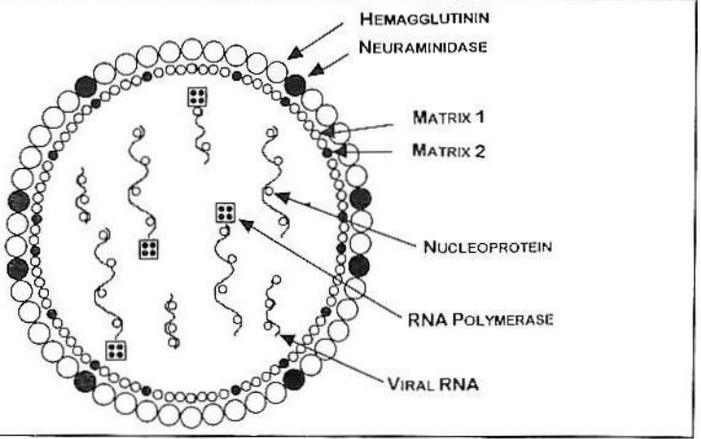
2) Capsid. Viral nucleic acid is always surrounded by a protein capsid. In viruses like polio or adenovirus that lack an envelope, the capsid proteins must play a role in attachment to target cells in addition to their role in viral assembly. Not surprisingly, capsid proteins in poliovirus are a major target of neutralizing antibodies, whereas antibodies to the influenza capsid protein called, M or Matrix protein, do not usually neutralize viral infectivity even though the M protein is the most abundant protein in the viral particle.

TABLE 1

VIRUS-IMMUNE SYSTEM INTERACTIONS

IMMUNITY		VIRUS CHARACTERISTICS		
		ONE/FEW SEROTYPES	MULTIPLE SEROTYPES	CONTINUALLY CHANGING
LONG-TERM	ONCE IN A LIFETIME	MEASLES, POLIO	ENTEROVIRUS	HIV?
SHORT-LIVED	INITIAL SEVERE, LESS SEVERE SECONDARY	RESPIRATORY SYNCYTIAL VIRUS	ROTAVIRUS	INFLUENZA

FIGURE 1. STRUCTURE OF INFLUENZA VIRUS



3) Nucleoprotein. The nucleoprotein neutralizes the negative charge on nucleic acids and allows them to be packaged compactly in the viral particle. In influenza virus this protein plays an important role in the control of viral synthesis and interplays both with the host cellular synthesis machinery as well as with the viral capsid protein. Interestingly the influenza nucleoprotein is a major target of the host's cellular immune response even though antibodies to the nucleoprotein do not neutralize viral infectivity. New strategies are currently being developed to induce cellular immunity to the nucleoprotein which might offer better protection to humans than vaccines that induce antibodies (Ulmer *et al.*, 1993).

4) Enzymes, control proteins. Viruses, especially viruses made of RNA, often direct synthesis of polymerases and a helicase (enzyme that unwinds nucleic acids) necessary to synthesize progeny nucleic acid. Many viruses also code for proteases and various control peptides. Little variation is usually found in these polypeptides.

II. Host immune response

There are three tiers of host defense against viral infection. The first defense mechanism to appear following viral infection is the production of interferon. Interferon reduces host cell synthesis of non-host protein. This mechanism slows virus production. The second step involves production of antibodies that neutralize free virus. Finally, cellular immunity in the form of T lymphocytes destroys virus-infected cells. Both antibodies and cellular immunity are primed by an initial infection or vaccination and, in many cases, can respond more quickly to stop a second infection before it produces symptoms. In some instances this immune memory lasts a lifetime and prevents a virus from causing a symptomatic infection more than once (see Table 1). Viruses of this category, like polio and measles can be effectively prevented by vaccines. Immune memory directed against other viruses may last only a few years so that after an initial infection that may be quite severe, milder subsequent infections may be contracted periodically. Viruses that exist as many different serotypes may provoke repeated infections. Influenza A viruses are continually changing so that they can outwit the immune system.

III. What spurs change?

Change in virus is provoked by errors in copying the viral genome. In viruses with DNA genomes these errors are rare, but in RNA viruses the genome co-

pying mechanism introduces frequent errors. If the error gives rise to a defective virus it will disappear, but if the error gives the progeny a selective advantage over the parent virus then it will rapidly replace the parent.

Normally viruses evolve toward an ideal situation for the virus (see Figure 2). Any subsequent change reduces the chance of the virus for survival. This usually involves a state where large amounts of infectious virus are produced over time. It is to the advantage of the virus not to incapacitate unduly its host. If the host is rapidly killed, virus production stops. In nature, even if the host is temporarily weakened, it will fall prey to a predator and again virus production will stop. Thus, ideally, the virus should produce minimal symptoms in its host while producing maximal progeny. Human viruses measles, rubella and polio have reached this equilibrium and no longer change. On the other hand influenza A virus is continually changing to escape the immune system. If it reaches a state where it can no longer change to a viable variant the lineage disappears.

