

# Johne's-Crohn's link gains ground

## KEY POINTS

- Studies recognize possible public health threat from MAP organism.
- Mycobacteria family also responsible for tuberculosis, leprosy.

By **SALLY SCHUFF**

**M**ORE refined tests for diagnosing Johne's disease in cattle — a disease that causes animal suffering and costs U.S. dairy producers more than \$200 million a year — appear to be on the way as the result of a newly completed genome map for the organism that causes the disease.

It is a development that will help battle the devastation of Johne's disease while also expected to advance knowledge about what scientists increasingly believe could be a public health threat from the organism, *Mycobacterium avium paratuberculosis* (MAP).

While the connection has yet to be proven, after years of research, scientists are, with increasing frequency, uncovering tantalizing circumstantial evidence that the organism that causes Johne's disease in cattle may play some role in Crohn's disease, a complex, chronic and disabling inflammatory bowel disease in humans. BOTH Johne's in cattle and Crohn's in humans are increasing worldwide in all industrialized countries, scientists reported. Several countries have initiated Johne's control programs.

For the first time, the newly reported genome map identifies gene sequences unique to MAP, a breakthrough that allows more specific tests for the disease and provides better targets for molecular testing, the researchers reported.

The work — led by Dr. Vivek Kapur, director of the University of Minnesota's Biomedical Genomic Center, with scientists from the University of Minnesota and the U.S. Department of Agriculture's National Animal Disease Center at Ames, Iowa — was completed through a USDA grant.

They sequenced the complete genome for MAP's K-10 clone, a clinical isolate of the disease-causing organism found in a dairy herd in Wisconsin in the mid-1970s. The work was reported Aug. 30 in *Proceedings of the National Academy of Sciences*.

## Basic prevention strategies for Johne's disease:

- Calves, lambs, kids, etc. should be born in clean environment.
- Reduce newborns' exposure to manure from adult animals by separation.
- Avoid manure contamination of feed and water sources.
- Use colostrum from Johne's-negative animals; do not pool colostrum.
- Avoid natural nursing and milk feeding when possible; feed artificial milk replacer or pasteurized milk instead.
- Thoroughly clean udder and teats before collecting colostrum.
- Till pastures contaminated with MAP, or graze using non-replacement feeder cattle.
- Identify all females in herd. Identify and remove or separate all positive animals.
- Cull or separate offspring of infected mothers as soon as possible to prevent spread.
- If purchasing herd additions, buy from low-risk herds.
- Work with veterinarian to develop a strategic plan for Johne's prevention and control.

Source: *Animal & Plant Health Inspection Service.*

The team already has developed a commercial test that will identify MAP in both fecal samples and milk.

## Studies raise alarm level

News of the completed MAP genome sequence came just as two other key studies heightened concern about the MAP organism's role in public health.

One study, conducted by the Marshfield Clinic in Marshfield, Wis., found viable MAP organisms in samples of pasteurized retail milk in California, Minnesota and Wisconsin — all among the nation's top five dairy states.

The second study, conducted by a University of Central Florida research team, was reported a year ago in the British medical journal *Lancet*. It reported the team's finding of viable MAP in the blood of 50% of the Crohn's disease patients sampled for the organism. The majority of these patients lived in Florida.

The Florida team, led by Dr. Saleh Naser, had earlier found MAP in the breast milk of three Crohn's patients, as reported in the *American Journal of Gastroenterology* in 2000.

Their research is continuing and is among a suite of projects on MAP, funded nationwide by the National Institutes of Health.

## Control stymied

Control of Johne's disease — a costly

disease that affects an estimated 22% of the nation's dairy herds and 8% of U.S. beef herds — has suffered in part, researchers believe, because it is hard to diagnose.

Mycobacterium are hard to detect and harder to kill. They differ from other bacteria in two key ways: "They survive very well in multiple environments. Secondly, when they infect a host, they know how to evade the immune system," one researcher explained.

Two devastating diseases known to humans — tuberculosis and leprosy — are caused by infectious agents from the mycobacteria family.

In the case of both tuberculosis and paratuberculosis, mycobacteria infect the host with the same method of action, Dr. Jay Ellingson, director of food safety services at the Marshfield Clinic, explained.

Both pathogens are "silent invaders," he said; once they invade their host, they can lie dormant for years, "waiting for a signal or a stress situation to cause active disease."

Cattle with Johne's disease suffer from chronic inflammation of the intestine, which leads to diarrhea, weight loss and death. Milk production and reproduction is lowered, and the infected animals are a danger to the herd.

Detecting the disease has been difficult up to now. Fecal cultures for MAP have been the "gold standard,"

but they can take up to 16 weeks to complete. Existing blood tests are not reliable until the disease is well advanced.

