Indian J Microbiol (December 2009) 49:365–369

SCIENTIFIC CORRESPONDENCE

Pandemic swine influenza virus (H1N1): A threatening evolution

Madhu Khanna · Binod Kumar · Neha Gupta · Prashant Kumar · Ankit Gupta · V K. Vijayan · Harpreet Kaur

Received: 21 October 2009 / Accepted: 26 October 2009

Abstract "Survival of the fittest" is an old axiom laid down by the great evolutionist Charles Darwin and micro-organisms seem to have exploited this statement to a great extent. The ability of viruses to adapt themselves to the changing environment has made it possible to inhabit itself in this vast world for the past millions of years. Experts are well versed with the fact that influenza viruses have the capability to trade genetic components from one to the other within animal and human population. In mid April 2009, the Centers for Disease Control and Prevention and the World Health Organization had recognized a dramatic increase in number of influenza cases. These current 2009 infections were found to be caused by a new strain of influenza type A H1N1 virus which is a re-assortment of several strains of influenza viruses commonly infecting human, avian, and swine population. This evolution is quite dependent on swine population which acts as a main reservoir for the re-assortment event in virus. With the current rate of progress and the efforts of heath authorities worldwide, we have still not lost the race against fighting this virus. This article gives an insight to the probable source of origin and the evolutionary progress it has gone through that makes it a potential threat in the future, the current scenario and the possible measures that may be explored to further strengthen the war against pandemic.

M. Khanna[1] (✉) · B. Kumar[1] · N. Gupta[1] · P. Kumar[1] · A. Gupta[1] · V. K. Vijayan[2] · H. Kaur[3]
[1]Department of Respiratory Virology, V. P. Chest Institute, University of Delhi, India
[2]Department of Respiratory Medicine, V. P. Chest Institute, University of Delhi, India
[3]Indian Council of Medical Research, Ansari Nagar, New Delhi, India

E-mail: madhukhanna_du@yahoo.com

Keywords Pandemic · Swine influenza virus · H1N1

Introduction

Influenza A virus, which belongs to the Orthomyxoviridae family of RNA viruses, mutate more rapidly showing more antigenic flexibility and hence are more virulent as compared to the other two subtypes of influenza virus (B and C) [1, 2]. It has been responsible for widescale pandemics since the 16th century causing at least 31 pandemics in the past 400 years [3]. The world had seen three major pandemics that have occurred in the 20th century, marked as the Spanish flu in 1918, Asian flu in 1957 and Hong Kong flu in 1968–69 leading to a large death toll [4–6]. Adding to this number is the novel swine (H1N1) 2009 virus recently spread globally with unprecedented speed that the World Health Organisation (WHO) raised its pandemic alert phase from Phase 3 to Phase 4 on 27 April 2009 and from Phase 4 to Phase 5 on 29 April 2009. On 11 June 2009 the pandemic level was escalated further from phase 5 to 6, which has made the health authorities conscious of its future potentials as the killer flu [6]. The attack rates for this A (H1N1) pandemic strain are expected to be higher than for seasonal strains because of the lower levels of pre-existing immunity in the population. Often, these new strains appear when an existing flu virus spreads to humans from other animal species, or when an existing human strain picks up new genes from a virus that usually infects birds. The sudden ability of this new swine flu virus to jump from pigs to humans and then to have sustained human to human transmission is an excellent example of evolution at work.

Being reported for the first time in United States and Mexico in March–April 2009, pandemic influenza H1N1 has emerged as deadly and virulent form of swine influenza virus that has infected human population at an

alarmingly high rate showing a sustained global pattern of human transmission[7, 8]. According to the WHO, a total of 3,99,232 laboratory confirmed cases of swine influenza have been reported, spreading over 175 countries, with a total of 4,735 deaths till the mid of October 2009 around the world [9]. The WHO-SEARO has reported 39,522 cases with 530 deaths while in India alone, out of 12,486 laboratory confirmed cases the number of deaths reported is 405 till mid of October 2009 [10].

Origin of influenza A (H1N1) virus

Genetic reassortment is one of the major reasons for a pandemic outbreak and occurs when genes from different species viz. human, avian and swine come together and are able to infect different host [11]. The rapid evolution of the influenza virus is an excellent example of natural selection. Influenza virus deserves particular attention as it undergoes a high rate of antigenic change giving rise to a new type of influenza strain for which there is no immunity in the population. H1N1 influenza A virus has persisted for more than 90 years since the 1918 pandemic. It is quite remarkable that the descendents of 1918 virus still circulate in humans as epidemic H1N1 and and in swine as epizootic H1N1 causing new pandemics, epidemics and epizootics. The novel H1N1 virus of the 2009 is the fourth generation descendent of the 1918 virus (Table 1) [12].

