Animal Studies Safeguard Food Supplies —and Human Health

Hundreds of different species of *Mycobacterium* are known to exist, and many of them have been infecting animals and humans—sometimes with deadly results—for thousands of years. Scientists at the Agricultural Research Service's National Animal Disease Center (NADC) in Ames, Iowa, are fighting back against two of its most damaging variants—Johne's disease and bovine tuberculosis (bovine TB).

M. avium subspecies *paratuberculosis* (MAP) is responsible for the onset of Johne's disease, which results in losses exceeding $200 million every year to the U.S. cattle industry. Experts believe that almost 70 percent of U.S. cattle herds are infected with MAP, and an animal can spread the disease soon after it is infected. But noticeable symptoms—including severe weight loss and diarrhea—can take up to 5 years to develop.

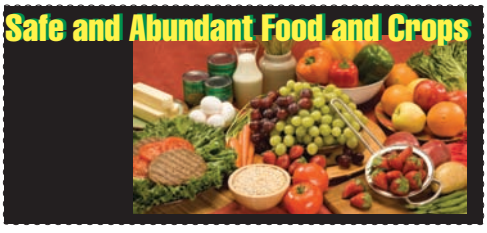
"MAP is like a stealth organism," says NADC microbiologist Judy Stabel. "It shelters in the host's white blood cells and stays at low levels until stress makes the disease apparent. And it's one of the hardest organisms to work with in the field."

"We need a better way to detect infected animals early on," adds NADC microbiologist John Bannantine.

Protein Prospecting

The MAP genome—which contains all its genes—has been sequenced, so researchers now have information about the different proteins that are made from those genes. Bannantine and colleagues took some of this genetic data and assembled an array of 96 proteins. They wanted to identify proteins that might be useful in confirming a diagnosis of Johne's disease or that might be targeted for therapeutic intervention.

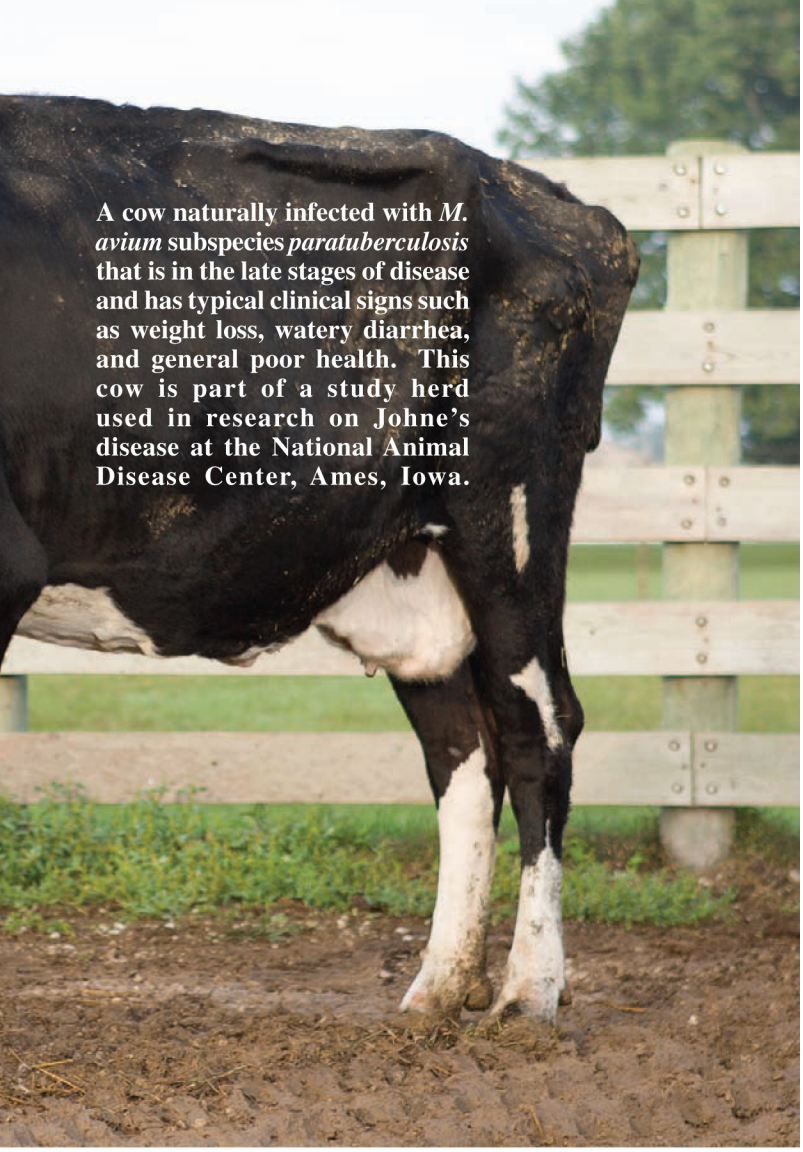
The researchers used the 96 proteins in the array to identify which ones prompted the most robust immune response from antibodies in the serum of infected cattle. They found three proteins that consistently drew the strongest attacks from serum antibodies—a level of immune response that clearly linked the three proteins with the onset of the disease. Bannantine believes that with additional work, these segments might provide crucial building blocks for development of a diagnostic tool for Johne's disease.



