Pandemic H5N1 Influenza

Lethal flu in birds caused by H5N1 virus is now nearly 10 years old, and has resulted in deaths directly or indirectly of more than 230 million birds worldwide. During that period there have been 251 laboratory confirmed cases in humans with an estimated case fatality rate of 50%. [1].

H5N1 virus has expanded its geographical distribution across countries in Asia, the Middle East, Africa and Europe causing fatal disease in domestic and migratory birds. The virus continues to mutate, and at present seems to have diverged into two genetically distinct clades. Clade 1 prevails in eastern China, South Korea and Japan, whereas clade 2 (together with its subclades 1, 2 and 3) has been reported from South and South-East Asia, the Middle East and Africa.

Influenza A viruses are well known for their ability to undergo antigenic change involving the haemagglutinin (HA) and neuraminidase (NA) antigens. So far 15 HA and 9 NA types are recognized, and individual virus strains may have any combination of HA and NA types. When the antigenic change is minor as a result of genetic mutations the process is referred to as antigenic shift. These minor year-to-year changes lead to continual low-level viral activity and is the reason why the flu vaccine needs to be changed annually according to the prevalent virus. When the antigenic change is major caused by reassortment in the genome of the virus antigenic shift is said to have occurred. Antigenic shift may result in pandemics of influenza. For the bird population H5N1 is a pandemic.

The prevalent strain of the virus is identified by a formula which indicates the type (i.e. A, B or C); the animal from which the strain was first identified (e.g. duck) but omitted if the host was human; the place where the strain was first isolated; number of isolates; the year of isolation; particulars of HA and N antigens.

Human influenza A is currently caused by H3N2 and H1N1, with H1N2 and H3N1 being encountered rarely. Other serotypes of influenza viruses can be found in a variety of mammals though the largest natural reservoir is in aquatic birds where all the HA and NA types can be found. Influenza B and C only affect humans.

Four pandemics occurred in the 20th century. ‘Spanish flu’ in 1918 was caused by an unusually virulent strain of influenza A/H1N1, and in a few months more people died of it than in the preceding 4 years of war. The pandemic of ‘Asian flu’ in 1957 was caused by the H2N2 strain [(A/Singapore/1/57(H2N2)], believed to be the result of human and avian viruses recombining in pigs. Approximately 40–50% of people worldwide were infected. The majority of clinical cases occurred in children and the impact on mortality was in the elderly. The pandemic of ‘Hong Kong flu’ in 1968, caused by the H3N2 strain [(A/Hong Kong/1/68(H3N2)], is also believed to have arisen through recombination of human and animal viruses. It coincided with the end of war in Vietnam, and was carried to the United States by troops returning home. Soon after, outbreaks occurred in Europe. Symptoms were mild and excess deaths negligible. In 1976 the H1N1 virus re-emerged causing outbreaks of ‘Red flu’. From the above description it would appear that pandemic strains emerge suddenly and spread globally within a year. At present H5N1 and H7N7 strains appear to be the candidate strains, but there is a likelihood that another unknown strain may also appear.

More recently an outbreak of human infection due to avian influenza occurred in Hong Kong in 1997 when influenza A H5N1 strain caused severe respiratory disease in 18 subjects of whom six died. The outbreak coincided with an epidemic in poultry of highly virulent illness caused by the same strain. Then from 2003 to early 2004 the largest epidemic of influenza affecting birds occurred in a number of South-East Asian countries extending up to Japan and South Korea in the north to parts of Africa and Europe. At present humans appear to be poor hosts. When human infections occur they tend to cluster in families and households, and in most cases there is a clear history of contact with infected poultry. There is a high case fatality characterised by fulminant pneumonia attributed to a marked inflammatory response, dubbed ‘cytokine storm’, to the infection.

Another major outbreak of human infection due to avian influenza occurred in the Netherlands in 2003. The causative virus was influenza A H7N7, and 89 virologically documented cases were reported with one death. The actual number of people could be higher as the seroprevalence of H7 antibody among household contacts was about 59%.

There is serological evidence that H5N1 and H7N7 are capable of human-to-human transmission but the process does not appear to be efficient. The possibility of genetic reassortment between human and avian viruses, or a variant in a virus that is...
mutating rapidly, is a constant threat to the occurrence of a highly pathogenic virus that is readily transmissible from person to person and cause a pandemic.