The genetic make-up of the novel 2009 H1N1 influenza A virus is that of a reassortant form which is constituted of gene segments from the swine, avian and human flu virus genes that does not share much similarity with the earlier known swine viruses. The current novel H1N1 virus shows it is a "quadruple reassortant" virus having 2 genes of Eurasian origin and 6 genes of North American origin which has infected the swine population. It is a reassortant with three potential host source with HA, NA, MP, NP and NS originating from swine influenza virus, PB2 and PA originating from bird influenza virus and PB1 from human influenza virus (figure 1) [13, 14].

Mutational changes in pandemic H1N1 influenza virus

The applicability of comparative sequence analysis technique for the molecular determinants of virulence in viruses has a profound effect on recent viewing in systematics and evolution of the virus. This type of molecular screening and phylogenetic assessment helps in understanding evolution of influenza viruses and for the early detection of emerging novel viruses which can lead to influenza pandemics. The emphasis on the seasonal influenza strains have recently been suppressed by emergence of even more virulent swine influenza virus 2009 (H1N1) strain. To gain an insight into the possible origin of 2009 outbreak of new influenza A (H1N1), protein homology and phylogenetic analysis has been used to study the sequence of proteins encoded by PB1, PB2, PA, HA, NP, MP1 and NS1 of the new H1N1 virus. These

Table 1 Influenza pandemics, associated strains and mortality

Years	Circulating virus strains	Number of deaths (no. per 1,00,000 people/year)
1918–1919	H1N1, Ist major pandemic	598.0
1928–1929	H1N1 (antigenic drift)	96.7
1934–1936	H1N1 (antigenic drift)	52.0
1947–1948	H1N1 A'	8.9
1951–1953	H1N1 (intrasubtypic reassortment)	34.1
1957–1958	H2N2 (antigenic shift)	40.6
1968–1969	H3N2 (antigenic shift)	16.9
1972–1973	H3N2 (antigenic drift)	11.8
1975–1976	H3N2 (drift) and H1N1 (swine outbreak)	12.4
1977–1978	H3N2 and H1N1	21.0
2003–2004	H3N2 A Fujian (intrasubtypic reassortment) and H1N1 (drift)	17.1
2009 till mid Oct. 09	H3N2 and H1N1 and swine origin H1N1 pandemic	Still continuing

Different circulating virus strains in different pandemic years with the mortality data [11]

