

# The Wisconsin State Laboratory of Hygiene and Emerging Enteric Pathogens

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## ABSTRACT

At the turn of the 20th century, typhoid fever was common in Wisconsin, and was a major impetus for the establishment of the Wisconsin State Laboratory of Hygiene (WSLH) in 1903. By the 1940s, typhoid was virtually eliminated in the United States due to public health measures such as disinfection of drinking water, sewage treatment, pasteurization, and shellfish bed sanitation.<sup>1</sup> However, new food and waterborne pathogens have emerged to take the place of *Salmonella* Typhi. Infections with non-typhoidal *Salmonella* strains in the United States have increased almost 10-fold since the 1950s.<sup>1</sup> In the last 20 years, the emergence of foodborne pathogens, such as *Escherichia coli* O157:H7, *Cyclospora cayatanensis*, *Noroviruses* (Norwalk-like viruses), *Cryptosporidium parvum*, *Campylobacter jejuni*, *Yersinia enterocolitica*, and multi-drug-resistant *Salmonella*, has identified a need for accurate laboratory diagnosis of enteric disease and outbreaks.<sup>2-6</sup>

## INTRODUCTION

Testing for food- and waterborne disease pathogens at WSLH has evolved over time in concert with the development of laboratory testing availability in the private sector. In the early years, WSLH performed large volumes of routine tests for enteric pathogens for medical facilities located throughout the state. During this time, testing at WSLH consisted of routine bacterial culture techniques and microscopic examination for ova and parasites. In the 1930s, three-quarters of the state's physicians utilized the services of WSLH for enteric microbiology. As clinical laboratories developed throughout the state, the role of the WSLH evolved from one of routine diagnostic testing to that of a specialized refer-

ence laboratory. Its focus is now on the laboratory investigation of food- and waterborne infection outbreaks in partnership with the Wisconsin Division of Public Health (WDPH), epidemiologic-related reference testing (identification confirmation, serotyping/serogrouping), national and global surveillance of foodborne pathogens directed by the Centers for Disease Control and Prevention (CDC), using molecular techniques for sub-typing, monitoring for antimicrobial resistance of enteric pathogens, and the utilization of state-of-the-art technologies for the detection and identification of unusual or emerging pathogens. Table 1 lists some of the bacterial, viral, and parasitic agents that cause sporadic infections and outbreaks of foodborne disease.

In this article, we describe the public health role of the food- and waterborne disease program (FWP) at WSLH, technological advancements in the diagnosis of enteric pathogens, and a few of the emerging pathogens that plague us in the 21st century.

## ROLE OF THE WSLH FOOD AND WATERBORNE DISEASE PROGRAM IN OUTBREAK INVESTIGATION AND SURVEILLANCE

A major role of the FWP is the laboratory investigation of food- and waterborne outbreaks in conjunction with the investigations of the epidemiologists at the WDPH. In 2002, 33 outbreaks were investigated in Wisconsin, with infectious etiologies identified in 31 (Table 2). A variety of bacterial pathogens were implicated in 15 outbreaks, including non-typhoidal *Salmonella*, *E. coli* O157:H7, *Staphylococcus aureus*, *Campylobacter coli*, and *Clostridium perfringens*. *Norovirus* accounted for 16 outbreaks, many of which were associated with restaurants. In these outbreak investigations, the FWP works with clinical laboratories and local health departments to obtain patient specimens and bacterial isolates to help define the etiology and extent of the outbreak.

The FWP provides high-complexity and public

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health-related testing not readily available to the private sector hospitals, clinics, and laboratories in Wisconsin. *Salmonella* isolates are serotyped. Pulsed-field gel electrophoresis (PFGE) is utilized to subtype *E. coli* O157:H7, *Salmonella*, *Listeria monocytogenes*, and *Campylobacter jejuni*. Nucleic acid amplification assays are used to probe stool specimens for *Norovirus*, and *Norovirus* sub-typing is performed using nucleic acid sequencing techniques. Many of these methods are developed and validated with the cooperation and support of CDC, with the ultimate goal of providing rapid and timely laboratory results to help limit outbreaks of enteric infections.

