Cracking the Hard Cases

A "mystery virus" begins killing residents of a small village in Central America. Thousands of people become ill in Milwaukee, Wisconsin, from an unknown contaminant in the drinking water. A leading medical research facility asks for help in checking the spread of disease-causing ticks. Military personnel need new weapons—not against human enemies—but against winged purveyors of malaria and dengue fever.

No, these aren't the ingredients for a new international fiction thriller—they're all actual incidents in which the Agricultural Research Service, chief research agency of the U.S. Department of Agriculture, has put its world-class scientific expertise to work for broad public good.

When 403,000 residents of Milwaukee, Wisconsin, became ill in 1993 from an unknown cause, the epidemic was front-page news nationwide. The culprit was soon unmasked: *Cryptosporidium parvum,* single-celled parasites measuring at most about 0.0002 inch, had contaminated the city's water supply.

But identifying the troublemaker wasn't enough. With one out of every two Milwaukee citizens sickened, city leaders and others wanted to know what had happened, why, and how to prevent it from ever happening again.

The American Water Works Association (AWWA), a national group representing more than 400 municipal water treatment facilities, came to USDA's Working Group on Water Quality for assistance. AWWA is greatly concerned with water quality and safety—especially with respect to cryptosporidium. The water quality working group knew firsthand about one prime source of information on cryptosporidium: ARS parasitologist Ronald Fayer.

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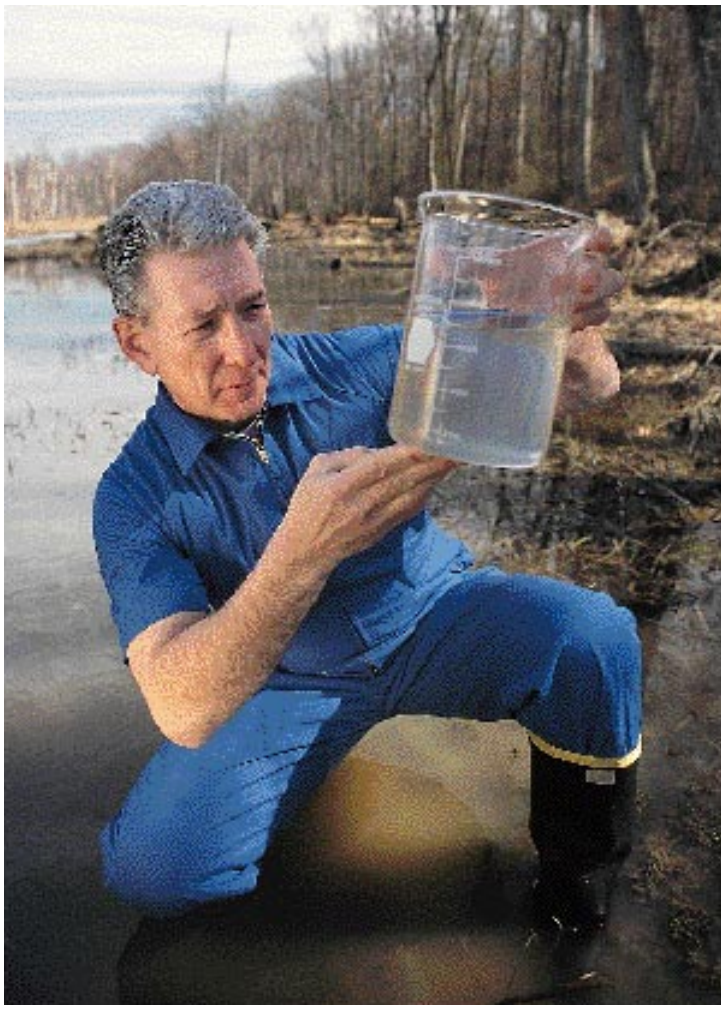

Parasitologist Ronald Fayer leads a project to determine the sources of cryptosporidium in surface waters and develop techniques for accurate detection of these organisms that infect humans, livestock, and wild animals. (K7269-1)

"Cryptosporidium has probably been around for many millennia," explains Fayer, who is with the ARS Immunology and Disease Resistance Laboratory at Beltsville, Maryland.

