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FOCUS on Field Epidemiology

Laboratory Diagnosis in Outbreak Investigations

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In recent issues of FOCUS, you have learned about a variety of laboratory techniques for diagnosing infectious agents from patient specimens.

Now that you know more about these techniques than most of the world's population, you can astound others with your intricate knowledge. Surely they will ask you about mysterious matters of molecular biology when they learn that you work for the health department!

However, before you launch into a long discourse on the subtler points of pulsed-field gel electrophoresis, remember that people are easily bored. While you may have been riveted to the FOCUS issues on laboratory diagnostics, others might not find this technical material quite so fascinating.

Therefore, it is helpful to speak in general terms, using examples to illustrate. Tell the story of a little pathogen who enters the big world and causes a ruckus. This issue of FOCUS provides examples of how a variety of laboratory diagnostic techniques are used in investigational outbreak settings.

Laboratory diagnosis can be used to:

- identify the agent causing an outbreak;
- confirm cases in an outbreak;
- link cases to the same outbreak, even with cases that occur over wide geographic areas;
- identify the strain or serotype of an agent involved in an outbreak; and
- learn more about the epidemiology of infectious agents for research purposes (such as to identify new modes of transmission, to learn more about newly described or reemerging infectious diseases, or to evaluate prevention measures).

Each of these uses for laboratory diagnostics is illustrated below using an outbreak example. Keep in mind that the list is not exclusive. Each of these examples may feature multiple aspects of laboratory diagnosis, and innumerable other outbreaks could illustrate the same points.

Identifying the Agent Causing an Outbreak

An important function of laboratory diagnostic tests is to identify the agent causing an ongoing or recent outbreak. Correctly identifying the agent may allow more effective prevention.

In 1998-1999, 3 clusters of febrile encephalitis in Malaysia were reported to the Malaysian Ministry of Health. (1) By the end of the outbreak, there had been more than 200 cases and more than 100 deaths. During the same time period, 9 similar cases were reported in Singapore, including 1 death. Japanese Encephalitis (JE), a viral encephalitis transmitted through the bite of a mosquito, was endemic to the area. Investigators initially suspected the JE virus as the cause of the outbreak, and some specimens tested positive for this agent. However, when nervous system specimens were grown in tissue culture, a previously unknown virus grew.

The cases were mostly adult men who had had contact with swine through farming or other means. Because JE is not usually associated with swine contact, JE virus seemed less plausible, and further investigations were undertaken. Samples from 13 patients were sent to the U.S. Centers for Disease Control and Prevention (CDC) for testing. JE virus was identified from only 1 of the specimens, while the rest were negative for JE antibodies.

To identify the agent, the samples were then examined under an electron micro-

scope. The structure of the previously unknown virus was similar in shape to a paramyxovirus (a type of RNA virus). Additional laboratory tests were performed to identify the agent more precisely. The virus was found to be related to Hendra virus (first identified in Hendra, Australia). Tissues from patients who died were positive when tested with antibodies to the Hendra virus. Antibodies to the Hendra-like virus were also found in the serum of some patients, and the virus itself was found in tissues of other patients.

Similar investigations were performed among swine to examine the epidemiologic link. The Hendra-like virus was found in the central nervous system, lung, and kidney tissues from swine at affected farms in Malaysia. The Singapore cases were determined to have handled swine from Malaysia.

To prevent further infection, transport of swine within Malaysia was banned, use of personal protective measures (gloves, masks, etc.) was encouraged for those who worked with swine, and importation of swine from Malaysia was prohibited by neighboring countries. Research on the epidemiology and transmission of this virus among swine and humans is ongoing.

Confirming Cases in an Outbreak

In late December 2005, an outbreak of mumps began in Iowa. By May of 2006, the outbreak had spread to (at least) 10 additional states, resulting in 2,597 reported cases of the illness. (2) Mumps is clinically characterized by swelling of the parotid (a large salivary gland) or other salivary gland that lasts for more than 2 days and cannot be associated with another cause.