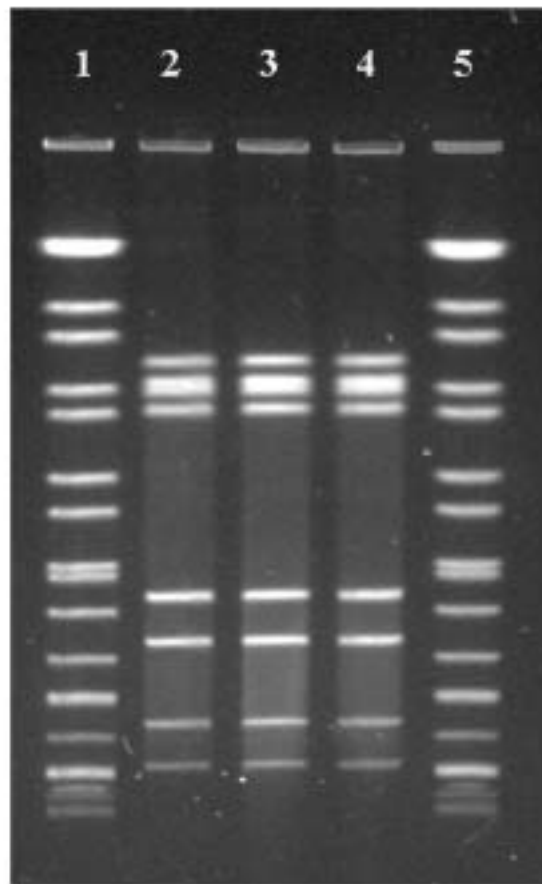
Surveillance is also an important function of the FWP. Antimicrobial resistance testing is performed on bacterial enteric pathogens submitted to WSLH from the clinical laboratories throughout the state to monitor for the emergence of antimicrobial resistance. The FWP also is part of the National Antimicrobial Resistance Monitoring program (NARMS), in which CDC monitors antimicrobial resistance of *Salmonella*, *Shigella*, *C. jejuni*, and *E. coli* O157:H7 at the national level. This program has followed trends in antimicrobial resistance since 1996. For example, the incidence of multi-resistant strains of *Salmonella* Newport has gone from 0% in 1996 to 25% in 2002, and ciprofloxacin-resistant *C. jejuni* has emerged, with 19% of strains resistant in 2002.

The WSLH also participates in surveillance of foodborne infections at a national and global level as a participant in PulseNet, the molecular sub-typing network for foodborne bacterial disease. This network was established in 1996 by CDC to facilitate sub-typing of bacterial foodborne pathogens for epidemiological purposes.<sup>7</sup> PulseNet currently includes all 50 state public health laboratories, several large local public health laboratories, Food and Drug Administration (FDA), and US Department of Agriculture (USDA) laboratories. PulseNet has recently joined forces with PulseNet North (Canada) and EuroNet to provide a global surveillance network. PulseNet participants utilize a rapid, standardized PFGE method for the DNA sub-typing (“fingerprinting”) of bacterial isolates.

Use of PFGE is a simple way of comparing the genetic material of bacterial isolates. It involves cutting bacterial DNA into pieces using specific enzymes (restriction endonucleases) and then separating the pieces on an agar gel under the force of an electrical field. The gel is then stained with a fluorescent dye that allows the DNA band pattern to be visualized under UV light. The separated pieces of DNA form a pattern of distinct

**Table 1.** Major Food- and Waterborne Enteric Pathogens

Bacterial	Viral
<i>Salmonella</i>	<i>Norovirus</i>
<i>Campylobacter jejuni</i>	Hepatitis A
<i>Shigella</i> species	<i>Rotavirus</i>
<i>Clostridium botulinum</i>	
<i>Clostridium perfringens</i>	
<i>Bacillus cereus</i>	<b>Parasitic</b>
<i>Staphylococcus aureus</i>	<i>Giardia lamblia</i>
<i>Vibrio cholerae</i>	<i>Entamoeba histolytica</i>
<i>Vibrio parahaemolyticus</i>	<i>Cyclospora cayatenensis</i>
<i>Vibrio vulnificus</i>	<i>Cryptosporidium parvum</i>
Shiga toxin-producing <i>E. coli</i>	<i>Trichinella spiralis</i>
Enterotoxigenic <i>E. coli</i>	
<i>Yersinia enterocolitica</i>	