"It was first named around the turn of this century, but it remained relatively obscure until the increase in Acquired Immune Deficiency Syndrome—AIDS—in the 1980's. Since then, there have been about 2,000 articles on this subject in scientific journals alone."

A healthy human who becomes infected with cryptosporidium may suffer symptoms ranging from diarrhea and dehydration to nausea, vomiting, abdominal pain, fatigue, and weakness. But a person whose immune system is somehow compromised, by AIDS or recent chemotherapy, for example, can also suffer damage to the liver, pancreas, or lungs.

Fayer notes that one University of Texas study showed humans can become infected by swallowing as few as 30 oocysts, the egglike stage of the parasite that is excreted in feces of infected people and animals. These oocysts are in turn infectious to people and other animals and can contaminate a water supply.

To make matters worse, cryptosporidium appears to be virtually everywhere. The parasite has been found on every continent and in 94 countries. Even more chilling, in one study of surface water near treatment plants at 66 sites in 14 states and one Canadian province, researchers found the parasite in raw water at 87 percent of sites—and in the treated water at 39 percent of sites.

In light of these alarming facts, the nation's waterworks operators needed a crash course in cryptosporidium. Ron Fayer supplied it.

"In May of 1995, we produced 1,000 copies of a 25-minute video about cryptosporidium that's been distributed to major water treatment facilities throughout the country," notes Fayer. "Many university departments of public health, microbiology, and veterinary medicine have copies, as do medical schools and state health departments. It's also gone to other countries, such as Mexico and Australia."

Fayer's video explains the life cycle of the parasite, its impact on humans, and methods for combating cryptosporidium, including freezing water for 24 hours or heating it to 162°F for 1 minute.

Fayer is also USDA's representative on the Working Group on Waterborne Cryptosporidium organized by the Department of Health and Human Services' Centers for Disease Control (CDC). He is a member of a cryptosporidium task force that's compiling a report on the status of analytical environmental methods for cryptosporidium to be used by the Environmental Protection Agency in deciding how water treatment facilities will screen for the parasite.

"Cryptosporidium is a problem that demands serious attention," Fayer emphasizes. "Everyone's heard about the Milwaukee outbreak, but there have been several others in recent years that didn't get as much attention. In Carroll County, Georgia, in 1987, 13,000 people out of a population of 39,000 were affected. Outbreaks have also occurred in Texas, New Mexico, Pennsylvania, and Oregon since the mid-1980's.

"There was a substantial outbreak in Clark County, Nevada, in 1995," says Fayer, "but the source of infection was not definitively established.

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Veterinary medical officer Carole Bolin examines a guinea pig for general condition and pulmonary health. These animals are being used to understand the pulmonary bleeding seen in the Nicaraguan outbreak of leptospirosis. (K7288-1)

They have a state-of-the-art water treatment plant with an excellent history and a water intake that is more than 100 feet below the surface of Lake Mead. If that plant—which operates better than many others in the country—was affected, we have a real dilemma to resolve."

Leptospirosis

A disease problem of a different sort landed on ARS veterinary medical officer Carole A. Bolin's desk in November 1995.

Bolin heads the Zoonotic Diseases Research Unit at the agency's National Animal Disease Center (NADC) at Ames, Iowa. Diseases that are communicable from animals to humans under natural conditions are said to be zoonotic. In her work, Bolin had often discussed diagnostic work on animal diseases with other federal agencies, such as the CDC in Atlanta, Georgia.

But when the CDC called Bolin on November 6, the questions raised were more than academic. Three people had died at the Achuapa Health Center in the State of León in Nicaragua. The people had suffered from profuse pulmonary hemorrhaging, initially raising fears of a pestilence similar to the Ebola virus that had ravaged Zaire during the summer.

By the time the CDC contacted Bolin, they had ruled out Ebola and identified the problem as leptospirosis, a disease found worldwide that affects domesticated livestock, wildlife, and humans. Now they hoped to draw on the NADC's widely recognized expertise in research and diagnosis of animal diseases such as leptospirosis to pinpoint the strain involved.

"There are more than 200 different strains of bacteria that can cause leptospirosis," Bolin explains. "Humans can become infected with the bacterium through indirect or direct exposure to infected urine, often through contaminated soil or water. Some animal species can be a host to several different bacterial strains. Identifying the specific strain of the bacterium involved is crucial to stopping the spread of an outbreak such as occurred in Nicaragua."

