

RECENT ADVANCES AND FUTURE PROSPECTS IN DIAGNOSIS OF H1N1 FLU VIRUS

Pooja Lal¹, Swati Arya², Akhilesh Kumar³, Vrushali Gadekar⁴, Ashok Kumar⁵

^{1,2,3,5}CSIR-Institute of Genomics and Integrative Biology, Delhi, India.

⁴ D. Y. Patil Biotechnology and Bioinformatics Institute, Pune, India.

Abstract: Swine flu, one of the major concerns in health and care sector since past few years is caused by influenza a subtype variant of H1N1, originated from pigs. The uniqueness about this virus is that after triple reassortment from avian, pig and human, can infect within human populations directly. This disease is quite severe as it is a new strain and the immune response against this is not that effective to normalize patients as seen in cases of seasonal flu. As a result of which, it affected almost all parts of the world thus, was declared as pandemic by WHO in 2009. Swine flu still prevails in several regions due to the drawbacks of current diagnosis and treatment. Therefore, an easy, efficient and appropriate method should come in practice to control the spread of it. This review will deal with the drawbacks of each method for diagnosis and the future work that can be employed in order to overcome these drawbacks.

Key words: RNA biosensor, H1N1, swine flu, diagnosis

I. INTRODUCTION

Pork flu virus belongs to influenza a type virus, a member of Orthomyxoviridae family. It contains single stranded negative strand of RNA encoding for 11 proteins. It emerged for the first time in Mexico in 2009 [1] and thus sometimes known as "Mexican flu". The same virus strain was found in other countries too causing several deaths as was reported to WHO[2] In India, western states showed more of influenza like illness, out of which maximum were cases of swine flu. H1N1 pandemic strain is a result of triple reassortment of genes from avian, human and swine influenza viruses[3]. Pig's tracheal epithelium has avian and human receptors and so it serves as a mixing vessel for the further reassortment. The transmission from avian influenza to human is restricted due to unavailability of receptor that bind to avian influenza viruses [4] All influenza subtypes are highly contagious and can spread to cause severe respiratory disease. Swine flu can even cause pneumonia in patients which can further lead to death. Therefore, it is a disease of global importance and need concern of scientific world.

II. HISTORY

Swine flu is not a new type of flu instead it was first diagnosed in the year 1918 in Spain and was thought to be transmitted from pigs to humans [5]. La Grippe or Spanish flu caused death of 20-40 million of population worldwide. In 1930, first swine influenza was isolated in a pig and classic SIH1N1 strain (A/Swine/Iowa/30) prototype was transmitted to other pigs for the experimental analysis and

characterization [6]. Serially, in 1950s to 1970s influenza A affected other regions of the world like Asian flu ,1957 and Hong Kong flu ,1968 [7] .In 1970, avian influenza (H3N2)and human influenza(H3N2) transmission to pig was observed. Then, in 1977, a landmark in the swine influenza history affecting Russia. In 2009, H1N1 was first diagnosed in two children of Mexico having no exposure to the pig by CDC. It was declared as pandemic by WHO in the same year because of several deaths worldwide [8]

III. VIRUS CHARACTERISTICS

Influenza viruses are well known for their characteristics of 'genetic shift' and 'genetic drift'. They have a segmented RNA genome, therefore, the reassortment is common, resulting in antigenic shift and forming new strain. The other way is slow mutation leading to evolution of these viruses, resulting in antigenic drift [9]

A. Antigenic drift

It is a continuous process and changes occur in surface protein encoding genes (HA and NA) gradually and slowly. It takes place due to point mutation and is an unpredictable phenomenon. Mutations include deletions, substitutions and insertion mechanisms. This causes not much of changes but a minor change in surface proteins. Antigenic drift causes a formation of unrecognized antigens. These antigens may not be determined by previously produced antibodies of earlier influenza

B. Antigenic shift

This is non-continuous, occasionally occurring process. This is due to exchange of gene from one virus to another which can cause virulence difference. Since influenza A has segmented genome so the segments from all the parent viruses are present [11]. This occurs in 10 to 30 years as reported in earlier publications [12]. In the case of this virus, it may jump from one organism to humans via intermediate animal host (mixing vessel).

