



Respiratory Virus Unit : Molecular Influenza Laboratory

BACKGROUND

The influenza laboratories at NICD comprise one of the few active WHO National Influenza Centres (NICs) in Africa and have been involved since the mid-1980s in isolating and characterizing virus strains circulating in parts of the country each year. The data is shared with the WHO Global Influenza network and contributes towards the annual decision made in September for updating the vaccine strains for the following southern hemisphere influenza season.

In addition to seasonal influenza, the ongoing panzootic of the Highly Pathogenic Avian Influenza (HPAI) H5N1 subtype is considered a serious public health risk with pandemic potential. While H5N1 is primarily an avian disease, the high mortality rate in those individuals which have been infected is of great concern. Pandemic preparedness is essential to control the spread of the disease in the event of this virus adapting to the human host and increasing its human-to-human transmissibility. Rapid identification of highly pathogenic influenza viruses is an essential prerequisite for effective clinical management, disease containment and implementation of infection control measures. The RVU has been working towards strengthening capacity for the rapid detection of HPAI H5N1 and other influenza subtypes with pandemic potential.

A number of the influenza A and B isolates were characterised by partial sequencing the HA1 subunit of the haemagglutinin (HA) gene and phylogenetic analysis to determine genetic drift from the vaccine strains. Sequence analysis of the HA1 subunit revealed the H1N1 viruses isolated during the season showed major genetic drift from A/New Caledonia/20/99, the southern hemisphere vaccine strain for 2007. The majority of the isolates were related to the A/Solomon Islands/3/06 northern hemisphere 2007/8 vaccine strain in clade 2 (Figure 1). Within this clade, the South African viruses clustered into two subclades represented by the reference A/Hong Kong/2652/06 and A/Brisbane/59/07 strains respectively. A 2007 isolate from Kenya was found to be similar to the South African isolates in the A/Brisbane/59/07 subcluster. In contrast to the rest of the South African H1 viruses, one isolate from Cape Town (A/Cape Town/106/07) grouped with the clade 1 H1N1 viruses which are more homologous to the A/New Caledonia/20/99 vaccine strain.

The H3N2 isolates had drifted substantially from the A/Wisconsin/67/05 vaccine strain with amino acid changes mapping to antigenic sites A, B and D. Many of the isolates analysed were related to the B/Brisbane/10/07 reference strain but some of the South African strains exhibited an unusual D7Y substitution while a few of the earlier H3 viruses had a distinctive change at K83R.

Phylogenetic analysis of representative South African 2007 influenza B viruses from the B/Yamagata/16/88 lineage revealed that they had many amino acid substitutions compared to the earlier isolates from this lineage. The influenza B isolates from the other lineage, however, showed little drift from the B/Malaysia/2506/04 vaccine strain.

The drift observed in the South African viruses from the respective vaccine strains was confirmed by the WHO Collaborating Centres for Reference and Research on Influenza (Melbourne and London). This trend was reported for many other countries in the southern hemisphere (WHO Consultation on the Composition of the Influenza Vaccine for the Southern Hemisphere, 2008, Geneva, September 2007). It was thus recommended that the H1N1, H3N2 and influenza B strains should all be updated for the 2008 southern hemisphere influenza vaccine to provide a better match to the circulating viruses.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

SEASONAL INFLUENZA: MOLECULAR EPIDEMIOLOGY OF THE 2007 INFLUENZA SEASON IN SOUTH AFRICA

Both subtypes of influenza A and B viruses circulated in South Africa during the influenza season with the majority of the isolates (390/533 or 73,2 %) being influenza A as determined by the Virus Isolation Laboratory. Many of the influenza A positives were subtyped by RT-PCR instead of by the traditional haemagglutination - inhibition assay (HAI) due to the fact that recent H3N2 viruses do not readily agglutinate erythrocytes. 148 of the influenza A isolates were subtyped as H1N1, 215 as H3N2 while the remainder could not be typed. Influenza B virus isolates from both the major genetic lineages (B/Yamagata/16/88 and B/Victoria/2/87) were identified.