At the Ames facility, Bolin and ARS molecular biologist Richard L. Zuerner have developed a new polymerase chain reaction (PCR) test that can identify exactly which strain of leptospirosis can be found in each host animal species. ARS is now in the process of establishing a cooperative research agreement with the CDC to support its future epidemiological investigations of the disease in humans and animals.

"We're using our new PCR diagnostic test to identify the specific

subtype of the bacterium that might have been the source of the Nicaraguan outbreak," Bolin says. "This new test lets us identify the subtype in a few days, rather than weeks or months. The ability to identify the specific subtype is invaluable to researchers performing epidemiological studies.

"With the PCR method," says Bolin, "we have the ability to pinpoint whether the infection was the result of transmission of the bacterium from cattle to humans, dogs to humans, or rats to humans. The major part of squelching the outbreak in Nicaragua is to eliminate the source of bacterial infection."

Ticks Bearing Diseases

John F. Carroll is interested in disease sources of a different kind—ticks. An entomologist at ARS' Parasite Biology and Epidemiology Laboratory in Beltsville, Maryland, Carroll began studies in 1990 with fellow ARS entomologist Edward T. Schmidtmann on the biology and control of *Ixodes scapularis*. These are the ticks that carry *Borrelia burgdorferi*, the organism that causes Lyme disease in humans.

"Ed and I looked at the distribution of ticks associated with horse pastures on Maryland's Eastern Shore," recalls Carroll. "The woods-pasture situation is a good model for high-risk suburban homesites. In recent years, the ticks have spread into this area—or at least have become much more noticeable since the 1970's."

So, unfortunately, have the cases of Lyme disease.

In 1994, the CDC reported 13,083 cases of Lyme disease in 44 states. Northeast and Middle Atlantic States generally account for about 80 percent of that total, compared with 10 to 15 percent for the upper Midwest and 5 to 6 percent in California.

Lyme disease in humans results in arthritic, cardiac, and neurologic problems. Although the disease can be treated with antibiotics if detected early, an established case is more difficult to combat, Carroll says.

"The tick that carries the disease organism is linked closely to the white-tailed deer population," he notes. "You apparently need the deer and white-footed mice to maintain a large population of these ticks. The deer population in the eastern United States has increased phenomenally since World War II because of regulated hunting and changes in land use. And when the woodlands are interspersed with farms where they obtain better feed, the deer tend to produce more twins."

"ARS researchers in the 1940's found that ticks would crawl to cloth that had the scent of dogs on it," Carroll says.

"Also, deer have glands on their legs that produce pheromones to mark their territory as they walk through the brush. We put some secretions from these glands on glass rods and found that ticks over a 24-hour period would remain on the secretions of the tarsal gland, which is halfway up the deer's leg. Ticks also responded to the secretions from a gland between the deer's toes."

As Carroll and Schmidtmann studied distribution patterns and movements of the ticks, they developed a tick-collecting device for field use. In the summer of 1994, research-

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A tick sweep device helps entomologist John Carroll estimate the tick population in brushy habitat. (K7292-5) Inset: Black-legged ticks collected during a tick sweep can carry the pathogen that causes Lyme disease. (K7293-1)

ers from the Walter Reed Army Institute of Research in Washington, D.C., decided to draw on Carroll and Schmidtmann's tick-collecting expertise for surveillance of medically important arthropods.

"The researchers from Walter Reed were evaluating methods for collecting host-seeking ticks and were impressed with our tick sweep," Carroll recalls.

"This is a device we developed in 1992—a long, angled metal handle with a square of rubberized flannel cloth that you could sweep through the brush to pick up ticks. The tick sweep can better access immature ticks in dense vegetation. Another nice thing about this device is that the adult ticks are big enough that you can see them as soon as they get on the cloth, so you know where you picked them up."

Mosquitoes are a worthy opponent for any researcher. They carry dengue fever, filariasis, yellow fever, and malaria.

Current models are constructed of lightweight aluminum by Paul Balsey, an ARS instrument maker at Beltsville.

In field trials, the Walter Reed researchers were pleased with the performance of the ARS tick sweep as a tool for checking pest populations at prospective maneuver sites or bases for U.S. military personnel.