IV. TRIPLE REASSORTMENT

The novel H1N1 is formed due the triple reassortment of gene segments from already existing influenza strains i.e. Swine influenza A strain from North America and circulating H1N1 strains among swine's from Europe and Asia. This triple reassortment virus contains eight segments, out of which six segments are from American origin and the rest two from Europe and Asian origin [13]. The reassortment analysis was previously done using automated reassortment finder on different genes isolates of swine influenza [14]. Swine has

receptors for both mammalian and avian influenza viruses and therefore, they serve as a mixing vessel for generation of new strain [15].

V. TRANSMISSION

Previous data has shown that the novel H1N1 is no longer endemic in swine populations and is seen to infect among humans too. Earlier, it was known to spread in pigs and the people in contact with them but later inter-species transmission was reported by [16]. The transmission of the different strains of influenza is through air and so, swine flu spread throughout the world due to migratory population [17]. Other viruses e.g. HIV gets inactive in air but influenza remains active and anyone in vicinity can catch infection. There are two types of transmissions routes: short range transmission and long range transmission according to the distance between the patient and the susceptible individual e.g. exhalation from patient to air and inhalation from infected air to susceptible population - short range route and populations between distant locations - long range route [18]. The isolation of swine origin virus from humans has been already reported by Tang et. al which confirms inter- species transmission [19].

The above diagram (Fig.1) states the transmission of influenza between different species due to the presence of the receptors of those influenza strains. Bird flu is common to happen in humans as reported in previous journals. Swine has receptors for both avian and human influenza thus; it serves as a mixing vessel for them. The gene segments transform and unite to form a completely new strain thereby cause more serious complications in diagnosis and recovery from disease.

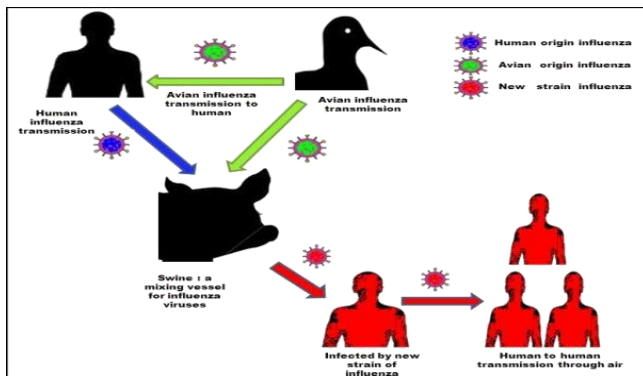


Fig.1. Development of new strain and transmission of influenza between species.

VI. SYMPTOMS

Pigs also show some common symptoms to that of humans like cough, fever, sneezing, breathlessness, etc. Humans include more symptoms like headache, chilling, sore throat, diarrhea, vomiting, congestion, stuffy nose and fatigue [20]. In some cases it can cause severe respiratory illness, pneumonia, gastrointestinal illness and even death at times. Children, aged group are more likely to spread this. Pregnant women [21], population above 65 years, respiratory illness patients and people with weak immunity are prone to catch

this infection. The progression of disease is sometimes seen within 24 hours but generally severe symptoms are seen within 3-5 days. These primary influenza symptoms lead to secondary infections of other bacterial and viral pathogens [22]. In previous reviews the difference in symptoms associated with cold and swine flu are well stated [23].

VII. PATHOGENESIS

After the entry of influenza into the respiratory tract through the different modes of transmission i.e. via aerosol or through contact with salivary or other respiratory secretions, it attaches to the epithelial cells on the lining of the tract and replicates. The replication of the virus and the action of immune cells together disrupts the cells on the lining of the respiratory tract. Since the virus replicates in both the upper and lower respiratory tract and so, complications are seen here [24].

