COMMUNICABLE DISEASES

1.2 Comparative study of Gene characterization of Influenza A pandemic (H1N1) 2009 viruses from virus isolates of 2009 pandemic and 2012 re-emerging viruses in western Rajasthan: Phylogenetic analysis and molecular characterization

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OBJECTIVES

1. Comparative study of Gene sequences of virus isolates of influenza A pandemic (H1N1) 2009 viruses caused pandemic in 2009-10 and re-emergence during 2012.
2. Genome analysis of sequences obtained in available data base to study regional strain specificity of viruses.
3. Extrapolation of sequences over clinical conditions of patients to study possible viral genomic basis of clinical conditions.

PROGRESS

An era of H1N1 virus amplification in emryogenated chicken eggs and its subsequent whole genome sequencing was started in the laboratory of the institute. A preliminary pilot trial of whole genome sequencing of the clinical samples and samples amplified in the chicken eggs was successfully accomplished with the technical guidance of National Institute of Virology, Pune.

The throat swab sample of a case of Pandemic Influenza A (H1N1) 2009 virus was taken and inoculated into the Allantoise cavity, Chorio allantoise membrane, yolk sac and amniotic sac of 10 days old embryogenated chicken eggs (Fig. 1).

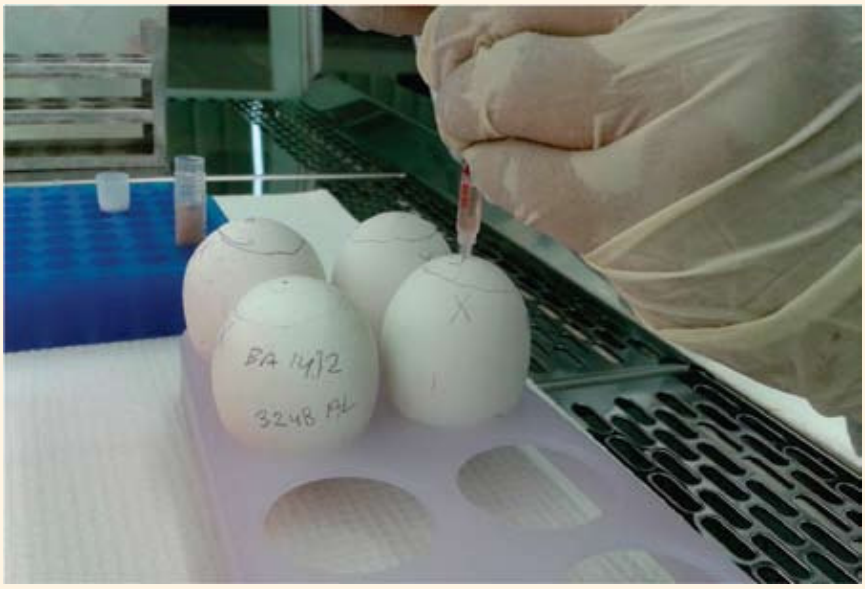

Fig. 1. Virus inoculation into eggs

After culture of virus for 24 hours, the yolk sac membrane and allanto-chorion membrane were processed for blocks preparation and subsequent study by the Electron Microscope (Commercially at All India Institute of Medical Sciences, New Delhi) to confirm the presence of virus in the membranes. The EM studies showed the presence of virus particles in the serial sections processed from the tissue blocks (Fig. 2).

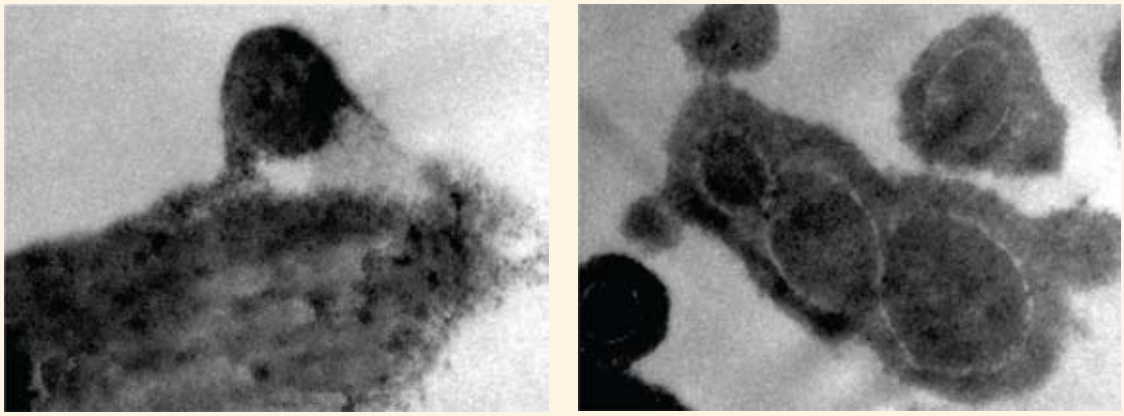

Fig.2. The Electron Microscopic picture of H1N1 virus particle grown in chorio-allantoise membrane of chicken eggs

The clinical samples proved to have H1N1 virus particle through EM studies and Real Time PCR, was subjected to further reactions for reverse transcription of viral RNA into c-DNA, denaturation, annealing and multiplication with about 94 primers belonging to all the eight gene segments of H1N1 virus genome. The c-DNA obtained were run on Agarose gel and bands of the some of the gene segments are shown in Fig.3