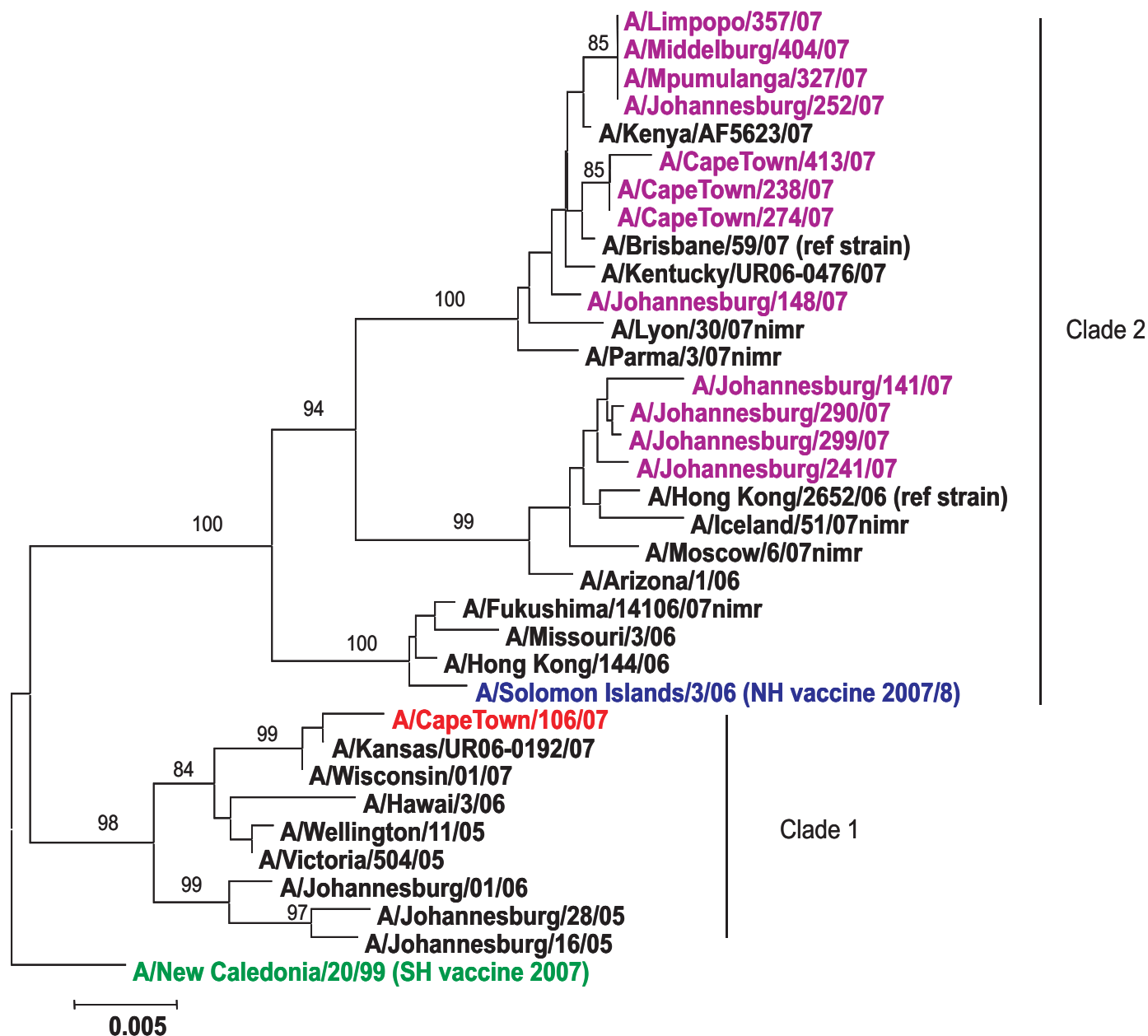


Figure 1: Phylogenetic tree of influenza H1N1 virus HA1 gene nucleotides (976 bp). Southern hemisphere 2007 vaccine strain is depicted in green and northern hemisphere 2007/8 vaccine strain in blue.

AVIAN H5N1 INFLUENZA AND PANDEMIC PREPAREDNESS

Significant progress has been made in building up laboratory capacity at NICD for the rapid detection of suspected cases of avian H5N1 influenza in humans. The evolution of different clades of H5N1 has made the detection of H5 viruses challenging. Methods for detection of H5N1 by real time PCR at NICD have been shown to be sensitive and specific and can detect viruses from the different clades that have evolved since 2003.

The RVU has participated in a number of External Quality Assurance panels for influenza virus detection by molecular techniques during 2007. IATA training for the shipment of dangerous goods has been completed by laboratory staff members involved in influenza/avian influenza work.

Links with the National Influenza laboratories in other African countries have been strengthened and laboratory assessments and training courses have been held for several SADC countries (Figure 2). The NICD



Figure 2: Influenza laboratory training of one of the scientists from the University Teaching Hospital in Lusaka, Zambia. (Photo courtesy of Dhamari Naidoo).

influenza laboratories are working towards becoming an accredited WHO Regional Reference Influenza Laboratory for southern Africa and are collaborating closely with WHO and CDC to achieve this status in the near future.

HIGHLIGHTS

These included participating in the activities of the NICD Influenza Symposium held on 19th- 20th February 2007. The laboratory demonstration of influenza diagnosis organized by the Virus Isolation Unit and the RVU as part of the Symposium was well received by the Viral Watch Doctor participants.

Highlights in terms of the avian/pandemic influenza preparedness included the involvement of RVU staff in the provincial 'Rapid Response Outbreak Training for Avian/Pandemic Influenza' carried out jointly by the Department of Health and NICD. The unit also participated in presenting and facilitating at the joint NICD/CDC workshop on 'Avian Influenza Rapid Response Team Training' held on 20 -23 August 2007 for participants from 12 SADC countries. Two scientists from the University Teaching Hospital, Lusaka, Zambia, were trained on molecular methods for the rapid detection of seasonal and avian influenza viruses in November 2007.

