COMMENTARY

The 2009 influenza pandemic

Shahid Jameel

A new influenza virus emerged in April 2009 and has spread efficiently, prompting the World Health Organization to declare a Phase 6 pandemic alert. In this commentary, I will discuss the biology of influenza viruses in general and the 2009 pandemic virus, especially their epidemiology, transmission, biology and pathogenesis, treatment and vaccines. I will also discuss the outbreak in India and the shameful lack of scientific data from our country.

A new strain of influenza virus, called swine-origin 2009 A (H1N1) influenza virus (hereafter called SO-IAV), emerged in Mexico and USA in April 2009, and has spread rapidly to 209 countries since then. As of 17 January 2010, the World Health Organization (WHO) reports at least 14,142 deaths[1]. On 11 June 2009, the WHO also raised the Flu Pandemic Alert Level to Phase 6, its highest level in over 40 years, officially designating a new global pandemic.

Influenza viruses and the 2009 pandemic virus

The influenza viruses (family Orthomyxoviridae) are enveloped viruses with segmented negative-stranded RNA genomes. They are classified in three genera – A, B and C, of which only viruses belonging to the first two cause any significant disease in humans[2]. The influenza A viruses contain eight genome segments that encode ten different viral proteins, of which nine are part of the virus structure (Figure 1). These include the surface haemagglutinin (HA), neuraminidase (NA) and M2 ion channel proteins, the M1 matrix protein, the nucleocapsid protein (NP) that packages the RNA genome, and the replication complex comprising the PA, PB1 and PB2 proteins. The NS1 protein is a virulence factor that modulates host innate immunity and is produced during infection. Some viruses also encode a protein called PB1-F2 from an alternate reading frame within the PB1 gene; this protein is also produced during infection and is associated with increased virulence and pathogenicity.

Influenza viruses are named on the basis of their surface proteins – HA, which is required for virus binding to the target cell, and NA, which is required for virus release from infected cells[2]. For influenza A viruses, 16 HA serotypes (H1–H16) and 9 NA serotypes (N1–N9) are known, of which only the H1, H2, H3 and H5 viruses, and rarely the H7 and H9 viruses have been found to infect humans[2].

Influenza viruses evolve through 'antigenic drift', and occasionally by 'antigenic shift'[2]. The viral RNA dependent RNA polymerase (replicase) lacks proofreading activity and is therefore unable to correct random errors introduced in the genome during replication. The effects of this are most obvious in the HA protein, which shows high rates of amino acid substitutions in its epitopes, and for which the ratio of non-synonymous to synonymous substitutions is >1. This indicates a positive selection, which is directly related to evasion of host immunity. 'Antigenic drift' changes the HA protein enough to render immunity acquired during an influenza season, either through infection or vaccination, ineffective in the next season. A more serious problem occurs when two different influenza viruses infect the same host. This leads to a reassortment of genome segments and the generation of novel progeny viruses. If this reassortment takes place between viruses originating from different species, it can generate viruses with pandemic potential, which include HA and/or NA proteins from the avian or swine influenza viruses against which humans have no preexisting immunity. This introduction of completely new HA serotypes into circulating human influenza viruses is called 'antigenic shift'.

The 20th century witnessed three influenza pandemics – the 'Spanish flu' of 1918–20 that was caused by an H1N1