So far, such existing tests have not been helpful in screening subclinical animals for the disease or from eliminating infected animals from the herd in a routine control program.

Rapid diagnosis is important because once infected animals shed MAP in feces, the organisms can persist for some time in soil and water and infect new animals.

Even before the new test from Kapur's group, the Marshfield Clinic had developed test protocols with a turnaround time of 24 hours. One is based on a gene sequence of MAP first identified and patented several years ago by Ellingson, who was, at the time, a post-doctoral scientist at USDA's National Animal Disease Center.

### Next generation of tests

News of the advance in MAP genomics is welcome. The National Academy of Sciences (NAS) called for such research in a 2003 comprehensive study of the diagnosis and control of Johne's disease.

USDA and other agencies should "seize the opportunity presented by the completion of the MAP genome project to accelerate progress in Johne's disease research, diagnostic test improvement and vaccine development," the NAS report noted two years before the genome was completely mapped.

That recommendation was one of 25 aimed at studying the gaps in understanding Johne's disease and its possible connection to Crohn's disease.

Kapur's team believes completion of the new genome sequence for the MAP organism will "enable the development of new generations of diagnostic tests for bovine Johne's disease."

ANDX Inc., a company identified as a University of Minnesota start-up, is commercializing the first such molecular assay test used to identify MAP in fecal samples and milk. Kapur said the test has been accredited by USDA and is ready to be commercialized.

(The *Proceedings of the National Academy of Sciences* noted that the research team applied for patents on the DNA sequences jointly owned by the University of Minnesota and USDA that have been licensed to ANDX.)

Unlike previous tests relying on laboratory culturing from fecal samples that could take weeks, new tests will get results to herd owners much sooner, Kapur explained.

The test is aimed at giving

## Johne's Q & A

**Q:** How do animal's get Johne's disease?

**A:** **Johne's typically enters a herd or flock when a healthy-looking but infected animal is purchased.**

**Individual animals get infected by close contact with other infected animals that shed the bacterium in their manure.**

**Young animals often acquire infection by eating material contaminated with *Mycobacterium paratuberculosis*.**

**Q:** How can you prevent your animals from getting Johne's?

**A:** **Be as certain as possible that animals brought into the herd are not infected with *M. paratuberculosis*. Some type of test is available for every animal. Test-negative herds are the best sources of animals for purchase.**

**Q:** How do you test animals for Johne's?

**A:** **There are three common ways: culture of fecal samples, DNA probe on fecal samples and blood tests for antibodies to *M. paratuberculosis*.**

**Q:** How do you control Johne's?

**A:** **In principle, two strategies must be employed at the same time: (1) newborn animals must be born and raised in a clean environment and fed milk free of *M. paratuberculosis*, and (2) adult animals carrying the *M. paratuberculosis* infection must be identified and removed from the herd.**

*Source: Johne's Information Center, University of Wisconsin.*

veterinarians in the field a new tool to more rapidly identify infected animals and eliminate them from breeding herds.

"We recognize that MAP is an important economic disease for dairy production. The increasing incidence and prevalence of this disease is by no means a good sign. Our strategy is to identify and eliminate infected animals and break the transmission of the disease," Kapur told *Feedstuffs*.

Scientists around the nation and worldwide are sharing information on Johne's as part of a USDA Cooperative Research, Extension & Education National Research Initiative program, Kapur reported. He hopes future research leads to development of a vaccine that will enable protection of uninfected animals.

### Looking for MAP in milk

The new polymerase chain reaction-based test can also identify MAP in milk, Kapur told *Feedstuffs*. "Whatever we can do to mitigate MAP from being found in milk will go a long way toward improving milk quality."

When asked to comment on the association of MAP and Crohn's disease, Kapur said, "There is increasing evidence that MAP is present in a certain subset of Crohn's patients. It is identifiable in tissue or blood of those patients." However, he cautioned, proving that MAP causes Crohn's disease "has not yet been achieved, and proving it conclusively will be extraordinarily difficult."

So far, Kapur believes "an association" between MAP and Crohn's disease rather than "a causal relationship" has been proven. He describes Crohn's as a complex, autoimmune disease.

Other researchers noted that a single cause is difficult to identify because genetic and immunological differences in humans play a role in who gets or does not get Crohn's.

Despite the controversy, scientific literature is full of research questioning the possibility of a link between MAP and Crohn's.

In July 2004, Dr. Robert Greenstein of New York's Veteran's Administration and Dr. Michael Collins, University of Wisconsin professor of microbiology, asked openly, "Is (MAP) zoonotic?" Zoonotic diseases are infectious and spread from animals to humans.