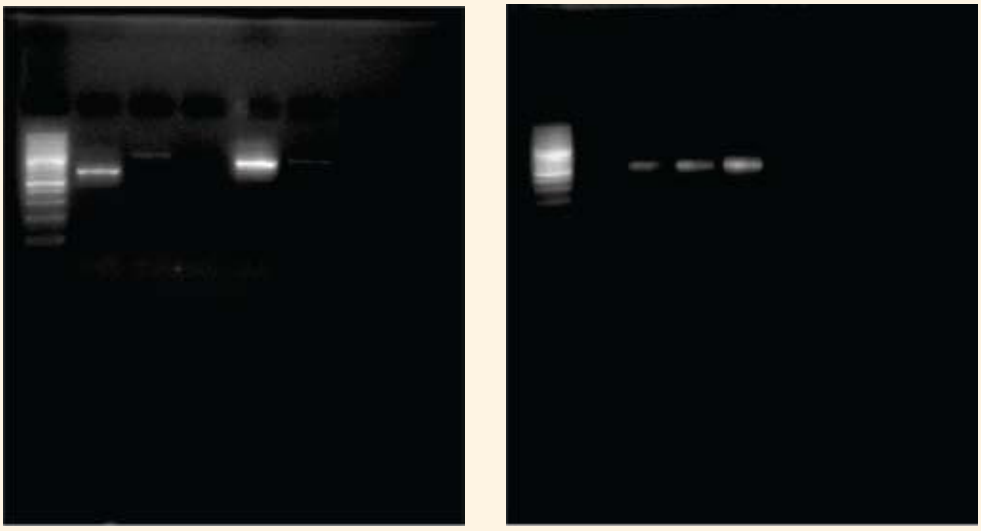

Fig.3. Agarose gel assay of some of the c-DNA of H1N1 virus.

3. Cycle Sequencing of c-DNA, purification and gene segment sequencing

c-DNA obtained from viral RNA and amplified subsequently and simultaneously was subjected to cyclo sequencing, purification and sequencing of gene segments. The final product was subjected to gene sequencing (Fig. 4).

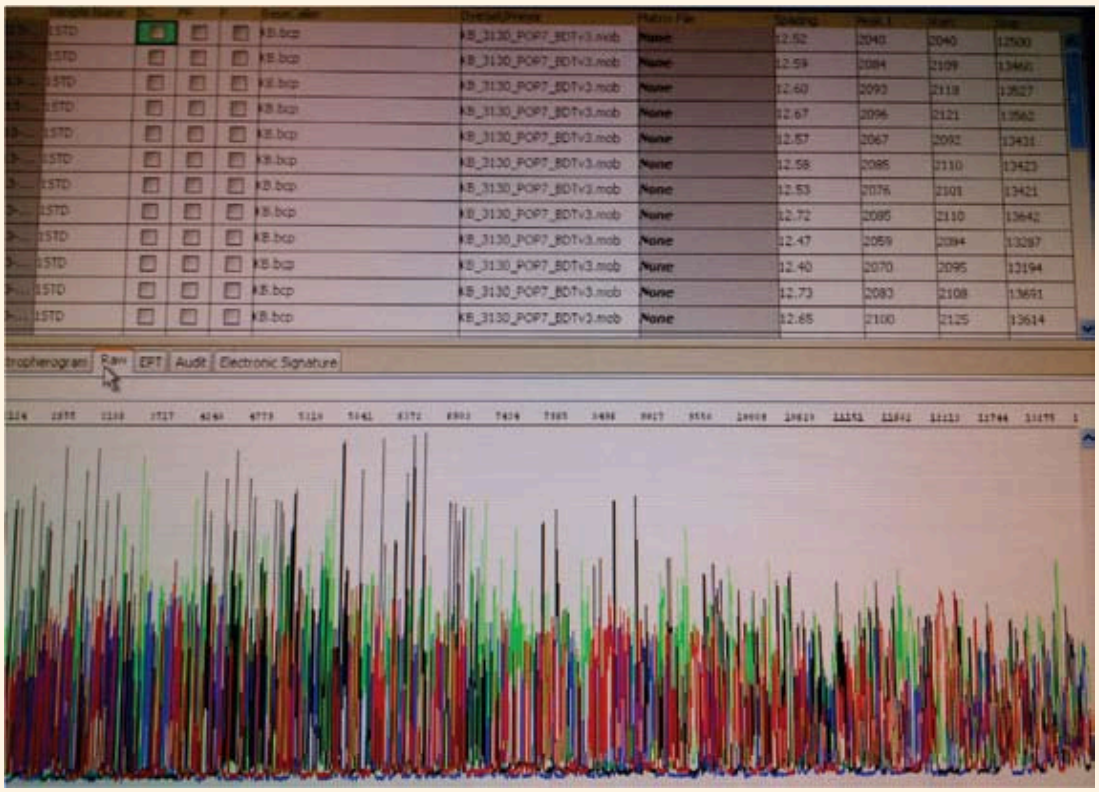

Fig. 4: Picture showing sequencing of one of gene segments of H1N1 virus

We expect to perform whole genome sequencing of H1N1 viruses and its agreement or dis-agreement pattern with reported genomes to explain the different clinical conditions of the patients.