Eight of the states involved in the outbreak (Illinois, Iowa, Kansas, Missouri, Nebraska, Pennsylvania, South Dakota, and Wisconsin) reported that the outbreak of mumps was observed as ongoing local transmission or case clusters. Three states (Colorado, Minnesota, and Mississippi) reported cases as being related to recent travel from an outbreak state. In this outbreak, infected individuals traveling by aircraft were implicated as the most likely source of disease transmission.

A majority of the mumps cases (1,487) reported from January 1 to May 2, 2006, came from Iowa. Kansas, Illinois, Nebraska and Wisconsin reported 371, 224, 201, and 176 cases, respectively. Of the 2,597 cases reported by the 11 states, 1,275 were ultimately classified as confirmed, 915 as probable, and 287 as suspect. The remaining 120 were classified as unknown. Thus a little less than half of the 2,597 reported cases were eventually characterized as "confirmed."

Why do the case numbers jump around? As you might remember from the discussion of case definitions in an earlier issue of *FOCUS,* some people who exhibit signs and symptoms of the disease you are investigating might in fact have a completely different disease. How do you know if they are truly a case in your outbreak or not? Laboratory diagnostics can help.

Many investigations use several levels of a case definition. In general, there can be "suspected" cases, who appear to have the illness; "probable" cases, who have the symptoms of the illness and perhaps an epidemiologic link to other cases or the source of infection; and "confirmed" cases, who have a laboratory-confirmed diagnosis of the disease in question and meet the other case criteria. We can see how the case definition was applied n the mumps investigation: those with negative mumps test results were among those who were excluded.

Cases can be confirmed using any of a number of laboratory tests. For example, the mumps virus could be cultured from a patient sample, polymerase chain reaction (PCR) could be used to prove mumps DNA was present in a clinical sample, electron microscopy could show the tell-tale shape of the virus, or an antibody stain specific for mumps could be used on a tissue sample from a case.

Linking Cases to the Same Outbreak

Listeriosis is a bacterial infection caused by the organism *Listeria monocytogenes.* The bacterium is found in soil and water, and can be present in apparently healthy animals such as cattle. Animal products, particularly unpasteurized foods, meats, and soft cheeses, can be contaminated with *Listeria*, causing fever, muscle ache, and nausea in individuals who consume them, with occasional serious complications. When pregnant women become infected, they risk having a premature birth or stillbirth. (3)

A 1998 outbreak of listeriosis illustrates how laboratory diagnostic techniques can be used to connect cases that occur over a wide, seemingly unrelated geographic area. In August 1998, cases of listeriosis were reported to the CDC by Connecticut, New York, Ohio, Tennessee, Massachusetts, West Virginia, Michigan, Oregon, Vermont, and Georgia. (4)

The reported cases all had the same serotype (strain) of *L. monocytogenes*. When isolates from patients were sub-typed using pulsed field gel electrophoresis (PFGE) or ribotyping, all the isolates shared the same pattern, and the pattern observed was one rarely seen in human infections. This fact was noted at the CDC, and a multi-state case-control study was conducted in conjunction with state health departments to identify the source of the infection.

As part of the investigation, 4-week food histories were taken from cases and controls, and it was found that cases were much more likely to have eaten hot dogs during that time than controls

Antigen detection methods

Determining the presence or absence of a particular pathogen can be accomplished through antigen detection methods (for more information, see *FOCUS Volume 4, Issue 3: Laboratory Diagnosis: An Overview*).

These methods test for the physical presence of parts of the viral or bacterial pathogen. Antigens are small parts of infectious organisms that are recognized by the immune system. The laboratory uses specially made antibodies to detect antigens just as the immune system would.