The RVU participated in various national and international meetings on avian/pandemic preparedness.

These included the 'Avian and Human Influenza Pandemic (AHI) Planning Meeting for International Agencies and Cooperating Partners to the SADC Region' held in May where Dr Besselaar gave a presentation on the 'Laboratory Preparedness in South Africa for the detection of Avian Influenza'. Dr Besselaar was invited to give a presentation on the 'Regional Preparedness of Africa for Avian Influenza' at the WHO National Influenza Centres meeting held in Toronto, 15-16 June 2007 and she presented at the 'Options for the Control of Influenza V1' which was held directly after the WHO meeting in Toronto. Dr Besselaar also participated in a closed meeting on the 'WHO Scientific Consultation on the use of human H5N1 influenza vaccines' in Geneva later in the year. Dhamari Naidoo gave a presentation at the 'International Symposium on Avian Influenza: Epidemiologic, Basic and Applied Research' meeting, New Delhi, India in October.

In terms of regional laboratory strengthening, Dr Besselaar visited Madagascar in July 2007 to provide scientific expertise for building up laboratory capacity in Madagascar for avian/pandemic influenza. In November 2007 Dr Besselaar visited the National Institute for Public Health laboratories in Luanda, Angola, to conduct a similar exercise as part of a CDC mission.

Dr Stefano Tempia (DVM, MSc, PhD), a CDC Avian and Human Influenza Technical Advisor, joined the NICD as an Attaché in October 2007.

Respiratory Virus Unit : Molecular Measles/Rubella Laboratory

BACKGROUND

It is important to identify which strains of measles virus are circulating to determine whether endemic transmission has been interrupted, to focus vaccination efforts on those countries where endemic transmission is still occurring, and to determine the origin of imported strains. This information is shared with the Global Measles Laboratory Network of the World Health Organization (WHO). The measles laboratory at NICD functions as a national laboratory (NL - identifying strains circulating in South Africa) as well as a regional reference laboratory (RRL - identifying strains circulating in the southern block countries of Africa). The laboratory also provides a service to other African countries that do not have access to nucleic acid sequencing technology.

When the incidence of measles is at a low level, it is known that the positive predictive value of the anti-measles IgM-antibody test is relatively low. Since South Africa is experiencing low measles incidence, molecular methods are being used in a diagnostic capacity, in conjunction with standard epidemiological information, to try and confirm whether measles IgM-reactive specimens are compatible with a diagnosis of measles.

Although rubella vaccination is not part of the EPI programs in South Africa or Africa, it is important to get a baseline measure of the strains circulating on the continent. This will help to demonstrate interruption of endemic transmission once vaccination programs are put in place. It will also help to define the global molecular epidemiology of rubella virus which is currently poorly described in Africa.

ACTIVITIES

Although 34 measles IgM-positive specimens were detected by the rash-surveillance program in South Africa in 2007, only 4 specimens contained measles virus nucleic acid. This low proportion of PCR-positive specimens is likely due to several factors:

- a) rash onset dates are often not provided, so the interval between onset and specimen collection is unknown (virus is usually only present for a few days after onset of rash)
- b) inappropriate specimen collection

- c) it is possible that there were false positive serology results, given that the positive predictive value of the test is not high in low measles incidence settings

Three of the four PCR-positive specimens contained wild-type virus (identified as clade B, genotype B3.1) an imported genotype. Genotype B3.1 is the predominant endemic strain of measles virus circulating on the African continent. Since the cases came from 3 different provinces, they most likely represent separate importation events. The other PCR-positive specimen contained vaccine virus; this was from a 10 month old child with a history of measles vaccination 6 days prior to rash onset.

Of the 44 national specimens with indeterminate measles IgM serology, only one contained measles virus nucleic acid that could be amplified and sequenced. Genotype B3.1 was detected which again represents an importation.

Regarding the NICD RRL function, 79 specimens (clinical material/viral isolates/PCR products) were received from 5 countries (Kenya, Zambia, Uganda, Democratic Republic of the Congo, Côte d'Ivoire) for molecular analysis. It was possible to obtain sequences for 75 (95%) of these specimens. With the exception of the DRC which had circulation of genotype B2 viruses, all the other countries had transmission of genotype B3.1 viruses.

As part of the WHO-EQA program for the NL of the southern block of countries, quarterly batches of sera were received by the serology laboratory for confirmatory testing; 47 of 227 sera were identified as having positive/indeterminate measles IgM results. These were tested for the presence of measles virus by PCR and 14 (30%) were PCR-positive. The amplicons were sequenced and genotypes B3.1 (Zambia, Angola) and D4 (Zambia) were identified.

A small number of specimens from confirmed rubella cases were investigated retrospectively - only genotype 2B viruses were identified. This is of interest because this genotype has been described as circulating endemically only in Asia. We thus need to increase the sample size to allow a more robust phylogenetic analysis.