Incorporating Marshfield Clinic's expertise in zoonotics, the clinic's physicians took part in a study published in the *Journal of Pediatrics* in 2003 that concluded that the pathogenesis of Crohn's disease and ulcerative colitis is believed to involve interplay of environmental factors acting on genetically susceptible people.

Crohn's was first reported to have similarity to animal paratuberculosis in 1913. It has confounded scientists' efforts to determine a cause or a cure ever since then.

In their *Lancet* letter, Greenstein and Collins noted that Crohn's disease, "which has no known cause, has remarkable clinical, epidemiological and pathological similarity to Johne's disease."

The authors said, "This similarity raises the reasonable concern, increasing among some veterinarians, governmental agencies and physicians, that MAP may indeed cause Crohn's disease."

### MAP found in patients

In a September 2004 article in *Lancet*,

Naser and his team reported finding MAP in the blood of 50% of patients they tested suffering from Crohn's disease. None of the controls were MAP positive.

Previously, Naser isolated the MAP organism from biopsy and surgical tissues from at least 87% of Crohn's patients tested in his lab. That compares to less than 25% from patients without the disease.

Naser, whose research team has investigated the MAP-Crohn's connection for 12 years, said finding MAP in the blood of patients and also in the breast milk of three Crohn's patients, with none found in seven normal controls "is very significant since human milk and blood are sterile."

"Since the MAP bacterium has been detected in the blood of Crohn's disease patients, it is an indication of the invasive capability of MAP infection in humans," Naser told *Feedstuffs*. "It is for sure an illustration of MAP systemic infection in humans. The significance of finding live MAP in human blood and milk illustrates a gross similarity with the disease picture in animals with Johne's disease."

Additionally, he reported, his team has completed blood tests on "more than 1,000 Crohn's patients for antibodies against MAP." More than "80% of the (Crohn's) patients had antibodies specific to MAP, and less than 25% of controls were positive," Naser said.

However, their research on cellular immunology indicated that some people have defective cells that normally would clear the pathogen from their bodies. Naser concluded, "In short, for somebody to develop Crohn's disease, the subject must be genetically or immunologically susceptible and exposed to MAP through contamination of food or drinks."

Fortunately, not everyone exposed to MAP is at risk of contracting disease.

# Time to be proactive

**C**ONGRESS has made a substantial investment in bovine paratuberculosis — commonly known as Johne's — a nasty cattle disease.

Dr. Michael Collins, a University of Wisconsin expert on the disease, pointed out that in the last six years, the U.S. has invested more than \$65 million in establishing a Johne's control program. The problem is that so far, many producers simply are not participating.

Earlier this year, the U.S. Department of Agriculture's National Animal Health Monitoring System reported that 11.2% of dairy herds it surveyed in 2002 were participating in Johne's disease certification, control or herd status programs. That was up from 0.9% in 1996 but still was a surprisingly low number.

Collins said, "The infrastructure is well in place to have a strong, reliable, scientifically based, national Johne's disease control program." He worried, though, that without more herd owner participation, USDA will have trouble justifying the program's continuation to Congress.

Hopefully, producers will find new enthusiasm for the program with new diagnostics (story, page 1).

That will make it more feasible for the dairy industry — and, to a lesser extent, the beef industry — to take advantage of what is a voluntary and subsidized disease control effort.

The *Mycobacterium avium subspecies paratuberculosis* (MAP) organism that causes Johne's is particularly persistent. When shed in the feces or milk, it can live in milk, drinking water and soil.

There's more. For decades,

## Inside Washington

with  
**SALLY SCHUFF**



scientists have been concerned that the MAP organism somehow may be implicated in Crohn's disease in humans who have a genetic predisposition for it. Crohn's is a devastating inflammatory bowel disease.

In 2001, scientists called for more studies on how pasteurization affects the MAP organism. That would underpin "effective hazard analysis critical control point programs should this agent be found to cause infection in humans," they wrote in a Council for Agricultural Science & Technology report.

So far, scientists have not made that link, and that has left dairy producers without a clear mandate for testing and control.

"If we could rationally accept that this disease is a biological accident, ... we could solve this problem," Collins said.

Impetus may come with news this spring that a Marshfield Clinic study of retail milk in California, Minnesota and Wisconsin reported finding viable MAP in 2.8% of pasteurized milk samples taken.

Moreover, MAP organisms remain in blood and lymph nodes when Johne's-positive animals are culled and slaughtered — another route for the organism to make it into the human food chain, scientists reported.

Collins and other scientists favor being proactive, but they warned that the time to start is now.