Researchers Study Microbial Threats to Shellfish Safety

Shellfish can remind us of treasured times—going clam digging with the family, slurping down oysters on the half shell at a raw bar with friends, or shucking them yourself in the hopes of finding a pearl. Unfortunately, eating them is often discouraged today because of environmental contamination.

In hopes of reversing this trend, scientists at the Microbial Safety of Aquaculture Products Center of Excellence in Dover, Delaware, are conducting research to provide safer shellfish. A field location of the ARS Eastern Regional Research Center's Microbial Food Safety Research Unit in Wyndmoor, Pennsylvania, the Center of Excellence is a partnership between ARS and Delaware State University, one of the 1890's Historically Black Colleges and Universities. ARS's Centers of Excellence are intended to foster complementary research on problems of national and regional concerns and to enhance cooperative research. Established in 1999 on the university's campus, the Dover worksite is the only ARS laboratory studying bacterial and viral safety of shellfish.

According to Gary P. Richards, a microbiologist and the center's lead scientist, shellfish are filter feeders, so they concentrate pollutants, including human pathogens, from seawater.

"The laboratory focuses on developing rapid, cost-effective, and practical methods to detect microorganisms in oysters, clams, and mussels and is evaluating processing strategies to eliminate these potentially harmful pathogens," Richards says.

Oysters, clams, and mussels are considered aquaculture species because of the amount of management that goes into maintaining productive molluscan shellfish beds. These shellfish are a concern to Richards and his colleague, microbiologist David H. Kingsley, because bacterial and viral pathogens can become concentrated within edible shellfish tissues. Many types of viral and bacterial pathogens that grow in the gut of infected people may contaminate water and food. Shellfish live along the shore, where they are subject to contamination with pathogens from improperly treated municipal waste, leaking septic systems, floodwaters, runoff, or overboard discharge of boat wastes. They may also become contaminated by unsanitized hands and surfaces at harvesting, processing, and preparation facilities.

From a food-safety standpoint, three groups of pathogens are of greatest concern to the molluscan shellfish trade: The noroviruses (formerly known as the Norwalk-like viruses), hepatitis A virus, and *Vibrio* bacteria.

Noroviruses, the leading cause of nonbacterial gastrointestinal illness in the United States, have gained recent notoriety because of several outbreaks on cruise ships. There are an estimated 9.2 million cases of norovirus infection caused by food in the United States annually. Norovirus illness comes on rapidly, lasts only a few days, and is rarely life threatening.

The second agent is hepatitis A virus. It is far less prevalent than norovirus—about 23,000 cases of hepatitis A are reported each year in the United States. But this virus infects the liver and can be more serious than norovirus infection. Although many illnesses are mild and asymptomatic, some require hospitalization or lead to death. Contamination of shellfish beds by direct exposure to human fecal wastes can readily lead to hepatitis A and norovirus infections.

The third group of human pathogens are bacteria of the genus *Vibrio*. Perhaps the best-known *Vibrio* infection is cholera, which sickens many people in underdeveloped countries through contaminated food and water. In the United States, *V. cholerae* is not a problem, but two other vibrios are of concern: *V. vulnificus* and *V. parahaemolyticus*. These bacteria are naturally found in shellfish and seawater, particularly when water temperatures are warm.

> Mortality rates for immunocompromised people infected with *Vibrio vulnificus* exceed 50 percent.

"Consumption of *V. vulnificus*-contaminated oysters is not a problem for healthy individuals," says Richards, "but that's not the case for sick, elderly, or immunocompromised people, especially those with liver disease or diabetes. These groups should avoid eating raw shellfish because of the widespread presence of *V. vulnificus* in the marine environment."

Mortality rates for those who acquire a *V. vulnificus* infection exceed 50 percent, with rapid disease onset and death often within 3-4 days. This bacterium is also a flesh-eating organism, which can produce major disfigurement in those who survive infection.

The other *Vibrio* pathogen found in the United States is *V. parahaemolyticus,* which causes a gastrointestinal illness that is generally not life threatening. Illnesses from *V. parahaemolyticus* have resulted in the closure of shellfish beds on the Atlantic, Pacific, and Gulf coasts of the United States and have led to major economic hardships for the shellfish industry.

Bacterial Virulence Factors

Deaths from *V. vulnificus* continue to occur among immunocompromised oyster consumers. To better understand how these and related bacteria invade the human host, Richards focused

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John Ewart (left), an aquaculture/fisheries specialist (University of Delaware), and ARS microbiologist Gary Richards examine freshly harvested oysters on board the Center for the Inland Bays' work boat before transport to the laboratory.

collaboratively with the Haskin Shellfish Research Laboratory, Rutgers University, in New Jersey to detect vibrios in oysters and seawater.

Virus Methods Development

Recently, Richards, working with the Centers for Disease Control and Prevention (CDC), developed a rapid method to detect a broad range of noroviruses. He combined a technique known as real-time reverse transcription-polymerase chain reaction (RT-PCR) with universal primers developed by CDC and was able to detect most norovirus types circulating in the world today.

According to Richards, "This method should be particularly useful to clinical, environmental, and food-testing laboratories, because, for the first time, analysts will be able to rapidly test for a wide spectrum of noroviruses in a single reaction tube."

