Catching a virus

A story about identifying viruses, finding them in nature and stopping them in their tracks.

Inaugural lecture by Professor FJ Burt

Emerging and re-emerging viruses have significant implications for public health. In recent years there have been outbreaks of disease of unprecedented magnitude with spread and establishment in distinct geographic regions. Many of these emerging viruses are transmitted by vectors or are spread to humans from animals. The viruses can cause significant human disease ranging from debilitating chronic arthritis to fulminant haemorrhagic disease with fatalities. The reasons for the emergence of these viruses are likely multi-factorial with global warming, human encroachment into forested areas, changes in agricultural practice, global travel and legal and illegal movement of animals playing a role in the spread of disease and infected vectors.

The most recent emerging virus to make the headlines was Zika virus (ZIKV). The virus was initially isolated from a rhesus monkey derived from the Zika Forest near Entebbe, Uganda, during yellow fever investigations in 1947 and subsequently isolated from wild caught *Aedes spp* collected in Uganda in 1948. Human disease was first reported in 1954 following the isolation of the virus from a patient during an epidemic of jaundice in eastern Nigeria. Subsequent serological studies and mosquito isolations confirmed that ZIKV was more widespread in Africa and Asia than initially presumed, although until the last decade, the incidence of human cases was mostly sporadic. Recent emergence of this virus as a cause of larger outbreaks of disease was first reported in 2007 when an outbreak of ZIKV was identified on Yap Island, Federated States of Micronesia, in southwestern Pacific Ocean. It was estimated that three quarters of the population of Yap Island were infected and patients presented with mild disease. In October 2013 the virus was identified in French Polynesia, located in the South Pacific with thousands of suspected cases of ZIKV infection. Although most patients presenting with mild disease, fever, arthralgia, maculopapular rash and conjunctivitis it was noted that there was an increase of neurological complications in the form of Guillain-Barré Syndrome in ZIKV infected patients. The current ongoing outbreak of ZIKV was initially identified in 2015 with cases occurring in Brazil. The outbreak, which was likely introduced from Polynesia, started in late 2014 and spread rapidly throughout Latin America and the

Caribbean. As with the outbreak in French Polynesia, an increase in occurrence of GBS was noted and, in addition, an increased frequency of microcephaly in new-born babies. A direct link between ZIKV and congenital defects was verified by detection of viral antigens and RNA in brain tissues of babies with intrauterine infection.

In 2016 yellow fever virus was in the news again. Yellow fever virus occurs in South America and sub Saharan Africa. The virus is transmitted by mosquitoes and causes severe disease in 15 to 20% of infected people who develop illness. Patients develop classic signs and symptoms with jaundice, renal failure and haemorrhage. The fatality rate in patients developing severe disease can be as high as 50%. The virus causes sporadic outbreaks, the most recent in Angola in 2016 and a concurrent outbreak occurring in the Democratic Republic of the Congo. The availability of a highly efficacious vaccine can significantly curtail the number of cases and the spread of an outbreak. In total there were 962 laboratory confirmed cases across the two countries and more than 7000 reported cases. Confirmed deaths were 121 with a further 377 reported deaths. An estimated 30 million people received the vaccine. Although endemic in Angola, this was the first outbreak in the country in 28 years.

Another arbovirus that has re-emerged after decades without notable activity is chikungunya virus. Chikungunya fever is a debilitating arthritic disease caused by chikungunya virus (CHIKV). The virus was initially isolated during an outbreak of disease occurring on the Makonde Plateau in Tanzania. 'Chikungunya' means, "that which bends up" in the Makonde language, describing the bending posture of patients affected by CHIKV infection. The virus usually circulates in a sylvatic cycle between non-human primates or mammalian reservoir hosts and *Aedes* species mosquitoes. After years of sporadic and infrequent outbreaks the virus caused major epidemics in Africa, Asia and the Indian Ocean. The virus emerged in the coastal region of Kenya in 2004 and spread rapidly through the Indian Ocean islands infecting hundreds of thousands of people. It was estimated that there were 215 000 cases, two thirds of the population, on Grande Comore, and by April 2006 the virus had spread to La Reunion causing 244 000 cases. The virus emerged in India in 2005 after a 32 year hiatus of viral activity with an estimated 1.3 million cases reported. In October 2013 the virus was identified for the first time in the western hemisphere on Saint Martin Island with subsequent autochotonous transmission of the virus in 48 countries or territories in the Caribbean, Central America, South America and North America causing an estimated 1 million cases.

However these outbreaks pale into, almost, insignificance compared with the Ebola virus outbreak in West Africa occurring from 2014 to 2016. Although the number of cases of Ebola disease were far fewer than the number of cases of disease caused by other recent emerging viruses, the fatality rate was significantly higher. There have been multiple outbreaks of this virus in other regions of Africa since it was first recognised in Zaire and Sudan in 1976, however previous outbreaks involved small numbers of cases with limited spread to other regions. Prior to 2014, the largest outbreak occurred in Gulu, Uganda with 425 cases and 224 fatalities. The outbreak in West Africa started in Guinea and spread to neighbouring countries, Sierra Leone and Liberia. There were an estimated 14124 cases in Sierra Leone, 10675 cases in Liberia and 3811 cases in Guinea. Case fatality rates varied from 28% to 67%. During the outbreak cases were imported into the United States, Europe, the United Kingdom, Mali, Senegal and Nigeria. Although bats are considered a possible source or reservoir of the virus it is still not clear how the virus is transmitted to humans.