Viruses that are introduced from a different species are not ideally suited to their host and may provoke serious epidemics. Human influenza follows this pattern. Whereas in waterfowl, influenza causes minimal symptoms and is produced in large quantities particularly by young birds, when it jumps to humans (probably after reassortment in pigs) it initiates serious epidemics. Subsequent annual epidemics are less severe partly because humans develop some immunity and partly because the virus evolves towards a less virulent form. In humans, influenza must continually change in search of ideal conditions because the immune system is continually pushing it to change. Interestingly human influenza B and C viruses do not have known reservoirs, provoke milder disease than influenza A, and change very little as if they are approaching an equilibrium situation.

IV. Potential viral reservoirs

1a) Most viruses infect only one species in nature. They are maintained year-round by infection of different individuals. There may be periods in the year when relatively few individuals are infected and then epidemics occur when conditions (climatic or other) for infection are favourable. In some cases asymptomatic or latent infections may predominate at certain times of the year.

1b) Although species-specific viruses infect only one species in nature, different members of the same virus family will infect other species. Only rarely does a species-specific virus mutate and cross between species. This is however the proposed mechanism for the appearance of human immunodeficiency virus (HIV) and the origin of highly virulent outbreaks of hog cholera virus, bovine viral diarrhoea virus, and influenza virus.

2) True animal reservoirs exist for arboviruses (arthropod-borne viruses, eg Dengue virus, hantaviruses) and zoonotic viruses (that are spread directly from animals without a vector, eg rabies). In the animal reservoir, often a rodent, infection may be asymptomatic or prolonged and mild while provoking a severe infection when transmitted to humans.

3) Influenza virus can also remain viable over winter in frozen lakes and then infect newborn migratory waterfowl in the Spring. A less natural and potentially more pernicious frozen reservoir is laboratory freezers from which smallpox virus or a variety of germ warfare virus strains could escape. It has been proposed that the 1977 reappearance and subsequent pandemic associated with an influenza strain identical to that from 1950 may have resulted from the escape of the 1950 strain from a laboratory (Webster *et al.*, 1992). Alternatively it has been shown that human influenza may be conserved for long periods with little change in a swine reservoir (Bikour *et al.*, 1995a).

Thus, in different species, influenza virus can be maintained by all three of these mechanisms. Whales and seals are infected from a true reservoir (mechanism 2) in waterfowl or shorebirds (Webster *et al.*, 1992). The natural reservoir of influenza A

FIGURE 2. WHERE DOES VIRAL CHANGE LEAD?

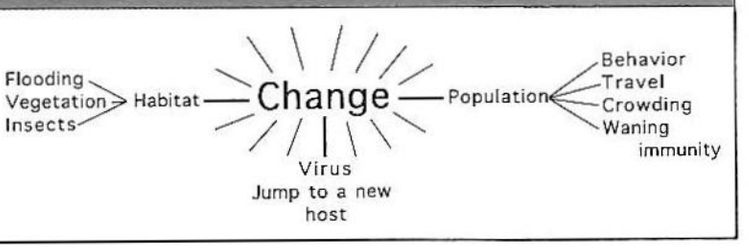
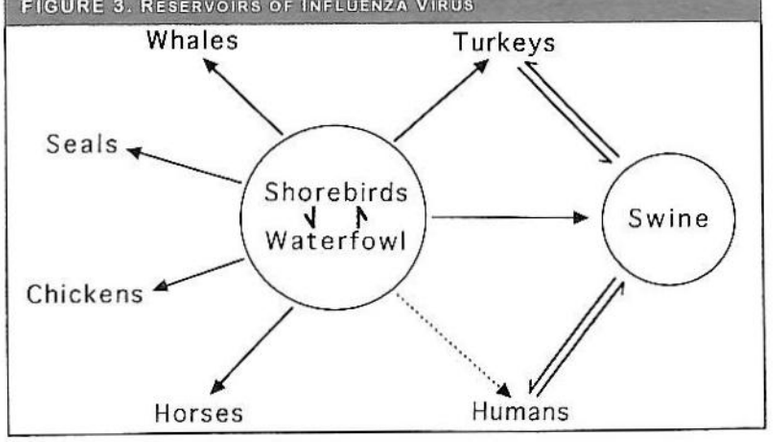


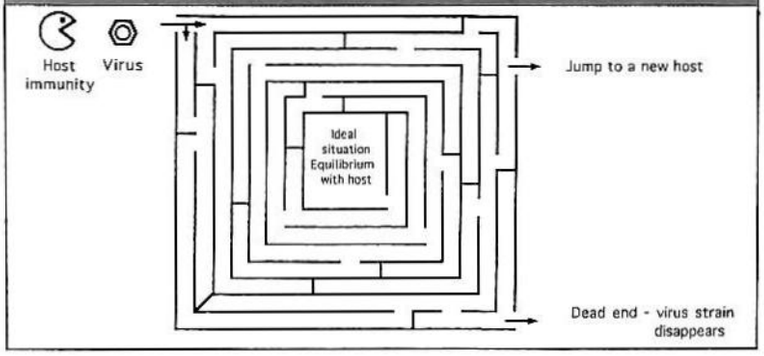
FIGURE 3. RESERVOIRS OF INFLUENZA VIRUS



virus is aquatic birds in which all virus subtypes can be found. From this reservoir (mechanism 1b) various subtypes have also been established in other species (see Figure 3). Swine are the only animals known to be receptive to influenza A viruses of avian and mammalian origin. Humans can readily be infected by swine viruses and vice versa. Turkeys are also naturally infected with swine viruses. Thus, swine also play a central role in the ecology of influenza virus and can be regarded as a "mixing vessel" for the introduction of influenza A viruses from birds into the human or turkey populations.