PEGGY GREB (D1441-1)

PEGGY GREB (D1442-1)

Microbiologist Judy Stabel bottle feeds young calves involved in a project to evaluate immune responses to a vaccine that protects against Johne's disease.



A cow naturally infected with *M. avium* subspecies *paratuberculosis* that is in the late stages of disease and has typical clinical signs such as weight loss, watery diarrhea, and general poor health. This cow is part of a study herd used in research on Johne's disease at the National Animal Disease Center, Ames, Iowa.

"This protein array is the only one like it in the world," Bannantine says. "Because we've been so careful in selecting the MAP proteins for our array, we're confident that the antibodies are responding to MAP proteins—not similar proteins produced by other *Mycobacterium* species."

Bannantine is also very pleased that their studies have cleared up another aspect of MAP infection.

"When an animal is first infected, there is a cell-mediated response to the bacterium," he says. "We thought that another type of immune response—the one that produces antibodies—developed much later. But in experimentally infected animals, we can use this array to detect exposure to MAP as early as 70 days after the animal is infected, much earlier than previously reported in field studies."

The next step is to determine whether these early-detected antigens are recognized by infected cattle in real-world dairy herds. "We also need to determine the extent of cross-reactivity these proteins have with other environmental mycobacteria, because one problem with current Johne's disease tests is the lack of specificity," Bannantine says.

Stabel has been studying more about the early stages of the cell-mediated response to MAP and finding ways to diagnose the disease in young animals. "We've found a way to use information about the cell-mediated response to detect MAP in naturally infected calves that are only 6 months old," she says. "When animals this young are diagnosed, then the producer can decide how best to respond—either by removing the animal from the herd or looking at other options."

Stabel has also helped evaluate animal models for MAP research and has concluded that a smaller ruminant model—like goats or sheep—shows promise. "These animals are slightly quicker to reach a clinical disease state," she says.

New Approaches to an Ancient Disease: Tuberculosis

The USDA Animal and Plant Health Inspection Service (APHIS) tests more than 1 million animals every year for bovine TB. A lot can ride on the outcome. For instance, from May 2002 through June 2004, 875,616 cows from 687 California herds were tested for bovine TB. As a result, 13,000 cows were culled.

During that time, dairy herd quarantines were enforced to ensure that contaminated milk from infected cows did not enter the human food supply and transmit the disease to people. This additional enforcement cost individual producers as much as $70,000 per month in lost income.

Finding effective diagnostics and vaccines for bovine TB is crucial for livestock and human health and producer profits. At NADC, around 80 white-tailed deer—some so tame that they try to steal food from the pockets of their caretakers—are key to this effort.

"White-tailed deer are a significant reservoir of bovine TB," says NADC veterinary medical officer Mitch Palmer. "When the deer jump fences and share livestock feed, they can contaminate the feed with TB bacteria. That's one way cattle herds become exposed to the disease and become infected."

