

Emerging Pathogens

Detecting waterborne microsporidia

By Susan McGinley

Water teems with millions of organisms most of us will never see, and some we wouldn't want to swallow, either. Good water quality depends on accurate testing and filtering methods to identify and remove pathogens, but sometimes a disease organism is so small that current testing methods don't pick it up. Then the pathogen becomes a floating time bomb, multiplying to a critical mass that will start making people sick.

Often the first to succumb are those with compromised immune systems—people with cancer who are undergoing chemotherapy, or AIDS patients. That was the case with microsporidia (see sidebar). No one had really paid much attention to this group of protozoan parasites until 30-40% of AIDS patients began developing microsporidial infections.

That caught the attention of two scientists in particular at the University of Arizona, who wondered what was going on.

"One of the things we do is look at emerging pathogens," says Chuck Gerba, an environmental microbiologist in the Department of Soil, Water and Environmental Sciences. "Here's a thing suddenly showing up in AIDS patients and it's widespread. We start looking at an environmental cause."

Two years ago Gerba and Ian Pepper, director of the university's Water Quality Center, began working on ways to identify the pathogen, because no detection methods had yet been developed. Scot Dowd, one of their Ph.D. candidates, developed a method for detecting microsporidia in the feces of

AIDS patients in 1997. Pepper and Gerba then needed to figure out how to find it in water, an ongoing strategy.

"It's one thing to detect it in a clinical sample and quite another to detect in an environmental sample," Pepper says. The trick was, and still is, to figure out how many microsporidia lurk in large bodies of water. Currently drinking water utilities don't monitor the water supply for microsporidia, and have no means for extracting the organism from the water.

Treating it as "an organized detective game," as Gerba says, they began by looking for a pattern. Was it waterborne, was it alive, how easily could it be killed through disinfection, and how well could it survive? These were things drinking water utilities need to know about a pathogen. In fact, this research is sponsored by the American Waterworks Association Research Foundation, the U.S. Environmental Protection Agency, sanitation districts, and even Amway.

The screening method the researchers devised for waterborne microsporidia involved nucleic acids—DNA.

"Just imagine you have one pathogen in an environmental sample surrounded by 100 million other pathogenic microorganisms," Pepper explains. "There's a way to amplify the DNA specific to the pathogen, to the equivalent of a million pathogenic cells, that makes the detection much easier." They worked out two DNA isolation methods and combined them with an assay method called polymerase chain reaction (PCR) where targeted sequences of DNA are amplified, sequenced and then checked against a known database.

"The buzz phrase is computer-based sequence analysis," Pepper says. While other methods used for routine screening of pathogens have failed to catch microsporidia, the PCR method "showed the ability to detect less than ten spores in purified water concentrates," according to the researchers. Not only that, it was able to identify and differentiate different species (thousands exist) of microsporidia that infect both humans and non-humans.

Pepper and Gerba were the first to detect microsporidia in groundwater in Arizona. They have been testing surface water samples from locations throughout the state, and some from California. So far, 50% of them have come back positive for human pathogenic microsporidia. The researchers add that it was detected first in Arizona only because that's where they started testing for it; other states have it, too.

The scientists maintain that more research is needed to determine how widespread human pathogenic microsporidia are in the water supply. A drinking water outbreak of microsporidiosis in France was just reported in the *Journal of Infectious Diseases*. Gerba and Pepper wonder about future outbreaks, and offer their detection methods as a way of avoiding them.

"Half of the time we cannot identify the agent causing waterborne disease. There are many pathogens out there to be discovered," Pepper says. "Every now and then you can fish an emerging pathogen out of that black box." ♦

Microsporidia

Microsporidia are protozoan parasites that infect animals, fish, insects and humans around the world, causing a wide variety of infections in the respiratory, urinary and gastrointestinal tracts. The bacteria-sized microsporidia are difficult to detect. The occurrence of microsporidial infections in humans was not well documented until AIDS patients began having problems with them. Currently 30-40% of AIDS patients suffer microsporidial attacks on the liver, lungs, and other organs throughout the body. Transplant recipients who get it can suffer from chronic diarrhea, vomiting, dyspepsia, and weight loss. In those with normal immune systems, microsporidia usually cause milder illnesses,

such as traveler's diarrhea, although corneal infections and other conditions are possible.

Microsporidia were first identified at the turn of the 20th century, but until recently no one had considered the environment as a source for infection of humans or other animals by these protozoa. The potential importance of these infectious agents, as well as the relative lack of suitable methods for their detection, drew the interest of scientists in the UA College of Agriculture and Life Sciences. Ian Pepper and Charles Gerba developed techniques for detection of microsporidia and used them to detect these parasites in groundwater in Arizona.