**Figure 1.** PFGE of *Campylobacter jejuni* isolates. Cut with restriction endonuclease Sma1. Lanes 1 and 5, *Salmonella* Braenderup control; Lane 2, *C. jejuni* from Patient 1; Lane 3, *C. jejuni* from Patient 2; Lane 4, *C. jejuni* from cat of patient 1.

bands that resembles a “bar code.” This “bar code” is the DNA fingerprint or pattern (Figure 1). Note that the three *C. jejuni* strains are indistinguishable with *Salmonella* Braenderup being used as a standard to normalize the patterns. The patterns are then entered into

**Table 2.** Outbreaks of Gastroenteritis—Wisconsin, 2002

Etiologic Agent	# Cases	Venue	Vehicle
<b>Bacterial</b>			
<i>Clostridium perfringens</i>	34	Restaurant	Beef tips and gravy
<i>Salmonella Hartford</i>	43	Restaurant	Refried beans
<i>Salmonella Enteritidis</i>	44	Restaurant	Chicken
<i>Clostridium perfringens</i>	13	Private home	Roast beef
<i>Salmonella Typhimurium</i>	14	Restaurant	Unknown
<i>Salmonella Newport</i>	10	Restaurant	Sub sandwiches
<i>Salmonella Enteritidis</i>	4	Restaurant	Unknown
<i>Campylobacter coli</i>	22	Golf course	Unknown
<i>E. coli</i> O157:H7	19	Multiple	Ground beef
<i>Salmonella</i> "i" Monophasic	16	Private home	Chicken
<i>E. coli</i> O157:H7	36	Multiple	Ground beef
<i>Staphylococcus aureus</i>	29	Church	Gravy
<i>Salmonella</i> "i" Monophasic	12	Restaurant	Unknown
<i>Salmonella Typhimurium</i>	20	Festival	Raw beef
<i>Clostridium perfringens</i>	12	Restaurant	Prime rib
<b>Total</b>	<b>328</b>		
<b>Viral</b>			
Norovirus	18	Restaurant	Chicken salad
Norovirus	17	Restaurant	Unknown
Norovirus	59	Restaurant	Sub sandwiches
Norovirus	2	Restaurant	Sub sandwiches
Norovirus	18	School picnic	Ice cream
Norovirus	37	Restaurant	Coffee, water
Norovirus	5	Restaurant	Unknown
Norovirus	6	Restaurant	Unknown
Norovirus	12	Private Home	Unknown
Norovirus	40	Camp	Unknown
Norovirus	6	Caterer	Unknown
Norovirus	20	Restaurant	Pastry
Norovirus	8	Private Home	Cake
Norovirus	28	Restaurant	Italian sausage
Norovirus	24	Private club	Unknown
Norovirus	24	Restaurant	Unknown
<b>Total</b>	<b>324</b>		

a local and national (CDC) electronic database enabling WSLH microbiologists to compare patterns of Wisconsin isolates to those seen in other states across the country. If a cluster of matching patterns is found, epidemiologists at the WDPH initiate an investigation. In this manner, a common food source may be determined as the source of the outbreak and the food recalled, if possible, thus limiting the extent of the outbreak. PulseNet

has identified many multi-state foodborne outbreaks that would likely have gone undetected because only a few people in each state had enteric illnesses. This public health activity has resulted in a significant reduction in the number of foodborne infections and has correspondingly reduced the associated human suffering and economic losses.

At present, PulseNet tracks five foodborne pathogens: *E. coli* O157:H7, *Salmonella*, *Shigella*, *Listeria monocytogenes*, and *C. jejuni*. Use of molecular sub-typing by PFGE is an integral part of our FWP and is supported by funds from the CDC Epidemiology and Laboratory Capacity grant. Its success is dependent upon the cooperation of all Wisconsin clinical laboratories for the submission of patient isolates for sub-typing, and upon the state and local public health agencies for the performance of thorough epidemiologic investigations.