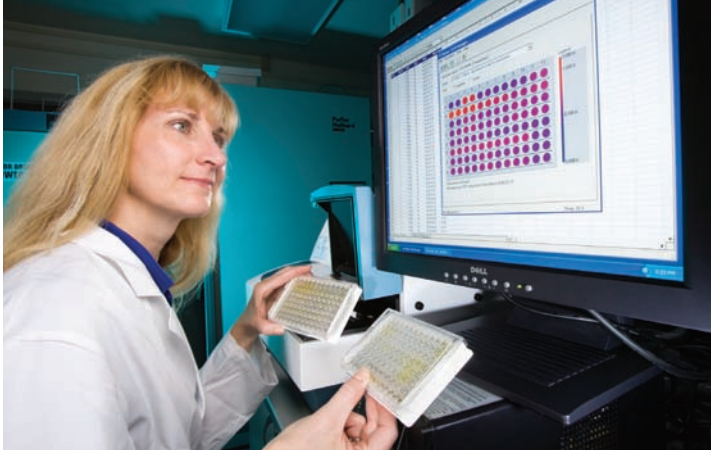
Vetting Vaccines

Because of the threat wild deer pose to domestic cattle, Palmer and his colleagues have been vaccinating white-tailed deer with the human TB vaccine *M. bovis* BCG. They want to see whether vaccination could be part of a successful effort to control the disease in wildlife.

"What we've found is that the vaccine is effective in decreasing the severity of the disease and that oral vaccination appears to be as effective as subcutaneous vaccination," Palmer says. "But we have some safety concerns. Because it's a live vaccine that stays in body tissues for up to 250 days, bacteria in the vaccine that prompt the protective immune response might also infect humans who consume venison from vaccinated deer. And vaccinated deer can shed the vaccine and expose other animals to the disease."

The vaccine research has yielded other significant findings as well—including results that could affect human health.

NADC molecular biologist Tyler Thacker led studies



PEGGY GREB (D1440-1)

Judy Stabel evaluates the results of the interferon-gamma blood test to diagnose cows infected with *M. avium* subspecies *paratuberculosis*.

indicating that in white-tailed deer, levels of interferon gamma (IFN-gamma)—a protein that is critical in the immune response to bovine TB infection—increase as the severity of the infection increases. This finding countered previous studies suggesting that levels of IFN-gamma rose when the immune system of a vaccinated animal was successfully fighting the pathogen.

Other results in the study suggested that elevated levels of IL-4, another immune protein, could be found in animals with lower infection levels. This also contradicted research with other animal species suggesting that IL-4 levels increase with disease severity.

"These results give us more accurate information about whether a trial vaccine is effective," Thacker says. "Vaccine testing is expensive, so it's helpful to have screening tools for interpreting results. Now that we know IFN-gamma is not a good predictor of vaccine efficacy, we can start looking for other indicators."

The NADC scientists have also been using neonatal calves to test human TB vaccines. This approach is cheaper and safer to use than testing in nonhuman primates.

"We've devised a very effective method for inoculating neonatal calves with TB by using an aerosol challenge," says NADC veterinary medical officer Ray Waters. "This gives us new options for testing human TB vaccines on animals with immune responses that closely resemble the human immune response."

In addition, the most common time for administering the vaccine to humans is soon after birth—timing that is mimicked when neonatal calves are used for tests. The neonatal calf tests are currently the most effective bridge between testing in mice and nonhuman primates. Results have so far been very similar to the findings in nonhuman primates.

Vaccines or Diagnostics—Which Comes First?

"Vaccine development and diagnostic development are intertwined," Thacker observes. "If we find indicators that predict immunity, then maybe we can refine those indicators and use them for diagnostic testing as well."

Thacker, Palmer, and Waters are working on improving bovine TB tests for cattle. "We have serum-based tests that are not approved for use in the TB eradication program yet," Palmer says. "But they are showing some potential for testing samples in the field, where time or temperature could affect the stability of the blood samples."