Another virus that is causing concern, particularly in European countries, is Crimean-Congo haemorrhagic fever virus. The virus is transmitted by ticks and is endemic in South Africa causing 1 to 10 cases annually. However in recent years it has emerged in southern European countries and in Turkey. Turkey has reported more than 10 000 cases since it was first identified in the country in 2002. The virus is transmitted by ticks belonging to the genus *Hyalomma*, which are found in Africa, Asia and southern regions of Europe, hence the potential to be a public health concern in European countries.

The availability of a highly efficacious vaccine for yellow fever virus, and an understanding of the source of the virus as a mosquito borne pathogen play significant roles in controlling outbreaks. Sanitation programs contribute to the control of outbreaks but the implementation of a vaccination program likely plays the more significant role in reducing case numbers and alleviating fear of the disease.

Yellow fever virus likely emerged in Africa more than 300 years ago and spread to the Americas in the 1600s on slave ships from West Africa causing disease on the West Coast of the United States. In the 1700s the virus spread to Europe. At the time it was believed to be spread by contact with infected individuals and it was only in the 1800s that two researchers, Josiah Nott (1848) and Carlos Finlay (1881), proposed that the virus was transmitted by a vector. Carlos Finlay performed an experiment in which mosquitoes that fed on an ill person were then allowed to feed on a healthy volunteer who subsequently became ill. However the

scientific community was not convinced that the virus was transmitted by a vector. The virus continued to cause outbreaks and as a result of deaths among the military, a Yellow Fever Commission was established by the US military. The Commission was chaired by Major Dr. Walter Reed, and assisted by members Dr James Carroll, Dr Jesse Lazaer and Dr Aristides Agramonte. Experiments were performed to determine the transmission of the virus. There were 30 volunteers and the experiments were carried out using two purpose built huts. In one hut a group of volunteers were exposed to the soiled bedding of ill patients and were protected from any exposure to mosquitoes. In a second hut the role of mosquitoes and infected air, was investigated by dividing the hut in two rooms separated by mosquito netting. In area A of the hut the volunteers were bitten by infected mosquitoes whereas in area B there were no mosquitoes but the volunteers shared the same air space as people in area A. Only volunteers exposed to mosquito bites became ill confirming that the virus was transmitted by mosquitoes and not through contact with infected persons or fomites. However the experiments were not without fatalities including the death of Dr Jesse Lazaer a member of the organising committee.

As a direct consequence of the findings, sanitation programs to reduce mosquito populations eradicated the disease in Panama and Cuba allowing the Panama Canal to be completed. After World War 1 a second Yellow Fever Commission was established in West Africa in 1925 to investigate the virus in Africa and determine if it was similar to the virus circulating in America. The virus was isolated for the first time in non-human primates from a Nigerian man who survived the disease. The virus strain was named after him, and is referred to as the Asibi strain. This was the first time an animal model was used for isolation attempts. The Asibi strain was later propagated in mice and chicken embryo tissue by Dr Max Theiler, a South African born medical doctor working in the United States. He developed an assay that could be used to determine the immune status of an individual, heralding the beginning of epidemiological surveillance for yellow fever virus in humans and animals. He was also responsible for passaging the virus in chicken embryo tissue deprived of nervous tissue to develop an attenuated vaccine that did not have the neurotropic side effects of the French vaccine. A total of 176 passages resulted in an attenuated strain that was non virulent but highly immunogenic and has been heralded as one of the most successful vaccines developed to date. In 1951 Dr Theiler was awarded the Nobel Prize in Physiology and Medicine for his role in the development of the vaccine.

In the 1900s there were few tools available for virus research. The introduction of animal models and cell culture have facilitated the isolation of viruses. Understanding immune

responses and interactions have prompted the development of indirect methods to look for evidence of infection such as IgG antibody responses. The advent of the electron microscope allowed visualisation of viruses and identification based on morphological features. However molecular techniques can be considered to have revolutionized how we perform research in virology today. The amplification of targeted regions of the genome using nucleic acid amplification techniques facilitated sequencing of small regions of the genome. The development of next generation sequencing techniques has allowed researchers to sequence entire viral genomes. Sequence data has been used to investigate the genetic diversity of viruses, to identify which species are circulating in distinct geographic regions, to develop assays for detection and to develop reverse genetic systems for pathogenicity studies. Amplification techniques have changed diagnostic approaches allowing a more rapid result to be generated and, using sophisticated assays, a result can provide concomitant information such as strain, species and viral load to mention a few. Sequence data has facilitated genetic engineering of viral proteins for preparation of safe reagents that do not require handling infectious pathogens and therefore development of safe assays building diagnostic and surveillance capacity. Molecular techniques have revolutionised laboratory research and diagnosis, although perhaps in the 1950s, researchers would have been exclaiming that cell culture and animal models revolutionised the way that research could be performed.

Reading the history of yellow fever virus teaches us that understanding where the virus circulated in nature and how the virus was transmitted to humans contributed to control of outbreaks. Developing tools that can be used to look for indirect evidence of infection contributed to understanding the epidemiology of the disease and where the virus was circulating. Passaging the virus through chicken embryo tissue allowed the development of a highly efficacious attenuated vaccine that is still used today. The application of improved laboratory assays and molecular techniques that are available today will contribute to our ability to catch and identify novel viruses and our understanding of emerging and re-emerging viruses of public health concern.