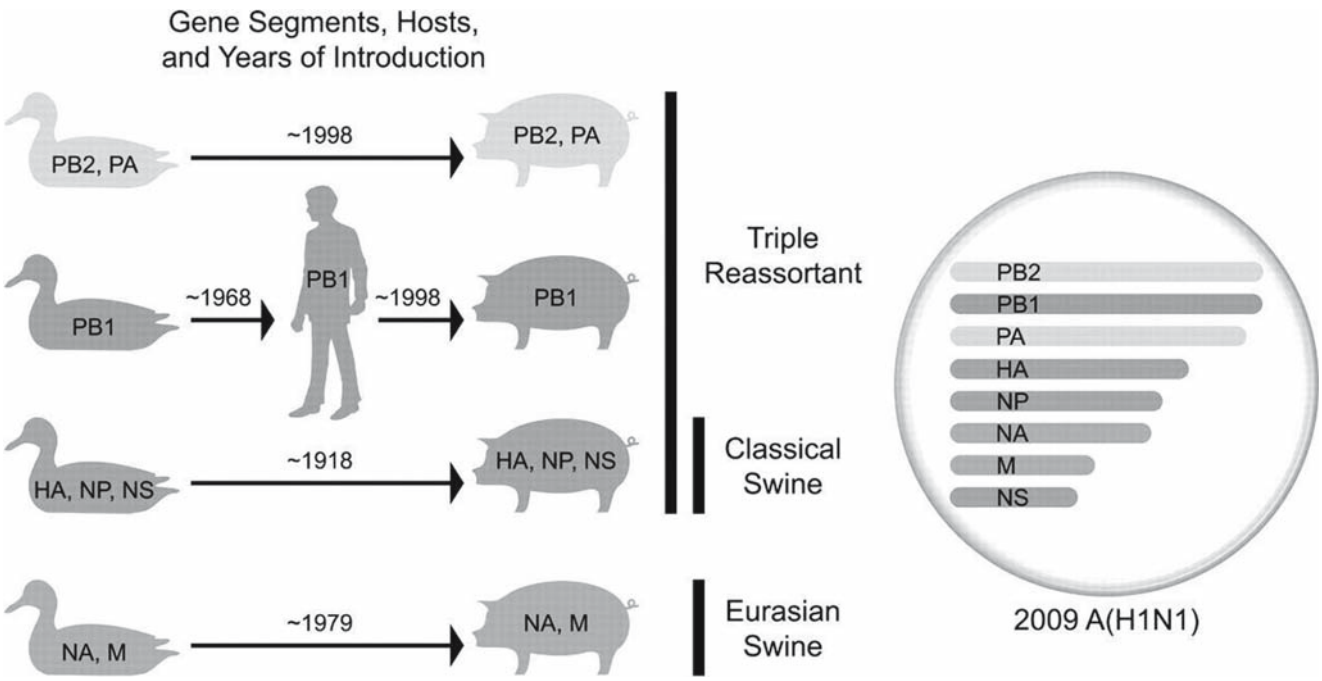

Fig. 1 Host and lineage origins for the gene segments of the 2009 A(H1N1) virus
PB2: polymerase basic 2; *PB1*: polymerase basic 1; PA: polymerase acidic; HA: hemagglutinin; NP: nucleoprotein; NA: neuraminidase; M: matrix gene; NS: non-structural gene. Gene segment in circle indicates host [13].

studies revealed that the virus has most likely evolved from recent swine viruses and that the new influenza A (H1N1) virus possesses a distinctive evolutionary trait to the influenza A viruses circulating in North America for the last two decades (1989–2009). These analyses also contribute to the role of pig population as the mixing vessels for influenza H1N1 viruses.

Looking in detail of some of the important genes of the virus, studies reveal the HA and the NA gene of the recent swine H1N1 virus, both coding for the surface antigens, has undergone a change. The HA gene has acquired a proline to serine substitution at position 200 thus has become slightly less virulent as compared to the 1918 (H1N1) virus. The NA gene, is also quite novel differing by 18.2% from the seasonal H1N1 virus which may be a possible reason that the available vaccines may not work effectively against the virus thereby increasing the demand for newly formulated vaccines in a short period of time [15, 16].

NS1 protein, the non-structural protein encoded by the NS gene is also truncated by stop codon at position 220 hereby creating a deletion in the PDZ domain involved in variety of cell signaling pathways as evident in 1918 H1N1 and H5N1 flu viruses [17] .One of the other important genes PB1–F2, associated with the pathogenicity of 1918 pandemic virus and H5N1, has been found to be truncated with a stop codon at three positions of 12, 55 and 88 indicating that further genetic analysis study is required to fully associate this gene functionality to the virulence of this novel H1N1 virus [18].

Antiviral and vaccination strategy

The clinical manifestations of the current 2009 influenza A (H1N1) virus are quite similar to the seasonal influenza virus, that makes it very difficult to differentially diagnose the swine H1N1 infection from the seasonal one, if precise and reliable molecular tests such as the real time PCR are not used. The incubation period of seasonal influenza in human is generally 1–4 days, with an average of 2 days which is similar for swine H1N1 except that few patients experience a sudden and rapid deterioration in their clinical condition, usually on day 5 or 6 following the onset of symptoms [19].

The new influenza A (H1N1) are currently susceptible to neuraminidase inhibitors such as oseltamivir (Tamiflu) and zanamivir (Relenza), but are found to be resistant to M2 inhibitors such as Amantadine and Rimantidine [20]. Use of the present available antiviral drugs for prophylaxis is also not recommended, since in many countries viz. Japan, Denmark, and Hong Kong, till now, 21 cases of

oseltamivir resistance have been reported . The mutation in NA gene of the new virus, shows that the antiviral drug, Tamiflu (oseltamivir), may not be able to control the virus effectively and further enhance the possibility of generation of drug resistant viruses as seen in some countries [15].

Vaccination with a strain-specific pandemic vaccine is considered to be one of the most effective measures for protecting individuals in the event of a pandemic. Thus, vaccines are considered to be the best approach towards preventing spread of the swine influenza infection. The new vaccine candidate for the 2009 pandemic influenza A has been influenza A /California /7 /2009, that is being produced as an inactivated surface antigen, since the existing sequences of 2009 influenza A are similar to this strain. Vaccine development against the current virus has started with the use of adjuvant for which trials have been started with M59 adjuvant and non-adjuvant forms [21]. In India also, three pharmaceutical companies, Serum Institute of India, Bharat Biotech, Hyderabad and Panacea Biotec, New Delhi have joined in the race for development of vaccine and are working diligently on its development [22]

Since, this novel swine H1N1 is highly unpredictable at this point of time, and it is pretty difficult to forecast about its pathogenicity well in advance. The virus may lose its virulence or even become more virulent in its second wave. It may acquire spontaneous mutations which may make it more pathogenic or the virus may even swap genes with the highly pathogenic avian influenza virus (H5N1) through gene re-assortment and lead to a formation of even deadlier strain capable of sustained human to human transmission. If these events continue to occur in the second wave, the impact of the virus may become more severe depending on the population density, and the ability of the virus to mutate and take even more virulent forms.

