Pathogen discovery in infectious disease investigation

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Pathogen discovery is and always has been an integral part of our fight against infectious diseases. However, as technology continues to develop, enabling the identification of an increasing number of previously unknown agents, this process of discovery and characterisation of pathogenic agents is becoming even more important. From novel diagnostics to vaccines and therapeutics, pathogenic discovery plays a key role in shaping the overall control and prevention strategy. Due to the nature of the author's work area, the focus of this brief review will be on viral pathogens. The tools and strategies presented should be largely applicable to other types of pathogens.

Hendra versus SARS: very different stories of pathogen discovery

In September 1994 a disease outbreak, with primarily respiratory presentation, occurred in a horse stable in Hendra, Brisbane, which claimed the life of the horse trainer and 13 of his high-value horses. Three days after receiving specimen samples at the CSIRO Australian Animal Health Laboratory (AAHL), a novel virus (later named Hendra virus) was isolated. Within two weeks, morphological and partial sequence characterisation was completed and a challenge experiment confirmed that the newly isolated virus killed horses four to five days after inoculation, thus fulfilling Koch's postulate[1]. This rapid identification of causative agents prevented the unnecessary quarantine measures that were being planned for and the potential great economic losses that would have resulted due to the banning of horse racing and export of livestock products if the aetiological agent was not promptly identified and the host specificity of the pathogen not defined.

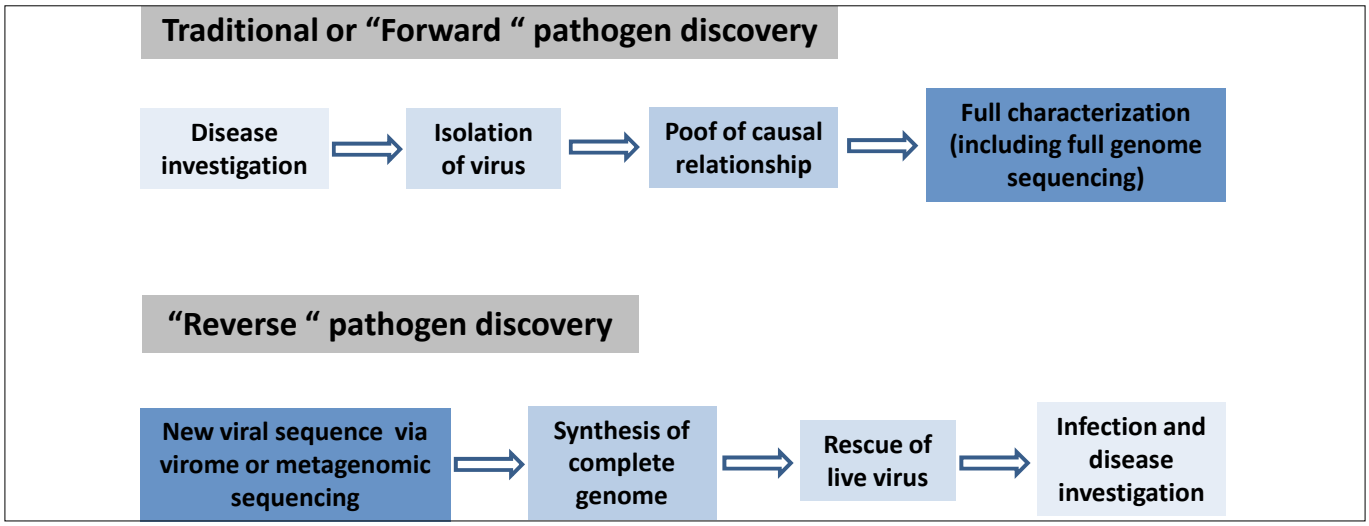
From November 2002, a mysterious disease known as "atypical pneumonia" was rapidly spreading in Southern China, exacerbated by several intensive nosocomial transmissions. It took almost six months before a novel coronavirus was isolated by the joint effort of the World Health Organization (WHO) SARS Collaborative Network[2-4]. By then, the SARS coronavirus had already spread to five continents and 25 countries, eventually resulting in more than 8000 human infections and 774 fatalities[5].