VIII. DIAGNOSIS

The diagnosis of swine flu virus is essential in patients because the symptoms are almost common to all influenza infections. It is difficult to distinguish between normal cold and severe influenza infections. Therefore, swine flu can be diagnosed by few tests and are as follows:

- Molecular based diagnostic methods
- Antigen- antibody based diagnostic methods.

The virus is collected during its shedding i.e. 2-5 days, the shedding of influenza starts just after 24 hours. The specimens used for diagnosis are nasal swabs, throat swabs and tracheal swabs dipped in transport media for transportation to diagnostic centers.

A. Molecular methods

1. Hybridization

It is the basic method employed for detection of viruses. Using hybridization, we can directly detect specific RNA and DNA from samples [25]. Hybridization sensitivity is more as compared to that of culturing and immune-fluorescence [26].

2. Polymerase chain reaction (PCR)

This is one of the currently used methods of detection of swine flu. The viral RNA is first to be transcribed by reverse transcriptase to cDNA using random hexamers, universal complementary nucleotides to the 3' end of all influenza viruses, this increases the chances of amplification of number of target regions. Matrix gene and nucleoprotein gene are highly conserved regions and are used as target for influenza type. The surface proteins encoding genes are targeted for the detection of its subtype. Nested primers are also used in diagnostic methods for influenza. WHO has given protocols for conventional and real-time RT-PCR [27]. RT-PCR utilizes reverse transcriptase a type of polymerase that synthesizes cDNA from RNA. For pandemic H1N1 the targets include HA and matrix gene. For extraction of cDNA, professionals use conventional PCR with their specific gene

primers which can be further sequenced to confirm amplification.

The advantages and disadvantages of molecular methods are as follows [28]. Molecular methods are more specific and sensitive than other diagnostic methods. The chances for false negative and false positive results are low. RT-PCR takes longer time as samples are sent to the public health laboratory and so causes delay in informing to the clinical authorities for the treatment. These tests have off-site special centers for diagnosis and may not be present in hospitals for emergency cases. These are generally more expensive methods than those of other methods.

B. Antigen – antibody based methods

1. Haemagglutination inhibition test

H1N1 has haemagglutinin (HA) envelope protein which binds to sialic acid receptors. The test utilizes the HA protein on the surface of the virus that binds to circulating antibodies. This prevents the virus to bind to the erythrocytes, forming -

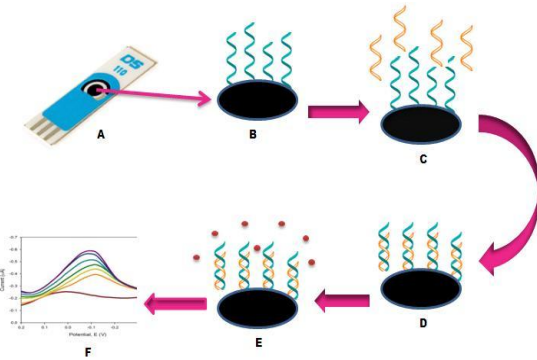


Fig.2 Fabrication of amperometric biosensor: (A) screen printed electrode (B) working electrode immobilized with probe (C) hybridization with target DNA/RNA (D) hybridized probe-target (E) reaction with redox reagent (F) amperometric analysis.

erythrocyte haemagglutinin lattice. This property is known as haemagglutination [29]. The serum from patients is allowed to mix with virus of known concentration and left for some time for the binding of virus envelope protein with the antibodies in the serum. The erythrocytes washed with PBS are then added to them in order to check whether the HA antigen is still free to bind with the erythrocytes. The comparisons are made using negative and highly positive controls, sera control and RBC control. This detects the presence or absence of antibodies corresponding to particular virus antigens. The titer with the highest dilution of serum that inhibits the virus induced haemagglutination is recorded. The titration is repeated three to four times and the geometric mean is calculated.