(odds ratio = 17.3). On December 19, an opened package of hot dogs from a case patient's home was tested and found to be contaminated with the outbreak strain of *L. monocytogenes*. The patient had eaten these hot dogs several weeks before becoming ill. The manufacturer of the hot dogs was informed, and the hot dogs were voluntarily recalled along with other food products that might have been contaminated. (4)

Identifying Specific Strains of an Agent Involved in an Outbreak

Aseptic meningitis, or viral meningitis, is a considerable health problem in the United States. It can occur sporadically (single cases) or in outbreaks. The viral infection can spread by direct contact with the respiratory secretions of someone who is ill or through fecal contact (for instance, when changing the diaper of an infected infant).

Outbreaks of meningitis are caused by enteroviruses, particularly echoviruses 5, 7, 9, and 30; coxsackieviruses B1, B4, and A9; and enterovirus 71. Symptoms caused by aseptic meningitis are similar to symptoms caused by encephalitis viruses, such as West Nile Virus and St. Louis encephalitis, and these viruses should be considered when making a diagnosis for aseptic meningitis.

Although most cases are asymptomatic, the virus can become a central nervous system infection, with fever, headache, stiff neck, and photosensitivity. (5) Occasionally life-threatening consequences such as encephalitis, myopericarditis, and paralysis can occur. Reporting of aseptic meningitis is not required on a national level, although voluntary reporting can alert officials to clusters of cases. The CDC maintains a voluntary reporting system for surveillance (the National Enterovirus Surveillance System, or NESS).

In the spring of 2003, 7 states reported outbreaks of aseptic meningitis. (6) Arizona reported 465 cases, more than 4 times the number reported for the same time period in 2002. Dozens of isolates were tested; 76% were positive for echovirus 30 (E30) and 1 (2%) was positive for echovirus 9 (E9).

In California, aseptic meningitis rates in 2003 were also above the rates seen in previous years. From more than 1,700 cases, about 150 specimens were submitted to the health department for diagnostic testing. The specimens were tested for both enteroviruses (which cause aseptic meningitis) and arboviruses (which cause encephalitis). More than half of the specimens (55%) had evidence of enterovirus by PCR or culture. Of these, most cases (85%) were identified as E30 infections, while a few (12%) were E9 infections.

Between March and July 2003, 320 cases of aseptic meningitis were reported from Augusta, Georgia, compared to 227 cases statewide for the entire previous year. Twenty-four throat and rectal swab and cerebrospinal fluid specimens tested positive for E9. (Enteroviruses were identified by PCR from 52 additional samples, but had not been typed at the time of publication in the *MMWR*.)

In Idaho, 38 cases of viral meningitis occurred between May and July 2003, compared with 4 the previous year. The 4 cases were investigated, and E30 was identified in 2 of them.

In South Carolina, an outbreak of aseptic meningitis peaked in May 2003, with 82 cases reported to the Aiken County Health Department and more cases subsequently identified in neighboring counties and across the state border in Georgia. By the end of July, 130 cases had been reported. E9 was identified from 20 specimens in 8 different counties; no additional virus was identified.

From an epidemiological standpoint, it is important to determine which viruses are causing a particular disease. In every outbreak across these different states, only E9 and/or E30 were identified, usually by PCR. E30 was involved with outbreaks in the western part of the U.S., while E9 was more active in the east. The enteroviruses are frequently associated with aseptic meningitis outbreaks, but in the years preceding 2003, there was very little activity from these particular viruses.

When trends over the last few decades are examined, a cyclical pattern can be observed. It has been suggested that during years of low E9 and E30 activity, the population susceptible to these viruses (generally children born in the period) grows until it is large enough for an outbreak to occur. After an outbreak, enough people have been exposed to the virus and have an immune response so that an outbreak does not occur again until enough new people enter the population.

Why Isn't the Bug Identified from ALL Specimens?

If a particular pathogen is causing an outbreak, it would be nice to find genetic material from the causative bacterium, virus or fungus in every clinical specimen. That, however, is not always the case.

There are many reasons why a pathogen may not be found in a specimen, even if it was the cause of disease. Sometimes pathogens are present at such low levels that they cannot be detected. The ill person may have recovered by the time the specimen was taken, so there is no longer evidence of the infection. Sometimes pathogens do not survive the trip from person to specimen container to laboratory, and the DNA or RNA is in such poor condition that the laboratory cannot detect anything at all. Another possibility, of course, is that the specific organism being tested was not the pathogen responsible for disease!