"If military entomologists need to go to a particular place and find what ticks are there—or if someone contracts a disease that could be tick-related—they can use this device to check the tick population in a specific spot," Carroll concludes. "Now other people are interested in using these devices, as well."

Repelling Skeeters and Other Pests

ARS chemist Albert B. DeMilo also helps protect U.S. military personnel from the dangers of the wild. At the agency's Insect Chemical Ecology Laboratory at Beltsville, Maryland, DeMilo conducts an ongoing search for just the right repellent chemicals to keep disease-bearing pests at bay, specifically mosquitoes.

"The Agricultural Research Service has had a longstanding memorandum of understanding with the Department of Defense for development of insect repellants," DeMilo notes. "We've been involved in that work going back to the early 1950's. Traditionally, we've supplied 95 percent of the compounds that are ultimately evaluated in the Defense Department's insect repellant development program."

Mosquitoes are a worthy opponent for any researcher. Various mosquito species carry dengue fever, filariasis, yellow fever, and malaria, to cite a few examples. DeMilo spends his days concocting possible new weapons against these winged marauders.

"There are several leads in the scientific literature that we can follow, looking at things that have worked in the past and developing analogs based on chemical structure and volatility," he explains.

"One compound we look at very closely is deet—diethyl toluamide—which has been on the scene for some 40 years. Deet is an excellent repellant, but we're looking for even better ones with enhanced activity—ones that perhaps repel insects that deet doesn't do much for. For example, deet doesn't repel malaria-transmitting mosquitoes as well as it repels those carrying other diseases.

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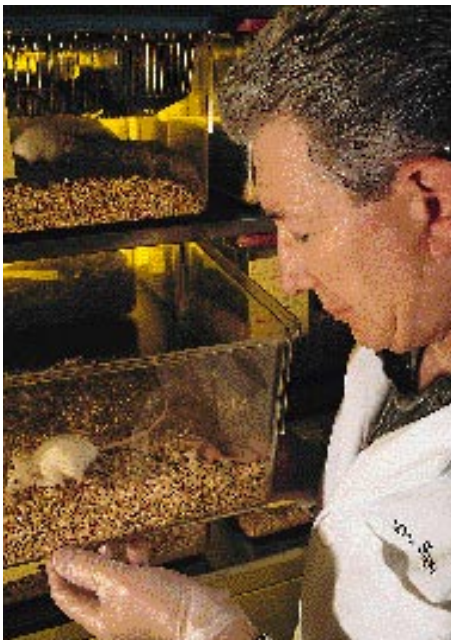

BALB/c, a special strain of lab mice being examined by parasitologist Ron Fayer, are providing unique insights into the functioning of cytokines protein secreted by T-cells that can prevent infection with cryptosporidium. (K7270-2)

"Besides that, we don't have a backup for deet," DeMilo points out. "And it must be noted that most of the repellants that were used in the past have been withdrawn from the market in the last 10 years because of environmental or health concerns, so we really need to plan ahead and devise alternatives."

DeMilo produces about 50 to 100 candidate compounds annually that go for preliminary screening to ARS' Medical and Veterinary Entomology Research Laboratory at Gainesville, Florida. If the prospective repellant performs well in the Gainesville tests, it graduates to toxicological testing at the U.S. Army Center for Health Promotion and Preventive Medicine at Aberdeen, Maryland; then, back to the Gainesville research