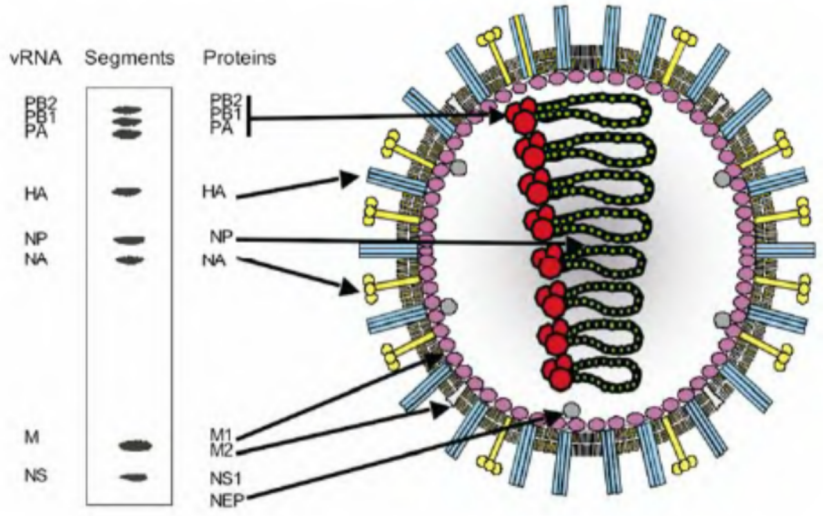

Figure 1. The architecture of influenza A viruses. The viral RNA (vRNA) in descending size, the proteins encoded by these and their location in the virus particle are shown.

virus and killed about 40 million people, the 'Asian flu' of 1957–58 that was caused by an H2N2 virus and killed about 1.5 million people, and the 'Hong Kong flu' of 1968–69 that was caused by an H3N2 virus and killed about 1 million people. In all of these viruses, the HA gene was from the avian virus lineage. The 2009 SO-IAV is also an H1N1 virus, but is a 'triple reassortant' with its HA gene from the swine lineage[3]. It contains a combination of gene segments that has previously not been reported in human or swine influenza viruses (Figure 2).

The lineages of various gene segments in the 2009 pandemic virus have been traced to viruses that have circulated in swine populations[3]. The HA, NP and NS genes are from the classical swine lineage, which entered pigs from birds around 1918 and have circulated since then. The M and NA genes are from an avian virus, which entered pigs around 1979 and has circulated since then only in Eurasia. The PA, PB1 and PB2 genetic lineages were traced to triple reassortant swine viruses. Of these, PB1 came into pigs from humans around 1998, but was seeded into humans from birds around 1968. The PA and PB2 lineages were also seeded into the swine population from birds around 1998. Thus, the SO-IAV is a complex mixture of influenza viruses that have circulated in avian, human and swine populations for many years. Genetic analyses of multiple virus isolates have also shown low diversity, suggesting that its introduction into humans is recent and through either a single event or multiple events involving similar viruses[4]. The molecular markers of adaptation to humans are also not observed in SO-IAV, suggesting that unrecognized determinants might be responsible for its efficient transmission in the human population. These viruses are antigenically homogenous and are similar to the H1N1 North American swine A viruses, but are distinct from the seasonal H1N1 human A viruses.

