

RESEARCH NOTE

Emerging Infectious Diseases 1997-1998: The Role of Molecular Epidemiology

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In the last year, the global public health community has continued to be confronted and challenged by emerging infectious diseases. As evident from studying a list of emerging diseases, almost every year at least one new human pathogen is recognized or the microbial cause of a previously identified disease is determined. The last year has proven to be no exception, with the first human illnesses due to an avian strain of influenza [influenza A (H5N1)] recognized in the latter half of 1997 in Hong Kong (K Subbarao et al. 1998 *Science* 351: 393-396, KY Yuen et al. 1998 *Lancet* 351: 467-471). Other firsts during 1997-1998 were the initial reports of nosocomial infections caused by strains of *Staphylococcus aureus* with reduced susceptibility to glycopeptides (i.e. vancomycin) from Japan (K Hiramatsu et al. 1997 *Lancet* 350: 1670-1673) and the United States (CDC 1997a *MMWR* 46: 813-815) and an outbreak of hantavirus pulmonary syndrome identified in Chile (CDC 1997b *MMWR* 46: 949-951).

Other prominent emerging infections continued to garner attention. In the United States, a cluster of *Escherichia coli* O157:H7 cases generated a massive recall of ground beef (CDC 1997c *MMWR* 46: 777-778), and for the second year in a row widespread outbreaks of cyclosporiasis linked to imported fresh raspberries, and other produce sources, were recognized (CDC 1997d *MMWR* 46: 461-462).

Reemergence of infectious diseases thought to be under control also occurred in the last year. Large outbreaks of measles were identified in São Paulo and Rio de Janeiro, Brazil, representing the first significant outbreaks of measles in South America in several years (WHO 1997 *Week Epidemiol Rec* 72: 349-353). Dengue fever continued to spread to many urban centers of the Americas and Asia (PAHO 1997 *Epidemiol Bull* 18: 1-6, AL Richards et al. 1997 *Am J Trop Med Hyg* 57: 49-55, G Kouri et al. 1998 *Emerg Infect Dis* 4: 89-92). In Africa, a large outbreak of Rift Valley fever occurred in Somalia and Kenya (CDC 1998 *MMWR* 47: 261-264). In the United States, a multistate outbreak of hepatitis A linked to frozen imported strawberries was reported (CDC 1997e *MMWR* 46: 288, 295).

Despite the many newly recognized pathogens and the increasing complexity of infectious diseases, in most parts of the world the infrastructure to control and prevent these diseases has eroded over the last 20 years (Ciset 1995 Report of the NSTC Ciset Working Group on emerging and re-emerging infectious disease). However, now increasing attention is being given to the problem of emerging infectious diseases, culminating in emerging infectious diseases being chosen as the theme for 1997's World Health Day (AAWH 1997 Resource Booklet for World Health Day). Slowly this increased attention is beginning to translate into long overdue improvements in global surveillance and response capacity (JW LeDuc 1996 *JAMA* 275: 3318-3320).

Enhanced capacity is crucial, since the underlying causes of disease emergence show no signs of abatement. These factors include global population growth and demographic change; human behaviors which promote microbial proliferation; technologic innovation with unintended consequences; changes in land use patterns and the environment; global commerce and population movement; microbial adaptability; and failure to effectively utilize public health and clinical tools (SS Morse 1995 *Emerg Infect Dis* 1: 7-15). In general, it is the interplay of these factors rather than any single one which results in most emerging infections.

To successfully address emerging infectious diseases, public health must develop and harness new tools. Examples include computerization and electronic transmission of surveillance data, new and rapid diagnostic laboratory assays, and utilization of satellite-based global positioning systems. One of the most powerful tools which has developed in recent years is the field of molecular epidemiology. This growing discipline has revolutionized our understanding of disease transmission

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and has allowed us to recognize and investigate outbreaks in ways never before possible. This paper will examine how this discipline is being applied to enhance routine surveillance activities in 1997-1998, and how it was applied to some of the outbreaks recognized in the past year.

ROUTINE SURVEILLANCE ACTIVITIES

In the United States, molecular epidemiology is being increasingly applied to routine surveillance activities. Subtyping technologies are being standardized for pathogens responsible for selected reportable diseases, and these technologies are being applied at the state and local level. In some instances, molecular subtyping is being done in the state public health laboratory, in other instances regional subtyping laboratories have been developed and provide assistance to a network of jurisdictions. Probably the two best examples are the regional subtyping network for tuberculosis, employing restriction fragment length polymorphism (RFLP) and the network of state laboratories performing pulsed field gel electrophoresis (PFGE) of *E. coli* O157:H7 isolates. In both instances, national databases have been developed to collect and disseminate fingerprint patterns. For the *E. coli* O157:H7 subtype network, efforts are underway to transmit the patterns electronically, a system which goes by the name PulseNet.

For tuberculosis, routine subtyping using RFLP has allowed elucidation of the patterns of tuberculosis transmission within defined geographic areas. This allowed a determination of the proportion of disease which is due to recent transmission, and uncovered chains of unsuspected transmission (PD Butcher et al. 1996 *Soc Applied Bacteriol Symp Series* 25: 53S-71S, TR Frieden et al. 1996 *Tubercle & Lung Disease* 77: 407-412, CR Braden et al. 1997 *J Infect Dis* 175: 1446-1452, D Wilkinson et al. 1997 *Trop Med & Internl Healt* 28: 747-753, PN Suffys et al. 1997 *Mem Inst Oswaldo Cruz* 92: 297-316, H van Deutekom et al. 1997 *Clin Infect Dis* 25: 1071-1077, H Safi et al. 1997 *J Clin Microbiol* 35: 2472-2476). It has also facilitated the ability to rapidly recognize strains of *Mycobacterium tuberculosis* with high levels of antimicrobial resistance even before the results of susceptibility testing have become available.