H5N1 is close to meeting the criteria for pandemic virus—new, can cause human illness, and can be transmitted from human-to-human. It is currently in phase 3 of the WHO phases of alerts for pandemic influenza. It is not yet a full-blown pandemic strain because of a single factor: inefficiency of human-to-human transmission. Influenza viruses attach to host cells by binding of the haemagglutinin to sialosaccharides on the surface of the host cell. Human influenza viruses prefer sialic acid (SA)-α-2, 6 Gal-terminated saccharides, whereas avian influenza viruses prefer those terminating in SA-α-2, 3 Gal. [2, 3]. Other variables that influence the binding avidity are type of SA and glycosylation and sialylation of the haemagglutinin close to the receptor binding site.

Histological data show that H5N1 virus attaches predominantly to type II pneumocytes, alveolar macrophages and non-ciliated cuboidal epithelial cells in terminal bronchioles. Attachment is progressively rarer towards the trachea. Predilection of H5N1 virus for type II pneumocytes and alveolar macrophages may contribute to the severity of the pulmonary lesion. Type II pneumocytes are metabolically active and are the most numerous cell types lining the alveoli. Damage to type II pneumocytes may impair their functions including re-epithelization after alveolar damage, ion transport and surfactant production and so may inhibit tissue repair. Failure to attach to upper respiratory tract may be a limiting factor in human-to-human transmissibility of H5N1 virus [4].

Challenges in Vaccine Production

Flu vaccine in current use is grown in eggs, a process that takes up to 9 months and annual rounds of vaccination are needed because the flu strain changes year to year. Even then regular flu vaccine matches the circulating strain only 80–90% of the time, and often does not work that well in the elderly whose immune systems are not good at making new antibodies.

Since H5N1 virus is highly lethal to chick embryo current method of vaccine production will not work and alternative approaches are needed. Inactivated split virus vaccine or viral haemagglutinins expressed in baculovirus (viruses of insects) have performed below expectation. [5, 6]. Clinical trial of a vaccine employing the haemagglutinin and neuraminidase genes from H5N1 inserted into an egg adapted vaccine virus strain (A/PR/8/34[H1N1]) gave seroconversion rate of 54% after a two dose regimen at 90 μg/dose. [7] This is not considered satisfactory for dealing with a pandemic.

Another approach has been to excise the amino acid sequences in haemagglutinins responsible for virulence in the H5N1 virus A/Vietnam/1194/2004 and then combining with influenza viruses that grow well in eggs [A/PR/8/34 (H1N1)]. The seed virus is grown in embryonated eggs, inactivated and further processed to produce vaccines containing purified haemagglutinin [8]. In a clinical trial the highest immune response of 78% seropositivity was obtained with 10 μg/dose after two doses. These are early days and more studies are needed for selecting appropriate adjuvant, duration of immunity and more importantly to check whether the antibodies induced would be sufficient to protect in the event of a pandemic. This is a clade 1 vaccine and evidence must be gathered to find out whether it would be effective against clade 1 variants that have genetic and antigenic differences. Also antibodies generated by clade 1 vaccines may not cross react against clade 2 viruses.

A universal flu vaccine can avoid such problems as well as those of recurrent ‘drift’ challenges. Such a universal vaccine would be based on conserved flu proteins that do not mutate year to year. A hotly pursued strategy is based on flu protein called M2. This protein forms an ion channel crossing the membrane of a virus particle or infected cell, very slightly jutting out from the surface. The protruding part of M2 (M2 ectodomain, M2e) is made up of 23 amino acids which scarcely vary from one human flu strain to the next right back to the 1918 Spanish flu. A fusion protein made from M2e fused to Hepatitis B virus (three copies of M2e fused to Hep B core) can boost immune response in laboratory animals. However M2 antibodies seem to work by binding to infected cells and hastening their clearance instead of binding to the invading virus. They have a potential to prevent death rather than keep people from getting infected. This and similar other approaches are currently being pursued by scientists. For example, a vaccine based on H5 DNA delivered by means of an adenovirus has been shown to protect against the 1997 Hong Kong strain and the 2004 Vietnam strain.

The typical farm environment of South-East Asia and its wet markets provide a bubbling crucible out of which future ‘drift’ and ‘shift’ strains of influenza A would doubtless arise. It should not be difficult to develop appropriate strategies of protection with currently available techniques.

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References