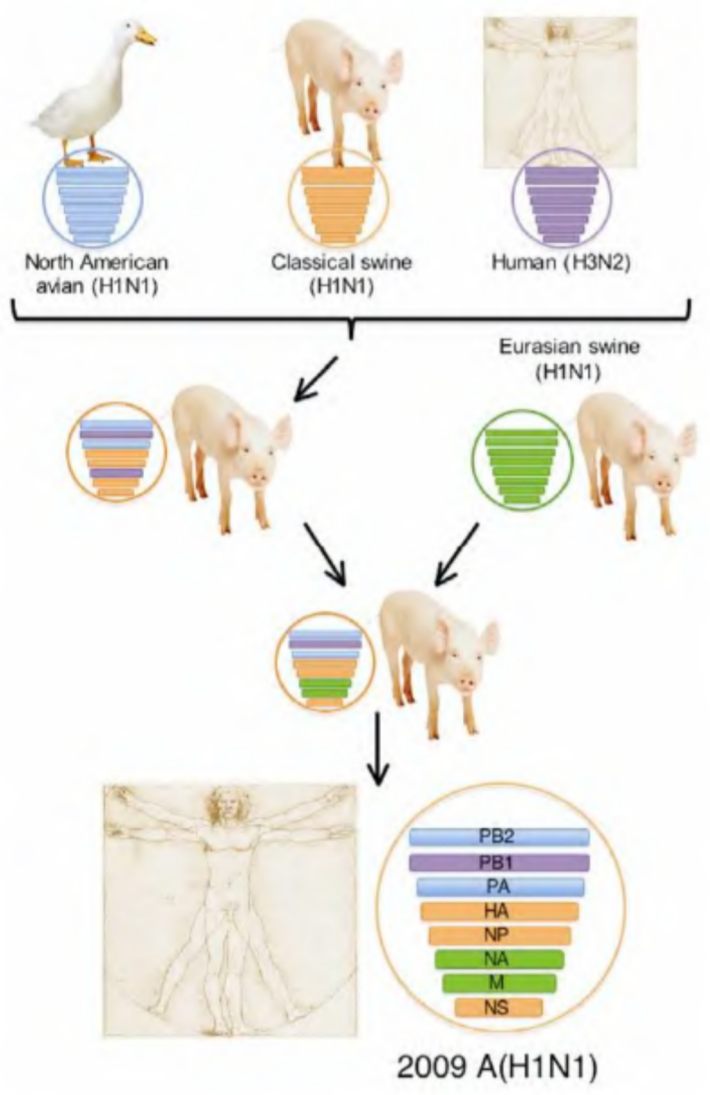

Figure 2. The lineage of SO-IAV. See text for details.

Epidemiology and transmission

The SO-IAV has efficiently transmitted between humans since its first detection in April 2009. So far it has spread in the Northern hemisphere outside of the flu season and in the Southern hemisphere during its flu season. It has caused mild disease, and that is in accordance with absence of the pathogenicity marker PB1-F2. As seasonal influenza A(H1N1) viruses are also circulating in humans since 1977, mild disease could also be due to partial immunity in the population. It will now be critical to watch how the virus behaves as it comes back to the Northern hemisphere in the next wave with the approaching flu season.

All previously characterized pandemics have been due to viruses generated by antigenic shift involving the HA gene of avian lineage – H1 for 1918 virus, H2 for 1957 virus and H3 for 1968 virus[2]. Though still of the H1 serotype, the HA of SO-IAV is different from the H1 HAs of seasonal influenza viruses; the inclusion of a porcine H1 in human influenza A viruses has been considered an antigenic pseudo-shift[5]. Although SO-IAV is of zoonotic origin, its HA may not be sufficiently divergent to call it a true antigenic shift[5]. Compared to viruses that caused previous pandemics, SO-IAV is at present not sufficiently virulent. But, it is already transmitting like a pandemic virus and is undergoing adaptive mutations. Whether it will remain mild or develop into a highly pathogenic fully pandemic virus remains to be seen.

Biology and pathogenesis

Influenza viruses enter their target cells by binding to sialic acids present on the surface of these cells[2]. These sialyl glycans occur as two main types, with the terminating *N*-acetylneuraminic acid linked to galactose either through a α2-3

or a α2-6 linkage, called Neu5Aα2-3Gal or Neu5Aα2-6Gal respectively. The sialyl glycans vary in their tissue and species distribution, and this determines the host range, tissue tropism and the ability of animal-origin influenza viruses to initiate a human pandemic. The Neu5Aα2-3Gal is the preferred receptor for 'avian-type' viruses, while the 'human-type' viruses have adapted to use Neu5Aα2-6Gal glycans. Using *SO-IAV* gene sequences, a modelling study has predicted these viruses to bind with high affinity to α2-6 linked glycans, and to also bind α2-3 linked glycans with increased affinity compared to other human H1N1 HA proteins[6]. This analysis also found novel substitutions (Lys145, Ser186, Thr187 and Ala189) in the HA protein of SO-IAV, suggesting that new epitopes may be generated, which would have implications for binding and neutralization. A study using carbohydrate microarrays compared the receptor specificities of two pandemic isolates of SO-IAV to seasonal and swine H1N1 viruses[7]. The results confirm that SO-IAV utilizes α2-6 as well as α2-3 glycans, which agrees with its increased virulence in animal models[8–10]. Since there is a higher proportion of α2-3-linked glycans in the human lower respiratory tract, viruses that utilize these receptors are thought to cause more severe infections. This may partly explain increased viral replication and pathology observed with pandemic viruses in the lungs of ferrets, mice and non-human primates compared to seasonal viruses[8–10].