2. Virus neutralization test

It is a reaction between a living virus mixed in serum and the susceptible host cells. To a 96 well plate, the heat inactivated test sera incubated with a set amount of virus (TCID₅₀ determined) are added, this allows an appropriate time for

binding of virus antigens to any antibodies. The susceptible host cells, in case of H1N1, MDCK cells are added to the serum virus mixture [30]. The analysis of plate is done by microscope for the cytopathic effect or by immunofluorescence for the presence of viral antigens in the monolayer of MDCK cells. The antibodies in the serum which are able to neutralize the viral antigens will cause no infection to the cells.

3. Rapid influenza detection tests (RIDTs)

This method is useful for patient's right time diagnosis of a disease. This allows a timely treatment to patients. It takes less than 30 minutes in diagnosing a disease and is commercially available. This is also known as point of care tests (POCTs). There are more than 15 FDA approved commercially available test in markets for the diagnosis of influenza infections. These tests are beneficial for rapid diagnosis but are known to produce false results. Therefore, the results are needed to be further confirmed by other diagnostic methods. The sensitivities of RIDTs is 50-70 % as compared to real time diagnosis. These tests can diagnose only influenza A type viruses or both A and B types but cannot distinguish between them or both A and B types and can differentiate between them. The subtypes of these cannot be determined using RIDTs and so, diagnosis of swine flu is a tough task for them [31].

IX. FUTURE PROSPECTS

Biosensors will prove to be an important tool for diagnosis of swine flu. They are remarkably known to convert biological signal into electrical, optical signals. Analysis is based on affinity reactions including DNA-DNA, antibody-antigen, enzyme substrate and protein-DNA. Biosensors include a biological component (enzyme, cells, organelles, microbes, etc) and the sensing material like optical, electrochemical sensors; encompasses the transducer component of a biosensor [32]. The first biosensor was developed in 1962 by Clark and Lyon for the detection of glucose (diabetes) in blood by immobilizing glucose oxidase enzyme [33]. Electrochemical transducers are most commonly used in biosensors. Amperometric sensors are already being developed for diagnosis of dreadful diseases like meningitis, etc. [34]. Amperometric biosensors consist of two and three electrode system. The disadvantage of two electrode system is the limitation of handling potential range on the working surface with higher current. This limitation led to employment of third counter electrode. In the three electrode system the potential is applied between the reference and working electrode and the current is measured between the working and counter [35]. To the surface of the working electrode, the single stranded oligo-DNA probe is attached which hybridizes with the sample on the basis of complementary sequence present. This hybridization in the presence of redox indicator, such as methylene blue generates current which is measured by potentiostat and compared with the reading obtained from the probe. In the case of swine flu, the probe designed is complementary to the RNA in patient's swab. This will make the analysis rapid and sensitive. RNA

sensors for diagnostics will prove to be an important tool for viral diseases which show severe consequences and threatens life of several. Cyclic voltammetry and differential pulse voltammetry studies confirm the presence or absence of swine flu infection by current measurement as a function of potential using potentiostat. The schematic representation of proposed amperometric biosensor is shown in Fig. 2.

X. CONCLUSIONS

Since 2009, hundreds of patients have died due to swine flu infection as H1N1 influenza was newly evolved. The symptoms are same to that of common cold and other viral infections and so often mistaken to be treated on time. Other diagnostic methods take longer time and have their own drawbacks and therefore, a new method should come into practice for the detection. Biosensors can prove to be more economical, faster and specific for molecular diagnosis of swine flu.