*E. coli* O157:H7 has been responsible for many well-publicized large outbreaks in Wisconsin and worldwide, and PFGE sub-typing was essential in the investigations of several Wisconsin outbreak investigations. It was utilized in an outbreak of *E. coli* O157:H7 associated with a Milwaukee restaurant in 2000 in which 1 young girl died of hemolytic-uremic syndrome (HUS) complications. PFGE helped link a commercial source of hamburger to a multi-state outbreak in 2000 that resulted in a recall of 1.1 million pounds of ground beef.

#### EMERGING ENTERIC PATHOGENS

##### *E. coli* O157:H7 and Other Shiga Toxin-Producing *E. coli* (STEC)

*E. coli* O157:H7 was first recognized as a human pathogen in 1982, and is the most common cause of HUS in children.<sup>8</sup> *E. coli* O157:H7 accounts for approximately 20,000 cases of diarrhea annually in the United States, with a mortality rate of 1%-2%.<sup>9</sup> This organism has obtained the gene that allows for the production of Shiga toxin which accounts for its pathogenicity. The major source of *E. coli* O157:H7 is ground beef; however, it has been detected in a wide spectrum of foods, including lettuce, alfalfa sprouts, fresh apple juice and cider, venison jerky, and milk.<sup>10-12</sup> While the prevalence of this illness may be significantly lower than other foodborne pathogens, such as *Noroviruses*, *Campylobacter sp.*, and *Salmonella*, its morbidity and mortality may be much greater.

WSLH has responded to many outbreaks over the years, and has improved its testing capabilities and surveillance accordingly. WSLH has adopted specialized culture media and immunomagnetic separation techniques to increase the sensitivity of culture methods. In

addition, enzyme immunoassays and nucleic acid amplification (PCR) methods are being utilized for the detection of STEC from stool specimens. These techniques can detect non-O157:H7 STEC in addition to *E. coli* O157:H7. Non-O157:H7 strains may account for 25%-50% of Shiga toxin-producing *E. coli* infections.<sup>13</sup> These strains are not detected with the selective media used by most clinical laboratories in Wisconsin for the detection of *E. coli* O157:H7. In the past year, in cooperation with the WDPH Bureau of Communicable Disease and several clinical laboratories, WSLH has initiated a study to detect, isolate, identify, and sub-type strains of non-O157:H7 STEC. The goals of this study are to determine the prevalence of non-O157:H7 STEC in Wisconsin, and to encourage clinical laboratories to institute diagnostic methods that will detect infections with these organisms.

#### *Norovirus*

*Norovirus*, commonly known as Norwalk-like virus, is a member of the family *Caliciviridae*. It is comprised of 4 genera (G1 through G4) which, in turn, are divided into at least 20 genetic clusters. *Norovirus* is a single-stranded RNA, non-enveloped virus, also referred to as small round-structured viruses (SRSV) based on electron microscopic imaging.

*Noroviruses* are now the most common cause of viral gastroenteritis in the United States, with an estimated 23 million cases occurring annually.<sup>14</sup> The syndrome of acute nausea and vomiting, known as "intestinal flu" or "winter vomiting disease," occurs in winter months in temperate climates. *Norovirus* was first associated with gastroenteritis in an outbreak in Norwalk, Ohio, in 1972. Using immune electron microscopy, 27-nm-diameter viral particles were visualized in stool specimens from volunteers infected with stool filtrates from patients in the outbreak.<sup>15</sup> Viral transmission primarily takes place through the fecal-oral route and person-to-person spread, though transmission through aerosolization of vomitus has been reported. *Norovirus* is highly transmissible, with as few as 10 virus particles needed to elicit an infection.<sup>16</sup> Studies have shown that up to 30% of infections may be asymptomatic.<sup>17</sup> Distinguishing between bacterial and viral outbreaks of gastroenteritis is important in tracking and controlling outbreaks. *Norovirus* accounted for 16 outbreaks in Wisconsin in 2002 (Table 2), and there was widespread *Norovirus* activity worldwide in 2002, with outbreaks occurring in a variety of settings, including cruise ships, hospitals, long-term-care facilities, schools, restaurants, and catered events.