Once endocytosed into cells, influenza viruses undergo uncoating in the acidified endosomes, which is driven by the viral M2 ion (H+) channel protein. This is the target for adamantane drugs, such as Amantadine and Rimantadine (Figure 3). The M2 proteins of SO-IAV carry the Ser31Asn mutation, which provides resistance to this class of drugs[11]. Following genome replication and protein synthesis, processing and intracellular trafficking, the ribonucleoprotein (RNP) complexes are packaged into new virions. The release (budding) of these virions requires NA, and this is yet another target for anti-influenza drugs such as Oseltamivir (Tamiflu) and Zanamivir (Relenza) (Figure 3). Analysis of the NA protein from available genomic sequences showed four novel substitutions (at positions 189, 331, 369 and 398) in SO-IAV[6]. Oseltamivir-resistant seasonal influenza A(H1N1) viruses carrying a His274Tyr mutation in the NA active site have been found in Europe, Oceania, South East Asia and South Africa during the 2007/08 flu season[12]. This mutation has also been detected at very low frequency in SO-IAV from Hong Kong[13], USA[14] and other parts of the world[15]. Since the NA His274Tyr mutation is rare in SO-IAV, Tamiflu continues to be the drug of choice. In models with such a mutant virus, Zanamivir was found to still make optimal contacts with the NA active site[6], suggesting the possibility of treating patients infected with Oseltamivir-resistant viruses with Zanamivir (Relenza).

Three excellent studies have addressed the transmission and pathogenesis of SO-IAV in ferret, mice and non-human primate models[8–10]. Munster *et al.*[8] found SO-IAV to be more pathogenic and to replicate better than seasonal viruses in the respiratory tracts of ferrets. Whereas replication of the seasonal viruses was limited to the nasal cavity, pandemic viruses were also found in the trachea and lungs. Virus shedding from the upper respiratory tract was also more efficient for the pandemic viruses. Maines *et al.*[9] carried out similar studies in ferrets and mice and reached similar conclusions, except to also show recovery of the pandemic virus from the intestines of intranasally inoculated ferrets. Itoh *et al.*[10] also found SO-IAV to replicate more efficiently than a seasonal virus in cell cultures, and in mice and ferrets. They additionally tested the two viruses in cynomolgus macaques and found SO-IAV to cause more severe lesions in the lungs; in specific-pathogen-free pigs, SO-IAV was found to replicate without any clinical symptoms. Assessment of human sera from different age groups suggested that prior exposure to human H1N1 viruses antigenically related to the 1918 viruses is likely to confer protection against SO-IAV. This is supported by reduced mortality and morbidity observed in the old, who are normally more susceptible to seasonal influenza. Itoh *et al.*[10] also showed the efficacy of Oseltamivir and Zanamivir, as also two experimental NA inhibitors T-705 and CS-8958, against SO-IAV in cell cultures and in mice.

Detection, treatment and vaccines

The WHO has put together guidelines and protocols for a real time reverse transcription-polymerase chain reaction (RT-PCR) assay for SO-IAV[16]. This provided a rapid and sensitive, though expensive, assay that has proven critical in assessing the spread of the pandemic. This assay involves three primer and probe sets: 'InfA', which amplify a conserved part of the matrix gene from all influenza A viruses; 'SW H1', which detect an HA gene segment unique to SO-IAV; and 'SW InfA', which detect the nucleoprotein gene from all swine influenza viruses. A recent report has claimed that