Epidemiologists must weigh the evidence. If a particular pathogen is identified in several clinical specimens from the same outbreak, this is often enough evidence to conclude that it was the cause of the outbreak. However, the conclusion depends on the pathogen. If the pathogen is extremely common in the general population, it could just be coincidence that it is present in a number of ill cases. If the pathogen is rare, finding it in a number of specimens is more likely to mean that it caused the outbreak.

Learning More About the Epidemiology of Infectious Agents

Staphylococcus aureus is a bacterium commonly present on the skin and in the nose, and it can occasionally cause infection. "Staph" can infect wounds or even blood, but it can be treated with antibiotics such as methicillin. A serious concern, however, is the emergence of *S. aureus* that is resistant to the antibiotic methicillin (methicillin-resistant *Staphylococcus aureus*, or MRSA).

MRSA is often associated with hospital infections involving direct contact; a health care worker having contact with an infected patient can transmit the disease to a previously uninfected patient. (7) However, community-acquired (i.e., non-hospital) MRSA has recently been recognized in institutions such as daycare centers and prisons, as well among specific populations such as men who have sex with men.

In August 2003, the CDC described a new mode of transmission of community-acquired MRSA that was occurring in several different states. (8) Laboratory diagnostic techniques were used to identify MRSA, which was apparently transmitted among sports participants. The outbreak investigations found that athletes often sought medical care for skin lesions but were incorrectly diagnosed, leading to further medical visits and eventually hospitalization. Importantly, these outbreaks suggested that transmission could occur without skin-to-skin contact, through means such as sharing sports equipment or personal items.

In Colorado, 5 cases of MRSA were reported in February 2003 among members of a fencing club and their household contacts. A confirmed case was defined as a member of the fencing club or a household contact with signs and symptoms of MRSA infection, such as fever, pus, swelling, or pain, and MRSA cultured from a clinical isolate. A probable case was defined as one of these persons with a skin or soft tissue infection, but without clinical culture. Among the 70 club members, 3 confirmed and 2 probable cases were identified (1 case was a household contact).

How do we know that the several cases among the fencers were not simply coincidence? PFGE was used to verify that the cases were infected with the same strain of MRSA. Two cases with available specimens had identical PFGE patterns. (As you will recall from the last issue of *FOCUS*, PFGE provides a quick means of visualizing the unique sequences of DNA present in an organism, providing a "fingerprint" that can be used to identify an organism or distinguish between strains of the same organism.)

Although a definitive mode of transmission was not determined, investigators discovered that sensor wires worn under fencing uniforms were shared among the players and had no schedule for cleaning between uses. No common source of exposure was identified outside the fencing club. Protective measures recommended to fencing club members included washing after every practice and tournament, covering abrasions, cleaning sensor wires between uses, and consulting a healthcare professional for skin lesions.

In September 2000, the CDC and the Pennsylvania Department of Health investigated MRSA among 10 members of a college football team in Pennsylvania. Seven of the 10 cases were hospitalized. All isolates from patients had indistinguishable PFGE patterns. The investigators concluded that possible risk factors for infection could have been skin trauma due to turf burns or shaving, as well as sharing unwashed bath towels.

In Los Angeles County, California, 2 cases of MRSA were identified among members of a college football team in September 2002. The 2 players had indistinguishable PFGE patterns. Players on the team reported frequent skin trauma and said they covered wounds only about half of the time. Balms and lubricants were also identified as potential modes of transmission.

In Indiana, 2 wrestlers on a high school team with MRSA were reported to the Indiana Department of Health in January 2003. Isolates were not available for PFGE analysis. Since the players had never wrestled each other, investigators suspected that sharing items such as towels or equipment could have transmitted the infection. No other common sources were identified in this outbreak.