Theses two vastly contrasting examples are reviewed here to demonstrate the importance of rapid pathogen discovery in combating emerging infectious diseases.

Different approaches to pathogen discovery

Table 1 provides a list of emerging viruses discovered in the last two decades, some of which were discovered by our group

Figure 1. Diagram showing the difference between conventional approach to pathogen discovery and the 'reverse' approach, relying on complete genome sequence and *in vitro* creation of live viruses.



in collaboration with Australian and/or international groups. As one can see from the examples given, although culturing of viruses still remains one of the most used methods, novel strategies based on molecular approaches, such as microarrays[6,7], subtractive hybridisation[8] and unbiased high-throughput sequencing[9], are rapidly becoming the method of choice in early detection of potential aetiological agents without the need for live virus isolation. In this context, it is important to note that, although fulfilment of Koch's postulate is still considered the 'gold standard' of confirming the causal relationship of a new pathogen and disease, the scientific community is becoming more and more accustomed to accepting that in many cases molecular and serological evidences may be sufficient[10].

Pathogen discovery and vaccine development

It goes without saying that the proper knowledge of a given pathogen is essential for the development of any effective vaccine. Traditionally, this can only be done when successful isolation of live pathogens has been achieved. In this modern age, however, vaccine development can be initiated even in the absence of a live pathogen by using the following two strategies: 1) subunit vaccine development using synthetic genes derived from complete or partial genome sequences; 2) creation of live pathogen using 'reverse' pathogen discovery (Figure 1). Other techniques such as mapping of immunodominant epitopes using random peptide libraries[11] or protein arrays[12] will also greatly facilitate the rapid development of vaccines against novel pathogens of public health and veterinary importance.

Biography

Lin-Fa Wang is a CSIRO OCE Science Leader and Senior Principal Research Scientist, an honorary Professor at the University of Melbourne and an elected fellow of the Australian Academy of Technological Sciences and Engineering. His research interests are in emerging viruses and virus-host interaction.

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Table 1. Discovery of new viruses or new host range of previously known viruses.

Virus	Driver for discovery	Key step in discovery	Significance of the discovery	Fulfilment of Koch's postulate
Hendra	Investigation of diseases in horses and humans	Culture of virus and electron microscopic identification as a paramyxovirus	First zoonotic paramyxovirus lethal in human and livestock animals	Yes, within two weeks of discovery
Nipah	Investigation of diseases in humans and pigs	Culture of virus and cross reactivity with anti-Hendra antibodies	A major BSL4 agent continuing to cause human deaths in the world	Yes, a few years later
Tioman	Accidental discovery made during search for Nipah virus	Culture of virus and genome characterisation	Led to the confirmation of bat origin for Menangle virus	Not applicable
Pulau	Accidental discovery made during search for Nipah virus	Culture of virus and genome characterisation	Facilitated the discovery of Melaka virus	Not applicable
Menangle	Investigation of pig disease outbreak	Culture of virus and genome characterisation	A new pathogen of pig and zoonotic potential	No
SARS	Investigation of a major global pandemic outbreaks of an unknown human disease	Culture of virus, genome characterisation and microarray analysis	A totally new pathogen with great zoonotic potential and major global impact	Not fully fulfilled since the lethal disease was hard to reproduce in animal models
SARS-like	Search for origin of SARS virus	PCR detection and serology	Discovery of a new group of viruses in the absence of live virus culture	Not applicable
Melaka	Investigation of human diseases	Culture of virus, electron microscopy and genome sequence	Discovery of a new human pathogen with the help of previously identified 'orphan' viruses	No
New arenavirus	Investigation of human diseases	Unbiased high-throughput sequencing	Discovery made by direct sequencing of human samples without culture or PCR amplification	No
Reston Ebola	Investigation of pig disease outbreaks	Culture of virus and microarray analysis	First proof of Ebola virus infection in pigs	No