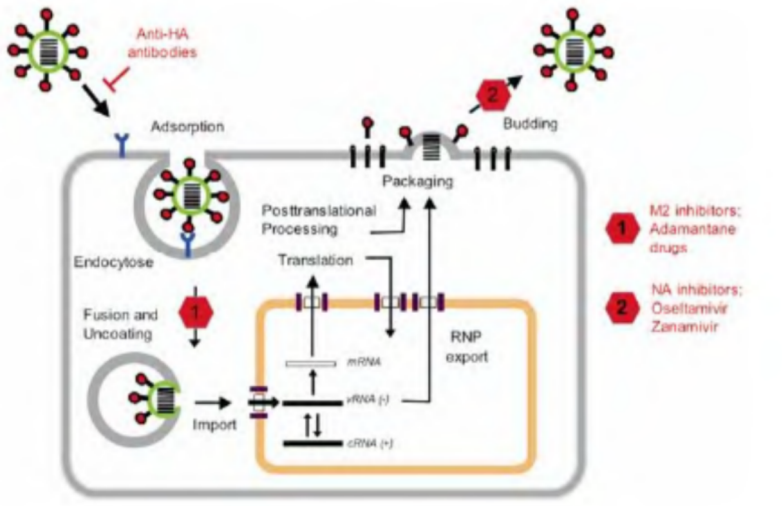

Figure 3. Influenza virus life cycle. Various steps in the entry, uncoating, replication and assembly of influenza viruses is shown. The targets for the two classes of drugs (1) M2 inhibitors and (2) NA inhibitors are also indicated.

the 'SW InfA' assay is not specific to swine-origin influenza viruses, but is also able to detect human and avian A(H5N1) influenza viruses[17]. A number of other real time RT-PCR assays have been developed and published during the past few months, but these have not yet been independently validated. The Centres for Disease Control and prevention (CDC, USA) carried out an assessment of various rapid influenza diagnostic tests (RIDT) and found sensitivity issues with these tests[18]. Thus, while a positive RIDT can be used for making treatment decisions, a negative RIDT cannot guide treatment options, which will have to be based on empirical clinical observations.

The standard treatment for SO-IAV infection is Tamiflu given as 75 mg tablets twice a day for 5 days to adults, and reduced doses to children, depending upon age and weight. The drug mitigates symptoms when administered within 48 h of infection, which is an impractical situation in most countries due to a 24–48 h turnaround time for testing. As a result, various countries have followed different strategies, but the antiviral is available everywhere through physicians and treatment centres. Its sale in the open market is generally banned due to the threat of misuse and development of resistant viruses. In rare cases of therapy failure, possibly due to Oseltamivir-resistant viruses, no further treatment or treatment with Relenza (Zanamivir) has shown positive results[14]. Seasonal influenza H1N1 viruses resistant to Zanamivir have also been detected in Australia at a frequency of about 2%. This resistance has been mapped to a previously undescribed Gln136Lys NA mutation that reduces Zanamivir susceptibility by about 300-fold, but the virus remains susceptible to Oseltamivir[19].

Who should be treated with Tamiflu? Physicians in western countries recommend treating everyone who presents with symptoms, especially those in high-risk groups, irrespective of a positive test[20]. If the test is positive, Tamiflu is continued, but if it is negative the treatment is abandoned. High-risk groups include those with underlying chronic conditions such as asthma, cardiovascular disease and diabetes, obese people and pregnant women.

Vaccines for influenza are based on the development of a vaccine strain, which is a reassortant between the outbreak strain and a strain that can grow well in chicken eggs or tissue culture cells. The reassortant virus with antigenic properties of the outbreak strain is then grown to high titer in embryonated eggs or cultured cells, purified and inactivated. It takes 5–6 months from the time the outbreak strain is identified to vaccine preparation. Vaccines for SO-IAV have been made and tested in humans. These trials have included a monovalent unadjuvanted, inactivated, split-virus vaccine produced in chicken eggs by CSL Biotherapies (Parkville, Australia)[21]; another inactivated vaccine produced in MDCK cells by Novartis (Marburg, Germany) was tested with and without the MF59 adjuvant[22]. Interim analyses showed that a single 7.5 μg dose or a 15 μg dose of the unadjuvanted vaccine raised optimal antibody responses in 14–21 days. The US National Institutes of Health is also carrying out five clinical trials of the vaccines from CSL Biotherapies and Sanofi-Pasteur[23].