REFERENCES

- [1] J. Gibbs, J. S. Armstrong and J. C. Drownie, "From where did the 2009 'swine-origin' influenza A virus (H1N1) emerge?", *Virology Journal*, Vol. 6, pp. 207, 2009.
- [2] T. Dikid, S. K. Jain, A. Sharma, A. Kumar and J. P. Narain, "Emerging and re-emerging infections in India: an overview", *Indian Journal Medical Research*, Vol. 138, pp. 19-31, 2013.
- [3] M. Babakir-Mina, S. Dimonte, C. F. Perno and M. Ciotte, "Origin of the 2009 Mexico influenza virus: a comparative phylogenetic analysis of the principal external antigens and matrix protein", *Archives of Virology*, Vol.154, pp. 1349-52, 2009.
- [4] H. Kida, T. Ito, J. Yasuda, Y. Shimizu, C. Itakura, K. F. Shortridge, Y. Kawaoka and R. G. Webster, "Potential for transmission of avian influenza viruses to pigs", *Journal of General Virology*, Vol. 75, pp. 2183-88, 1994.
- [5] J. K. Taubenberger and D. M. Morens, "1918 Influenza: the mother of all pandemics", *Emerging Infectious Diseases*, Vol 12, pp. 15-22, 2006.
- [6] W. Olsen, "The Emergence of Novel Swine Influenza Viruses in North America", *Virus Research*, Vol. 85, No. 2, pp.199-210, 2002.
- [7] E. D. Kilbourne, "Influenza pandemics of the 20th century", *Emerging Infectious Diseases*, Vol. 12, pp. 9-14, 2006.
- [8] S. S. I. Jafri, M. Iiyas and M. Idrees, "Swine flu: A threat to human health", *Biotechnology and Molecular biology Reviews*, Vol. 5, No. 3, pp. 46- 50, 2010.
- [9] Scholtissek C, "Molecular evolution of influenza viruses", *Virus genes*, Vol. 11, No. 2-3, pp. 209-15, 1995.
- [10] N. J. McDonald, C. B. Smith and N. J. Cox, "Antigenic drift in the evolution of H1N1 influenza A viruses resulting from deletion of a single amino acid in the haemagglutinin gene", *Journal of General Virology*, Vol. 88, pp. 3209-3213, 2007.
- [11] E. Domingo, R. G. Webster and J. F. Holland, "Antigenic variation in influenza viruses", *Origin and Evolution of Viruses*, pp. 377-390, 2009.
- [12] Weinberger and B. G. Loebenstein, "Vaccination in the elderly", *Immunosenescence*, pp. 157- 159, 2012.
- [13] R. G. Webster, V. S. Hinshaw and W. J. Bean Jr., "Antigenic shift in myxoviruses", *Medical Microbiology and Immunology*, Vol. 64, pp. 57-68, 1997.
- [14] F. S. Dawood, S. Jain, L. Finelli, M. W. Shaw, S. Lindstrom, R. J. Garten, L.V. Gubareva, X. Xu, C. M. Bridges and T. M. Uyeki, "Emergence of a Novel Swine-Origin Influenza A (H1N1) Virus in Humans", *The New England Journal of Medicine*, Vol.360, pp. 2605-2615, 2009.
- [15] Kingsford, N. Nagarajan and S. L. Salzberg, "2009 Swine-Origin Influenza a (H1N1) Resembles Previous Influenza Isolates", *Plosone*, Vol.4, No.7, pp. e6402, 2009.
- [16] Thacker and B. Janke, "Swine influenza virus: zoonotic potential and vaccination strategies for the control of avian and swine influenzas", *The Journal of Infectious Diseases*, Vol. 197 No.1, pp. 819-24, 2008.
- [17] R. J. Garten, C. T. Davis et al., "Antigenic and Genetic Characteristics of Swine-Origin 2009 A (H1N1) Influenza Viruses Circulating in Humans", *Science*, Vol. 325, No. 5937, pp. 197-201, 2009.
- [18] K. Khan, J. Arino, W. Hu, P. Raposo, J. Sears, F. Calderon, C. Heidebrecht, M. Macdonald, J. Liauw, A. Chan and M. Gardam, "Spread of a novel influenza A (H1N1) virus via global airline transportation", *The New England Journal of Medicine*, Vol. 361, No. 2, pp. 212-4, 2009.
- [19] J. W. Tang, Y. Li, I. Eames, P. K. Chan and G. L. Ridgway, "Factors involved in the aerosol transmission of infection and control of ventilation in healthcare premises", *Journal of Hospital Infection*, Vol. 64, No.2, pp.100-14, 2006.
- [20] S. Parmar, N. Shah, M. Kasarwala, M. Virpura and D. D. Prajapati, "A review on swine flu", *Journal of Pharmaceutical Science and Bioscientific Research*, Vol. 1, No. 1, pp. 11-17, 2011.
- [21] V. Wiwanitkit, "Swine flu: the present pandemic infectious disease", *Kulak Burun Bogaz Ihtis Derg.*, Vol. 19, No. 2, pp. 57-61, 2009.
- [22] K. P. Myers, C. W. Olsen and G. C. Gray, "Cases of swine influenza in humans: a review of the literature", *Clinical Infectious Diseases*, Vol. 44, No. 8, pp.1084-8, 2007.
- [23] K. R. S. Lade, S. D. Sawant and M. C. Singh, "Review on influenza with special emphasis on swine flu", *International Journal of Current Pharmaceutical Research*, Vol. 3, No. 1, pp. 97-107, 2011.
- [24] R. Wang and J. K. Taubenberger, "Method for molecular surveillance of influenza", *Expert Review of Anti-infective Therapy*, Vol. 8, No. 2, pp. 517-527, 2010.
- [25] J. S. Ellis and M. C. Zambon, "Molecular diagnosis of influenza", *Reviews in Medical Virology*, Vol. 12, No. 6, pp. 375-389, 2002.
- [26] F. Rimmelzwaan, M. Baars, E. C. J. Claas and A. D. M.