*Norovirus* fails to grow in all cell culture models,

therefore amplification of a segment of the *Norovirus* genome by use of reverse-transcriptase PCR is the method of choice for detection. This assay offers a highly sensitive approach for the detection of a broad range of *Noroviruses* in clinical samples, but does not detect all strains of the *Norovirus* genus. *Norovirus* can generally be detected in stool and vomitus (although less frequently due to acidity). WSLH provides *Norovirus* testing in support of outbreak investigations, but does not provide testing for sporadic cases. *Norovirus* disease is of short duration, usually 1-2 days, and diagnostic testing is of little value for management of the individual patient.

#### *Cryptosporidium*

The largest-recorded waterborne disease outbreak in the United States occurred in 1993, when an estimated 402,000 people in Milwaukee, Wis, developed gastroenteritis associated with the consumption of drinking water from a municipal water plant contaminated with *Cryptosporidium*. *Cryptosporidium* sp. has long been recognized as a pathogen of farm and domesticated animals, but was not recognized as a human pathogen until 1976. The organism is a protozoan parasite transmitted by ingestion of oocysts that have been excreted in the feces of infected humans or animals. Several species have been described, with *C. parvum* being the predominant pathogen in humans. Transmission can occur through person-to-person or animal-to-person contact, or ingestion of fecally contaminated food or water. Because *Cryptosporidium* oocysts are highly resistant to chemical disinfectants used to treat drinking water, physical removal by filtration is an important component of the municipal water treatment process.

In 2002, there were 515 cases of cryptosporidiosis reported in Wisconsin. In immunocompetent individuals, cryptosporidiosis manifests itself with profuse watery diarrhea, cramps, nausea, and anorexia, lasting 10-15 days. Relapses occur in up to 20% of patients. In immunocompromised patients, protracted disease may occur. In HIV-infected persons, cryptosporidiosis lasting more than 1 month is part of the CDC's case definition of AIDS.

Diagnosis of cryptosporidiosis has progressed from histologic identification in intestinal biopsies to microscopic examination of fecal specimens for oocysts, enzyme immunoassays (EIA) for parasite antigens, and nucleic acid amplification assays. At WSLH, the FWP utilizes several methods for the detection of *Cryptosporidium*. For microscopic examination of fecal specimens, microbiologists use special stains such as hot safranin, a modified acid-fast stain, or a direct fluo-

rescent antibody stain to detect oocysts. To increase sensitivity, FWP is in the process of developing a real-time PCR assay for the detection of *Cryptosporidium* nucleic acid in fecal specimens. In some clinical laboratories, EIAs for *Cryptosporidium parvum* and *Giardia lamblia* constitute the routine ova and parasite screen. Rapid membrane EIAs are being used as screening tests for cryptosporidiosis in many clinics and small laboratories. There have been documented problems with false-positive results using these assays, and the WDPH and WSLH have recommended that positive membrane EIA results be confirmed using an alternative non-EIA method.

## THE FUTURE OF ENTERIC PATHOGEN CONTROL

Wisconsin will continue to see the emergence of new enteric pathogens with the increasing global economy and large scale methods of food production. There is a need to enhance food safety through the development of global surveillance networks and the development of standardized molecular detection and sub-typing techniques that are rapid and can be implemented in state and local public health laboratories. At the state level in Wisconsin, all clinical laboratories are encouraged to submit enteric bacterial pathogen isolates to WSLH for appropriate identification, sub-typing, PFGE, and antimicrobial resistance testing, so that WSLH can perform thorough surveillance and monitor trends in antimicrobial resistance. In addition, efforts must be made to reduce the emergence of drug-resistant bacterial enteric pathogens through more prudent use of antibiotics in agriculture and human medicine. These measures will require continued partnerships and cooperation among clinical laboratories, local public health officials and laboratories, the WDPH, WSLH, food producers, and clinicians to ensure the safety of our food and water supply in Wisconsin.

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