Other recent research in this area involved detection of noroviruses in stools of individuals who had no symptoms of illness. The results suggest that healthcare workers and food handlers could unknowingly spread noroviruses in the workplace, and highlight the importance of good hygienic practices, particularly handwashing, to reduce the threat of food contamination and enteric virus illness.

Kingsley and Richards have also developed a way to extract viral RNA from shellfish. This method permits relatively rapid purification of hepatitis A virus and norovirus genetic material from within shellfish tissues. To detect the virus's genetic material, RT-PCR is then used to amplify the viral RNA. This extraction method is being evaluated by state, federal, and foreign laboratories to measure its performance, determine its cost-effectiveness, and consider it for possible adoption in virus-testing programs.

on identifying *Vibrio* enzymes that may enhance bacterial invasiveness. Leading a research group involving scientists from Delaware State University and the National Institutes of Health, Richards recently discovered and characterized an enzyme in *V. vulnificus* and identified it as phosphoglucose isomerase with a novel lysyl aminopeptidase activity.

The presence of this enzyme activity signifies a potential mechanism that may help the spread of *Vibrio.* He also detected the enzyme in virtually all species of *Vibrio* tested to date, but not in non-*Vibrio* pathogens. The enzyme is capable of metabolizing substances found in human tissues and the bloodstream. Such metabolism produces peptides that act on the blood vessels. These could account for the low blood pressure and rapid spread of *V. vulnificus*—a hallmark of *Vibrio* infection in humans.

Richards also developed a quick and simple enzyme-based assay that will allow vibrios to be readily detected in food, water, and clinical samples. This assay is being evaluated

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In studies to measure virus uptake and depletion rates, technician Gloria Meade inoculates a tank of oysters with hepatitis A virus.

Intervention Strategies

Some shellfish consumers prefer to eat shellfish raw or only lightly cooked, so the shellfish industry is interested in methods that can inactivate pathogens in their products without cooking. Kingsley is studying a way to sanitize raw shellfish and other virus-contaminated foods by using high-pressure processing (HPP), in which foods are subjected to extremely high pressure. The advantage of this technology is that no heat or chemicals are involved, permitting shellfish and other foods to retain their raw, uncooked flavor and character.

"HPP is being used commercially, for example, to pasteurize fruit juices in Japan and to treat sliced deli meats in Spain," Kingsley says.

In the United States, some in the oyster industry are already using HPP to facilitate shucking, eliminate *Vibrio* contamination, and extend product shelf life. These factors make HPP desirable to the processor and consumer, but the initial costs of the HPP units have prevented their widespread use.

Kingsley, working collaboratively with researchers from the U.S. Food and Drug Administration, tested the ability of HPP to inactivate hepatitis A virus from oysters. They found that 1-minute treatments of oysters greatly reduced hepatitis A virus populations.

Since it is not currently possible to replicate norovirus in the laboratory, Kingsley and Richards, in collaboration with researchers at the University of Delaware, used feline calicivirus, a cat virus that is genetically related to norovirus, to demonstrate that noroviruses may be sensitive to HPP. Kingsle hopes to find a way to directly study norovirus response to HPP in the future. Shellfish processors may be more willing to invest the capital needed to perform HPP once it's been conclusively shown to inactivate norovirus.

"Our research is of direct benefit to state and federal regulatory agencies who can use the improved methods and to the industry and seafood processors who can use new and innovative processing strategies to reduce contamination in seafoods," Richards says. "We will continue to evaluate these methods and seek partners to help validate them as we pursue new ways to enhance seafood safety."—By **Jim Core,** ARS.

This research is part of Food Safety (Animal and Plant Products), an ARS National Program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

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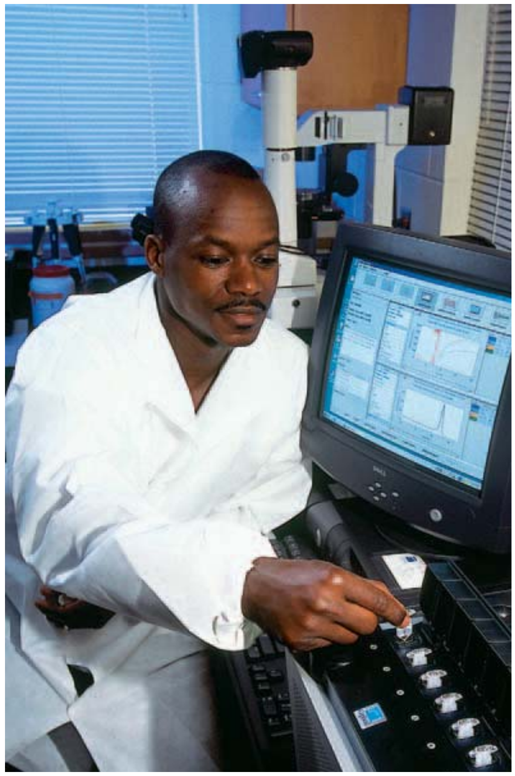

Technician Michael Watson loads shellfish extracts into a real-time RT-PCR machine to detect the presence of norovirus RNA.

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At the ARS Microbial Safety of Aquaculture Products Center of Excellence, microbiologist Gary Richards injects *Vibrio* extract into a chromatograph to isolate enzymes that may influence bacterial invasiveness.