How soon will the vaccine be available to the public-at-large in western countries and more so in poor countries, is another question. The current global capacity is estimated to be about 3 billion doses annually and many developed countries have already ordered advance supplies to cover their populations. There is also some reluctance among healthcare workers in western countries to take the vaccine due to an increase in paralytic events associated with a swine flu vaccine used in USA in 1976–77. The first stocks of the pandemic vaccine have been delivered and vaccinations of healthcare workers have started in USA and some other western countries.

The outbreak in India

Following the outbreak in Mexico and USA in April 2009, India initiated entry screening of people coming from swine flu-affected countries. A few testing centres were also set up quickly following availability of the WHO real time RT-PCR test. Most of the early cases were detected in travellers coming to India from affected countries or their contacts.

As of 20 January 2010, the Ministry of Health and Family Welfare (MoHFW) reports that over 10.5 million passengers have been screened at the country's airports, samples from 120,660 persons were tested, of which 28,401 (23.5%) were found to be positive[24]. The first SO-IAV death was on 3 August in Pune. Since that time 1152 deaths have been confirmed, giving a case fatality of 4%. The highest numbers of cases have been reported from Delhi (9625), Maharashtra (4943), Delhi (3364), Tamil Nadu (2079), Rajasthan (2073), Karnataka (2007) and Haryana (1930). Urban centres like Pune, Mumbai and Delhi have been the most affected in India.

Even though India has over 28,000 confirmed cases and 1152 deaths, which if you believe the infectious disease iceberg model, would translate into many-folds more, no epidemiological analysis of the Indian outbreak is found in the public domain. We do not know the risk factors for the Indian population, the reasons for a mortality rate that is about 3–4 times the global average, or any epidemiological details of the terrifying spread in cities like Pune. There are also no genomic sequences from India uploaded in public databases, making it difficult to analyse the virus circulating in India.

The MoHFW is releasing daily updates since 1 August 2009, and that remains the only source of information on SO-IAV spread in India. On the basis of this information, I have drawn the graph shown in Figure 4, which is updated till 20 January 2010. It is quite clear from this analysis that cases in India have shown no signs of levelling off from August till October, a period that is outside our regular flu season. As we enter the normal flu season, the cases did increase in what appears to be a second wave. Since the 'mortality curve' runs almost parallel to the 'cases curve', and as the number of cases increase, we should be prepared for more deaths.

How has India dealt with the outbreak? There was definitely value in early screening and the government did well to aggressively screen at ports of entry. This early screening and testing delayed the spread of SO-IAV in India by 2–3 weeks, but it has eventually followed an expected pattern of spread – first in large urban centres followed by small towns, and we do not even know the situation in rural areas. Public health experts now see no value in screening at ports of entry since the virus is spreading efficiently in the population[25]. Even in the presence of a Public Health Preparedness Plan, an early initiative to screen for the pandemic virus, and the government's generous spending on the testing initiatives, experts believe