In this series of outbreaks, PFGE was used to verify that MRSA was in fact being transmitted between members of the same athletic team. Because isolates from the infected members on a given team had indistinguishable PFGE patterns, we know that the infections were the same strain. The exact mode of transmission—whether sharing equipment or sharing bath towels—was not identified. However, these findings lay the groundwork for future studies of MRSA focusing on potential modes of transmission among team members.

Evaluation of Prevention Measures

In addition to the 5 common uses of laboratory results in outbreak investigations described above, another goal of public health research is to verify that protective measures employed to prevent the spread of disease are effective.

Types of Close Contacts Quarantined During the SARS Outbreak:

- Health care workers
- Family members
- Co-workers
- Classmates and teachers
- Friends
- Airplane passengers within 3 rows of a case
- Other passengers and drivers of public transportation vehicles when the trip lasted at least one hour
- People who had contact with a person in quarantine at a facility where a SARS case occurred

This is illustrated by the measures taken to curb the transmission of severe acute respiratory syndrome (SARS), the disease that paralyzed Taiwan and other countries in early 2003. SARS remains a serious cause of concern, even though the major epidemic has subsided.

Because SARS was difficult to differentiate from other respiratory illnesses and initially could not be diagnosed with standard laboratory techniques, Taiwan employed widespread use of quarantine. (9) A majority of the 131,000 people quarantined from March until July 2003 were close contacts of SARS patients and travelers from countries designated by the World Health Organization as SARS-affected.

Hospital staff and patients were usually quarantined in a health care facility, but most others were quarantined at home. Persons who were quarantined were required to take their temperatures 2 to 3 times a day and report immediately if fever or respiratory symptoms occurred. People under "Level A" quarantine could not leave the house for any reason unless deemed appropriate by the health authorities. Persons under "Level B" quarantine could leave to seek medical attention, exercise in an outdoor area, buy food, dispose of garbage, and perform other activities if approved by health authorities.

The quarantine program was very intensive, inconvenienced a large number of people, and was expensive. Obviously health authorities deemed the potential prevention of additional SARS cases to be worth the personal and financial costs. But how effective was the quarantine in preventing cases?

Since the idea was to keep people who might have SARS from making contact with other people, investigators evaluated how many of those persons quarantined actually developed SARS. Of the 50,319 people under Level A quarantine, 112 (0.22%) were diagnosed with suspected or probable SARS while under quarantine. Of the 80,813 people under Level B quarantine, 21 (0.03%) received this diagnosis. The highest rates were among health care workers, family members of SARS patients, and airplane passengers seated within 3 rows of a SARS patient. The lowest rates were among travelers arriving from SARS-affected countries.

Was this level of quarantine worth it? Only a small percentage of quarantined persons had suspected or probable SARS diagnosed, and an even smaller percentage of the persons quarantined had a laboratory-confirmed case of SARS. However, assuming that each case of laboratory-confirmed SARS might have led to another cluster of cases, a very large number of cases might have been prevented by implementing the quarantine.

At the very least, the epidemiologic and laboratory evaluation of quarantined persons showed which groups were most likely to develop SARS if they had contact with a patient (health care workers and family members). The investigators noted that further research needed to be done. SARS rates did decrease during the time of the quarantine, but because multiple prevention measures were put into effect, the role that quarantine played remains uncertain.

A later study in Beijing evaluated how quarantine could be made more efficient. (10) The report concluded that only persons coming into contact with actively ill SARS patients needed to be quarantined; those who had contact during the incubation period before symptoms became apparent were not at risk of developing SARS.

From these examples, we can see how laboratory diagnostic tests can be used to solve outbreak investigations, to identify agents, and to investigate remaining questions about infectious diseases. Laboratory diagnostic techniques are an integral part of public health surveillance, investigation and research; understanding the basics of how these tests work will improve your conduct of outbreak investigations.

Resources:

CDC-recommended case definitions can be found at http://www.cdc.gov/epo/dphsi/PHS/infdis.htm

Updated information on monkeypox can be found on the CDC website: http://www.cdc.gov/ncidod/monkeypox/index.htm

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