Presently, the Global Influenza Surveillance System of WHO is built upon a network of 112 national influenza centres in 83 countries working closely together with its four WHO collaborating centres. By participating in the Global Influenza Surveillance Network, a country can effectively contribute to information regarding the pandemic situation globally [23].

The recent pandemic alert indicates that it is time to take stringent action towards the establishment of a strong and systemic surveillance system that will integrate phylogenetic information of influenza in human and livestock. The re-assortment events in mammals often lead to the generation of pandemic influenza strains over a period of years and hence it is suggested that appropriate surveillance strategies should be developed for detection of precursor viruses that could help in aborting the future pandemic wave.

References

1. Wright P and Webster R (2001) Orthomyxoviruses In: Fields Virology, 4th edn, pp. 1533–1579. Edited by D. Knipe and P. Howley. Philadelphia: Lippincott Williams and Wilkins.
2. Khanna M and Kumar P (2002) Influenza: A serious global threat. J. Infect. Dis. Antimicro Agents 19:25
3. Khanna M, Kumar P, Choudhary K, Kumar B and Vijayan VK (2008) Emerging influenza virus: A global threat. J. Biosci. 33: 475–482
4. Reid AH and Taubenberger JK (2003) The origin of the 1918 pandemic influenza virus: a continuing enigma. Journal of General Virology 84:2285–2292
5. Schafer JR, Kawaoka Y, Bean WJ, Suss J, Senne D and Webster RG (1993) Origin of the pandemic 1957 H2 influenza A virus and the persistence of its possible progenitors in the avian reservoir. Virology 194:781–788.
6. Scholtissek, C, Koennecke I and Rott R (1978) Host range recombinants of fowl plague (influenza A) virus. Virology 91:79–85
7. ECDC Interim Risk Assessment: Influenza A (H1N1) 2009 pandemic. [Online]. 2009 July 20; Available from: http://ecdc.europa.eu/en/files/pdf/Health_topics/090720_Influenza_A(HINI)_Risk_Assessment.pdf.
8. Outbreak of swine-origin influenza A (H1N1) virus infection-Mexico, March–April 2009, MMWR Morb Mortal Wkly Rep, 58 (2009) 467–470
9. http://www.who.int/csr/don/2009_10_16/en/index.html.
10. Ministry of Health and Family Welfare, Information on swine flu; daily press release http://mohfw.nic.in/press-release-on-swine-flu.htm
11. Zhou NN, Senne DA, Landgraf JS, Swenson SL, Erickson G, Rossow K and Liu L (1999) Genetic reassortment of avian, swine, and human influenza A viruses in American pigs. J. Virol. 73:8851–8856
12. Morens DM, Taubenberger JK and Faucia AS The persistant legacy of 1918 influenza virus N Engl J Med 361:225–229
13. Trifonov V, Khiabanian H, Greenbaum B and Rabadan R (2009) The origin of recent swine influenza A (H1N1) virus infecting humans. Eurosurveillance 4:1
14. Zheng K, SongNian HU, TianXian LI (2009) Genome evolution of novel influenza A (H1N1) virus in human. Chinese Sci Bull 54:2159–2163
15. Khanna M, Gupta N, Gupta A and Vijayan VK (2009) Influenza A (H1N1) 2009: a pandemic alarm, J Biosci 34:481–489
16. MaurerStroh S, Ma J, Lee RTC, Sirota FL and Eisenhaber F (2009) Mapping the sequence mutations of 2009 H1N1 influenza A virus neuraminidase relative to drug and antibody binding sites. Biol Direct 4:18–47
17. Garten RJ, Davis CT, Russell CA, Shu B, Lindstrom S, Balish A et al (2009) antigenic and genetic characteristics of swine origin 2009 A (H1N1) influenza viruses circulating in humans. Science 325:197–201

18. Trifonov V, Racaniello V and Rabadan R, (2009) The contribution of the PB1–F2 protein to the fitness of Influenza A virus and its recent evolution in 2009 Influenza A (H1N1) pandemic virus. N Engl J Med 360, 115–119
19. Eccles R (2005) Understanding the symptoms of the common cold and influenza. Lancet Infect. Dis. 5: 718–725.
20. CDC, Antiviral Drugs and Swine Influenza, http://www.cdc.gov/swineflu/antiviral_swine.htm (Accessed at 28 August 2009)
21. Update: drug susceptibility of swine-origin influenza A (H1N1) viruses, April 2009. (2009) MMWR Morb Mortal Wkly Rep 58:433–435
22. Three Indian Companies working to develop swine flu vaccine, August 2009 http://www.zeenews.com/news554120.html
23. Global surveillance during an influenza pandemic, Geneva: World Health Organization 2009 http://www.who.int/csr/disease/swineflu/global_pandemic_influenza=surveillance_apr09.pdf