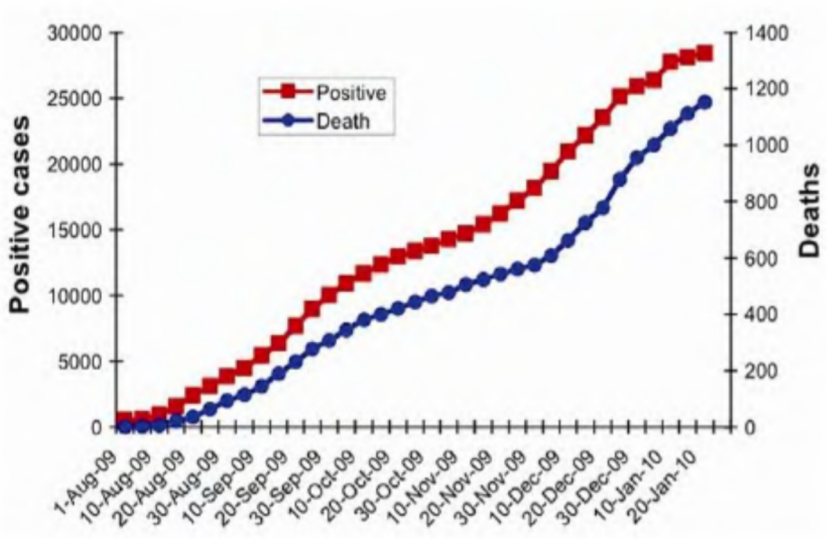

Figure 4. Swine flu in India. The plots show cumulative positive cases (red line) and mortality (blue line) due to SO-IAV infection. The source of data is the website of the Ministry of Health and Family Welfare, Government of India.

that a poor healthcare infrastructure has failed the country[25]. There is a clear need to strengthen that.

An over-zealous media coupled with the government's perceived lack of transparency and an inherent mistrust of the government system, created widespread panic and knee-jerk reactions. This is not good for dealing with any pandemic. There is a need for more transparency from government institutions and the media should be considered partners in disseminating the message. And the message is that this pandemic is serious, but we have the tools to manage it. There will be more cases and there will be more deaths, but a calm and sustained response (and not panic) is the only way to overcome it.

Various reports in the media have quoted the Indian Council of Medical Research (ICMR) and the MoHFW that a vaccine against the 2009 pandemic virus will soon undergo bridge trials in the country, and will hopefully be deployed by mid-2010. In the absence of any epidemiological analysis of the outbreak in India, who will receive it? Have we identified the risk groups, or will we give it to the same population subgroups as the Americans and the Europeans? Considering that ICMR has an entire national institute dedicated to disease epidemiology (National Institute of Epidemiology, Chennai), it is shameful that no epidemiological analysis of the outbreak is available. It is imperative that such information be in the public domain for all stakeholders to analyse it and participate in the vaccination strategy.

Epilogue

Pandemic influenza is unpredictable. While the world was focused on the H5N1 avian influenza virus, which has become endemic in wild birds and poultry in many regions, and which has shown about 60% mortality in the small numbers of infected humans, SO-IAV emerged to transmit efficiently between humans. Thankfully, the mortality is still low. But, will this virus return in a more virulent form in the next wave? The 1918 pandemic started that summer as a mild disease, but in the next wave during winter, the virus came back in a highly virulent form, eventually infecting about a third of the world population and killing an estimated 40–50 million persons. This history of pandemic flu is reason enough to exercise caution and limit virus transmission in the human population.

Can a new pandemic virus still cause similar levels of mortality? With increasing population density, large urban centres and airline travel that can take an asymptomatic carrier half way around the world in 24 hours, the potential for transmission is much greater today than any other time in human history. If about 30% of the world population, which is 6.8 billion, gets infected, it will amount to about 2 billion people. At the present low rate of about 1% mortality, we are still looking at roughly 20 million deaths. Are we ready to accept this in 2009?

Thankfully, this scenario is offset by immense progress in the prevention, detection and treatment of infectious diseases. The agent for the 1918 flu took 13 years to identify, but the 2009 pandemic virus was identified in days, characterized in weeks and a vaccine is ready in less than 6 months. Health systems are also better prepared today to deal with such exigencies than they were 90 years ago.

The biggest challenge for the world today is to remove global disparities in the availability of pandemic flu drugs and vaccines. In the early part of the last century, fire brigades would only go to properties that had fire insurance. The problem with this model was that if your neighbour did not have insurance, your house would burn too, even if you had fire insurance. Pandemic influenza is like that. It will continue to spread unless it is tackled at the origin, and for that supplies must be available to poorer countries as well.

This is the first major influenza pandemic since 1977 and the first in the era of biotechnology. It reflects in the speed with which the virus was characterized and vaccines were made. This is also a great example to showcase the benefits of investments in biomedical research. Scientists, physicians, administrators and politicians who fought for these resources deserve our gratitude.