PEGGY GREB (D1445-1)

Two of about 60 deer maintained at NADC in Ames, Iowa. The unique research herd is used to study vaccines for prevention of tuberculosis in white-tailed deer.

PEGGY GREB (D1447-1)

Microbiologist John Bannantine loads amylose resin affinity columns to purify recombinant *M. avium* subspecies *paratuberculosis* proteins produced in *Escherichia coli.*

PEGGY GREB (D1446-1)

Veterinary medical officers Ray Waters (left) and Mitch Palmer (right) prepare to collect blood to be used in developing improved tests for tuberculosis in cattle.

Meanwhile, producers and APHIS staff often use a commercial blood-based test called "Bovigam" to confirm a preliminary positive skin test diagnosis of bovine TB.

"Standardizing the protocol for using Bovigam is still a work in progress," says Waters. "Labs have been running different test protocols, so we're working on establishing standard measures for processing blood samples. For instance, we've found that using vessel plates to test samples is just as effective as using tubes, and with vessel plates we don't need to use as much of the reagent to obtain accurate results. We also need to determine optimal schedules for collecting and processing samples—and we need to determine a safe temperature range for maintaining those samples before they reach the lab."

Into the Wild

Animal health depends as much on meticulous fieldwork as it does on painstaking lab studies. So Waters, Palmer, and Thacker were part of a team that obtained blood samples from 760 wild deer and surveyed them for bovine TB, the largest such sampling to date. The team found that the blood tests accurately identified an infected animal about 70 percent of the time. On the other hand, the tests correctly identified a noninfected animal about 90 percent of the time.

Palmer explains, "With most tests of this type, there's typically a tradeoff between the accuracy of positive and negative results. We're continuing to work on this because we'd like to get both numbers in the high 80s or low 90s.

"We're approaching bovine TB research from a range of perspectives, but all of it helps us understand the immune response," Palmer continues. "When an animal is infected with TB, tissue destruction is caused by the host reacting to the TB pathogen. We want to understand what is causing the tissue destruction, which will help us find ways to treat the disease and intervene in the process of tissue destruction."—By **Ann Perry,** ARS.

This research is part of Animal Health, an ARS national program (#103) described on the World Wide Web at www.nps.ars.usda.gov.

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Rift Valley Fever

Rift Valley fever (RVF) was once confined to Africa—until cases were confirmed in Saudi Arabia and Yemen in 2000. These cases sparked concerns about the disease's ability to spread further. RVF has never reached the United States, but if it were to do so, researchers at the Arthropod-Borne Animal Diseases Research Laboratory (ABADRL) in Laramie, Wyoming, would be ready.

RVF is a potentially devastating zoonotic disease. Fortunately, ABADRL scientists are collaborating with colleagues in the United States and abroad to ensure that the nation will be prepared in the improbable event of a U.S. outbreak.

Under the leadership of microbiologist William Wilson, ABADRL scientists are pursuing RVF research with three main objectives: to determine which North American mosquito species are competent to transmit the RVF virus; to develop sensitive and specific diagnostic tools; and to develop and evaluate vaccines.

To meet the first objective, ABADRL scientists are investigating whether there is a vector genetic component to RVF transmission. Preliminary studies have examined which mosquito and midge species are more likely to act as virus vectors. The results suggest regional differences in the ability of mosquito populations to become infected and transmit the virus. To expand research in this area, ABADRL is increasing the size and scope of its existing mosquito colonies to include additional species.

The ABADRL scientists are working with the Canadian Food Inspection Agency and USDA's Animal and Plant Health Inspection Service to develop operator-safe, sensitive diagnostic tests for early detection. They are also cooperating with the U.S. Department of Homeland Security to evaluate the safety of potential commercial vaccines. ABADRL is collaborating with the Canadian agency to develop livestock challenge models to evaluate the efficacy of new vaccines as they are developed.

Such research ensures that the United States won't be caught off guard should an outbreak of RVF occur. The work also has immediate practical applications in countries currently affected by the disease.—By **Laura McGinnis,** ARS.