The initial introduction of influenza virus into humans is usually from avian sources probably via passage in swine (mechanism 1b). The predominant mechanism, however, for conservation of human influenza is sequential transmission in different individuals (mechanism 1a). This does not explain why human influenza usually persists in a community only 5 or 6 weeks during the winter and then disappears for a year. In tropical climates low levels of influenza persist year round and it has been postulated that this population serves as a reservoir for temperate climates (Webster *et al.*, 1992). An alternative hypothesis has recently been proposed where influenza is maintained locally in asymptomatic carriers who reactivate virus from a latent state and then transmit it to susceptible individuals (Hope-Simpson, 1992). This would explain better influenza outbreaks in geographic locations without contact one with the other, especially in the era prior to efficient global transportation, but would not readily explain why all human influenza isolates in one year have essentially the same sequence. Indeed in other species where isolated populations exist, influenza virus has evolved differently. For instance, ducks do not cross the Atlantic nor Pacific oceans, so American ducks are isolated from their Eurasian cousins. Duck influenza strains are quite different from those in Eurasia (Webster *et al.*, 1992). The practice of raising swine under rigidly separate conditions has been shown to give rise to distinct virus variants in different regions (Bikour *et al.*, 1995b).

FIGURE 4. WHEN DOES AN EPIDEMIC EXPLODE?



V. When does an epidemic explode?

The appearance of new virus variants may provoke epidemics, but this is only one element in the cascade that generates epidemics. Equally important are the changes that favour the appearance of virus variants: change in the environment; change in the population (see Figure 4).

History is replete with examples of changes in the ecosystem that trigger epidemics. Black death in Europe invariably followed flooding that drove rats and their fleas (vectors of black death) from the sewers to closer contact with humans. The American hantavirus episode followed unusual rains in a desert climate that favoured the multiplication of rodents. Insect control has always been a factor in the control of epidemics. Control of mosquitoes has limited spread of yellow fever virus and malaria. Deforestation causes increased contact of man and his domestic animals with new animals and obviously favours introduction of new viruses. When the environment changes the delicate equilibrium between virus and their hosts is unbalanced. When a virus changes in search of a new equilibrium there is an increased risk of generating a variant that is highly virulent. Thus change in the virus and increased contact with other species favour the appearance of epidemics. These are no doubt factors in the appearance of the AIDS virus and Ebola virus.

Change in the population is a powerful stimulus for epidemics. Human behavioral change such as increased promiscuity has brought upon us an epidemic of sexually transmitted diseases. Intravenous drug use and even uncontrolled transfusion has favoured hepatitis viruses. Crowding of humans or animals creates conditions where potential epidemic viruses may take hold and spread. Travel of humans or animals has spread many diseases in the past. Even today, travellers can often bring home exotic diseases. War, which associates travel, crowding and promiscuity, has fostered epidemics throughout history.

Agricultural practices have great potential to spur epidemics. Traditional farming or subsistence farming brings humans into close contact with several different species of animals. In China, the close contact of ducks, swine, and humans is considered to be instrumental in the appearance of influenza epidemics. Modern intensive farming methods for the meat and poultry industry house many animals together creating conditions of crowding. Furthermore, the short life spans of the animals result in minimal immunity. Together these conditions breed epidemics like the current

fowl influenza outbreak in Mexico. It has recently been reported that swine raised under intensive farming conditions can maintain human and swine influenza virus for long periods with minimal change (Bikour *et al.*, 1995a, b). This indicates the potential for reappearance of these viruses when human immunity wanes.

VI. Can we avoid epidemics?

The appearance of viruses capable of initiating epidemics is unavoidable. Progress is being made however, especially in the veterinary field, to control these viruses once they appear and limit their impact to localized outbreaks. Human epidemic viruses often appear when man perturbs the ecosystem and/or from his agricultural practices. Closer monitoring of intensive farming might prevent some epidemics. Limiting the contact not only between different herds of farm animals but also between different species of farm and wild animals would certainly reduce the risk of viruses jumping from a reservoir to initiate an epidemic. The case of influenza supports this proposition. Human, swine, and equine epidemics have been shown to originate from avian viruses. Limiting contact between birds (particularly ducks), pigs, and humans could reduce the risk of appearance of pandemic strains. Human pandemics might also originate from virus maintained in swine reservoirs. If this mechanism can be

confirmed it might be useful to attempt to eliminate influenza virus from swine farms through vaccination and isolation.

Faced with many contradictory theories, scientists wonder what would happen if influenza could be temporarily eradicated from humans. Would this be good or bad? Indeed it might render humanity even more susceptible to a new pandemic when, unexpectedly, a mutant avian influenza virus raised its ugly head? ◆

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