- E Osterhaus, "Comparison of RNA hybridization, hemagglutination assay, titration of infectious virus and immunofluorescence as methods for monitoring influenza virus replication in vitro", *Journal of Virological Methods*, Vol. 74, No. 1, pp. 57-66, 1998.
- [27] T. Jiang, X. P. Kang & Y. Deng et al., "Development of a real time RT-PCR assay for a novel influenza A (H1N1) virus", *Journal of Virological Methods*, Vol. 163, No. 2, pp. 470-473, 2009.
- [28] Wassenegger, "Advantages and disadvantages of using PCR technique to characterize transgenic plants", *Molecular Biotechnology*, Vol. 17, No. 1, pp. 73-82, 2001.
- [29] B. H. Janke, "Diagnosis of swine influenza", *Journal of Swine Health and Production*, Vol. 8, No. 2, pp. 79-84, 2000.
- [30] T. Rowe, R. A. Abernathy and J. H. Primmer et al., "Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assay", *Journal of Clinical Microbiology*, Vol. 37, No. 4, pp. 937-943, 1999.
- [31] C. Baas, I. G. Barr, R. A. Fouchier, A. Kelso and A. C. Hurt, "A comparison of rapid point-of-care tests for the detection of avian influenza A(H7N9) virus", *Eurosurveillance*, Vol. 18, No. 21, article 5, 2013.
- [32] G. Marrazza, I. Chianella and M. Mascini, "Disposable DNA electrochemical sensor for hybridization detection", *Biosensor Bioelectronics*, Vol. 14, pp. 43-51, 1999.
- [33] J. Wang, "Electrochemical Glucose Biosensors", *Chemical Reviews*, Vol. 108, pp. 814-825, 2008.
- [34] S. K. Dash, M. Sharma, S. Khare and A. Kumar, "Omp85 genosensor for detection of human brain bacterial meningitis", *Biotechnology Letters*, Vol. 35, No. 6, pp. 929-35, 2013.
- [35] M. Pohanka and P. Skládal, "Electrochemical biosensors – principles and applications", *Journal of Applied Biomedicine*, Vol. 6, pp. 57-64, 2008.