There are now 20 state public health laboratories, plus laboratories in the U.S. Department of Agriculture and the Food and Drug Administration, using standardized methods for PFGE of *E. coli* O157:H7 (J Stephenson 1997 *JAMA* 277: 1337-1340). In many of these jurisdictions, clinical laboratories are required to forward *E. coli* O157:H7 isolates to the referral laboratory, and these strains are routinely subtyped. During 1997,

while using such policies, the Colorado State Public Health Laboratory identified 12 isolates from seemingly sporadic cases of illness with indistinguishable PFGE patterns. Investigations performed by local and state epidemiologists disclosed that these persons had consumed frozen ground beef patties from the same company, eventually leading to the recall of almost 12 million kilograms of possibly tainted ground beef (CDC 1997c, *loc. cit.*). The unique PFGE pattern of the outbreak strain was distributed to the PFGE network, and comparisons to more than 300 recently subtyped *E. coli* O157:H7 isolates from elsewhere in the country found no matches. Thus the Colorado outbreak appeared to be localized. Although it is impossible to predict what might have happened, it is probably that the outbreak would not have been identified except for the routine molecular subtyping or until a large number of cases had occurred. It is also likely that the quick action in recalling the possibly contaminated ground beef prevented cases from occurring elsewhere, since this product had been widely distributed. This episode demonstrates the value of routine subtyping, as it is quite likely similar outbreaks will continue to be recognized in the future. In the coming year, the PFGE network will add routine testing of *Salmonella* subtype *typhimurium* isolates submitted to participating laboratories.

MOLECULAR SUBTYPING AND DISEASE OUTBREAKS

The value of molecular subtyping has become so well established that it plays a role in almost every disease outbreak investigation which has been conducted in recent years. In 1997, an outbreak of hepatitis A was reported among children in several school districts in Michigan (CDC 1997e, *loc. cit.*). Investigations demonstrated a strong association with consumption of food items containing strawberries. The implicated strawberries had been imported by a facility in southern California and then distributed frozen to schools in many parts of the United States. Genetic sequences of hepatitis A viruses from cases in different schools in Michigan showed they were identical, indicating that a food handler in Michigan was not responsible (since there were none in common among the schools) and that contamination was "upstream" from the item's distribution within the state. Case finding was initiated in areas where the implicated lots of frozen strawberries had been distributed, and small clusters of cases were detected in other states. In each instance, the number of cases was too small to conduct meaningful epidemiologic studies to implicate a source. However, genetic sequencing proved essential to deter-

mine that some of these cases were linked to the Michigan outbreak by virtue of having the same genetic sequence. Shortly after the outbreak investigation in Michigan, increased numbers of hepatitis A cases were recognized among school children in Maine, a state which trace backs showed had received frozen strawberries from the same company but not from the implicated lots. However, epidemiologic studies showed an association with consumption of strawberries at school. By obtaining genetic material from retained serum specimens, the CDC's hepatitis reference laboratory was able to link the Maine outbreak to the cases in Michigan by performing molecular subtyping. These remarkable laboratory studies are a testament to the power of this technique. Unfortunately, assays are lacking to detect hepatitis A in many types of food items, such as strawberries. Since there were no viral isolates from the implicated food to compare to those from the ill persons, the site and mode of contamination remains unclear.

Although the outbreak of human illness caused by influenza A(H5N1) in Hong Kong was the first which has been identified, even in this setting molecular epidemiology played an important role. Between May and December 1997, a total of 18 human cases were identified, and viral isolates were made from specimens from all but two of the human cases (Subbarao, *loc. cit.*). Sequencing of the hemagglutinin antigen was performed on these isolates, which showed that they were all tightly clustered but minor sequence differences could be determined. These human viral isolates were compared not only to isolates from poultry specimens obtained in Hong Kong during the same period, but to influenza A(H5N1) strains obtained from avian species in Asia in 1997. Such testing showed that the Hong Kong isolates were similar to an avirulent influenza A(H5N1) virus obtained outside of Singapore, raising the possibility that the Singapore strain could be used as a potential vaccine candidate for humans should one be necessary.

Similar genetic sequencing work on the hantavirus responsible for illness in Chile during 1997 was performed. This allowed the virus to be compared to hantaviral isolates responsible for outbreaks in neighboring Argentina in 1995 and 1996, allowing rapid determination of likely rodent reservoirs and thus implementation of control measures.

FUTURE ROLES FOR MOLECULAR EPIDEMIOLOGY

Molecular epidemiology has become an essential tool in the outbreak setting to define who is, and is not, a part of an outbreak, and to identify possible sources of infection. This discipline is also increasingly demonstrating its utility as part of routine disease surveillance, both in identifying likely disease outbreaks and in exploring the patterns of disease transmission within a population. To a large degree, the technology necessary to conduct molecular epidemiology is limited to certain centers. In addition, the technology works best when coupled with a well trained and responsive investigation infrastructure. The challenge for the future is to make this methodology more widely available, and to standardize techniques across international boundaries. In the same way the United States is routinely studying *E. coli* O157:H7 isolates, this should be done with global partners as well to better define the global epidemiology of this pathogen and to detect international outbreaks due to global commerce. Every country should have access to a facility which could perform necessary subtyping techniques, if not on a routine basis, but at the very least in the setting of a disease outbreak. Such capacity could be located within the country, or in some circumstances on a regional basis. Finally, subtyping techniques and standardized methodology need to be developed for those pathogens in which it does not currently exist; examples would include parasitic agents such as malaria and cyclospora, and gram-positive bacterial pathogens. In this way, we can better harness the true potential of this evolving discipline.