Note added in proof: The 2009 pandemic influenza virus continues to be mild even during the ongoing flu season in the Northern hemisphere. There are also no indications of mutations that are associated with increased virulence. Oseltamivir-resistant mutants have been detected but have not shown widespread transmission. Vaccines for the 2009 pandemic influenza are now available in western countries. India has reportedly placed orders for 1 million doses of the vaccine and bridge trials for the Sanofi-Pasteur vaccine are starting in 100 volunteers at three centres. There are also reports of Indian vaccine manufacturers coming up with indigenous vaccines, which are likely to be available by mid-2010.

1. World Health Organization, Pandemic (H1N1) 2009 – update 84. WHO, 2009; http://www.who.int/csr/don/2010_10_22/en/index.html
2. Wright, P. F., Neumann, G. and Kawaoka, Y., In *Field's Virology* (eds Knipe, D. M. and Howley, P. M.), Lippincott, Williams and Wilkins, Philadelphia, 2007, pp. 1691–1740.
3. Garten, R. J. *et al.*, *Science*, 2009, **325**, 197–201.
4. Schnitzler, S. U. and Schnitzler, P., *Virus Genes*, 2009; doi: 10.1007/s11262-009-0404-8.
5. Gatherer, D., *J. Clin. Virol.*, 2009, **45**, 174–178.
6. Soundararajan, V. *et al.*, *Nature Biotech.*, 2009, **27**, 510–513.
7. Childs, R. A. *et al.*, *Nature Biotech.*, 2009, **27**, 797–799.
8. Munster, V. J., *Science*, 2009, **325**, 481–483.
9. Maines, T. R. *et al.*, *Science*, 2009, **325**, 484–487.
10. Itoh, Y. *et al.*, *Nature*, 2009, **460**, 1021–1025.
11. Stouffer, A. L. *et al.*, *Nature*, 2008, **451**, 596–599.
12. Hurt, A. C. *et al.*, *Antiviral Res.*, 2009, **83**, 90–93.
13. Leung, T. W. C. *et al.*, *J. Clin. Virol.*, 2009, **46**, 298–299.
14. Garrison, M. *et al.*, *MMWR*, 2009, **58**, 969–972.
15. Influenza Virus Resource, GenBank sequences from pandemic (H1N1) 2009 viruses, 2009; http://www.ncbi.nlm.nih.gov/genomes/FLU/SwineFlu.html
16. Centres for Disease Control and Prevention, CDC protocol of realtime RTPCR for influenza A(H1N1), 30 April 2009; http://www.who.int/csr/resources/publications/swineflu/realtimeptpcr/en/
17. Peacey, M. *et al.*, *J. Clin. Microbiol.*, 2009, **47**, 3789–3790.
18. Balish, A. *et al.*, *MMWR*, 2009, **58**, 826–829.
19. Hurt, A. C. *et al.*, *J. Virol.*, 2009, **83**, 10366–10373.
20. Ellis, C. and McEwen, R., *Br. Med. J.*, 2009, **339**, b2698.
21. Greenberg, M. E. *et al.*, *N. Engl. J. Med.*, 10 September 2009 (Epub ahead of print).
22. Clark, T. W. *et al.*, *N. Engl. J. Med.*, 2009, **361**, 2405–2413.
23. Butler, D., *Nature*, 2009, **460**, 562.
24. Ministry of Health and Family Welfare, Government of India, Press releases on swine flu, 20 January 2010; http://mohfw.nic.in/press_releases_on_swine_flu.htm
25. John, J. T. and Muliyil, J., *Indian J. Med. Res.*, 2009, **130**, 101–104.

ACKNOWLEDGEMENTS. I thank Prof. T. Jacob John for his advice and encouragement to learn and write about influenza. Much of the modern diagnostic infrastructure that we see today in India was initiated in the mid-1990s after the Surat plague through the vision and leadership of (late) Prof. V. Ramalingaswami. This review is dedicated to his memory.

Shahid Jameel is in the Virology Group, International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi 110 067, India.
e-mail: shahid@icgeb.res.in