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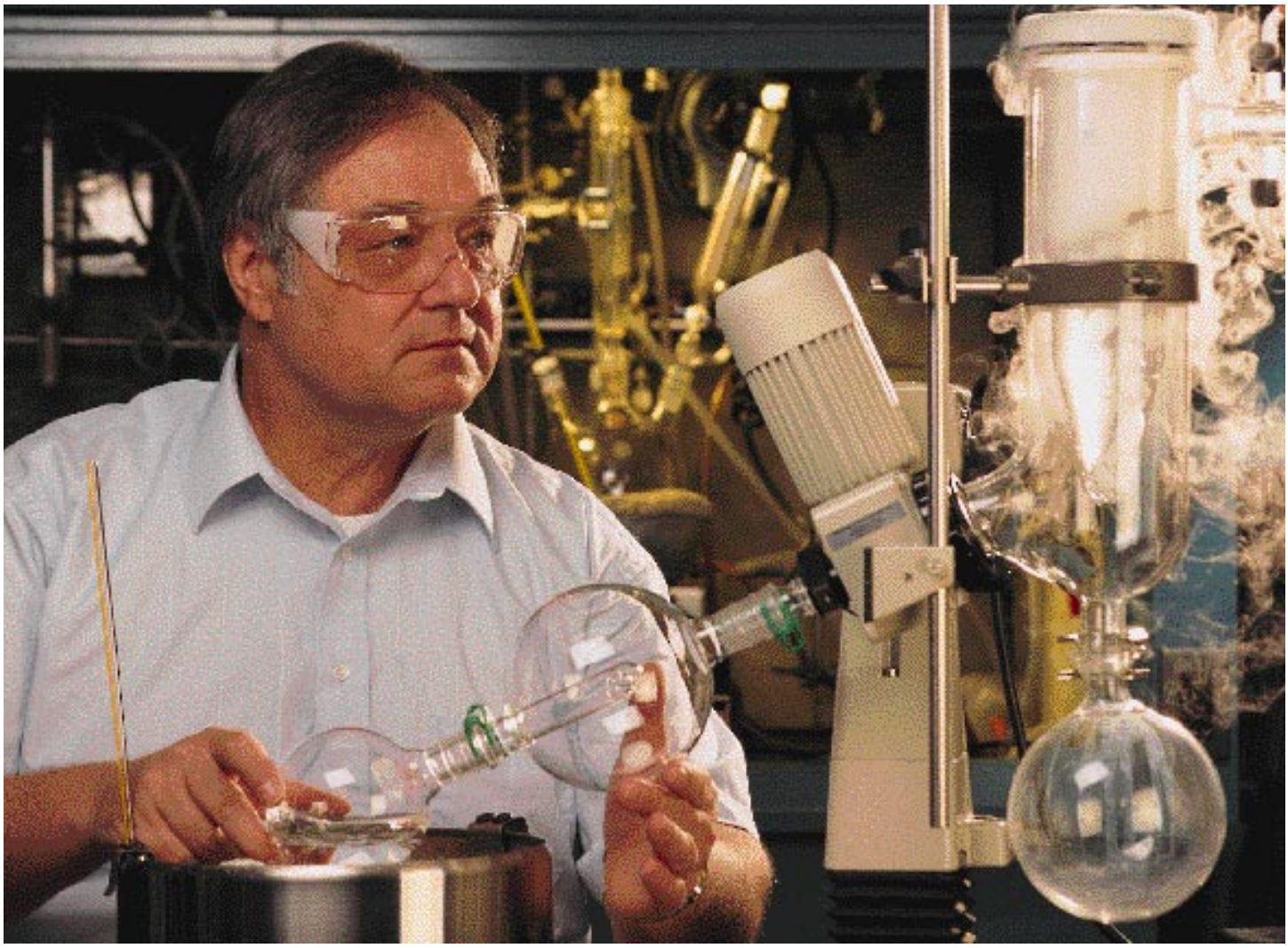

In his Beltsville (Maryland) lab, chemist Albert DeMilo synthesizes a possible insect repellant that will be sent to ARS' Gainesville, Florida, laboratory for tests and field evaluation. (K7294-1)

lab for more stringent tests on human volunteers.

"If it performs well on human skin and meets or exceeds the repellent performance of deet, it will go through a battery of field tests and then on to advanced toxicological tests back at Aberdeen," DeMilo says. "At that point, we have a compound that we might try to get companies interested in producing in volume, not only for military personnel, but also for the general public."

All told, the process could consume a full decade from DeMilo's initial brewing to completion of the advanced toxicological tests. How many survive the trip?

"At USDA, going back to the 1940's, we've looked at about 16,000 compounds," DeMilo says. "Only about a dozen of them have made it to the advanced toxicological testing stage. Even those few successes are worth the struggle," he says.

"It's been said that the U.S. military throughout history has actually lost more people to disease than to bullets, and a large percentage of those diseases were transmitted by arthropods," DeMilo says.

"The work that we do here in ARS could ultimately result in a product used by military personnel going into many situations where insects are likely to be a problem."—By **Sandy Miller Hays** and **Linda Cooke**, ARS.

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