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Dairy

December 2005

NEW TOOL TO SCREEN HERDS FOR BVD - PI

The Rocky Ford Lab, which is a Branch laboratory of Colorado State University has been using a new method of pooling samples to analyze by PCR for persistently infected (PI) BVD cattle. They have been using it for over two years and it appears to have great potential as a herd screening tool. They started it as a means to certify cow/calf beef herds as being free of BVD. Since then the feedlot industry has become very interested and some are beginning to use it. Five other states are also implementing similar efforts and the laboratories in those states are preparing to offer this type of test. By early December the Rocky Ford Lab had run over 40,000 samples on this system.

For the test they use either a skin snip (ear punch) or whole blood. The skin must not be placed in formalin but just in a red top tube, and if not submitted fresh, it should be frozen until ready to submit (freezing actually enhances). A small amount of a buffered solution is placed in the tube to immerse the skin sample, a microliter amount of the fluid is removed and combined with other samples up to a maximum of 100 samples. A PCR test is performed on this combined sample for BVD, along with a positive control, in case there is an inhibition of some type. The fee is \$50 per pooled test plus other small fees, so if all the samples were negative the test would only cost \$0.61 per head.

However, if the test is positive further testing is needed to identify the PI animal(s). The test used for this is the antigen capture ELISA and it costs about \$5 per sample. So, for the 100 sample example it could run the bill up to over \$500. But this would be to identify a positive animal(s) that is known to be present. It would certainly be worth the cost to get the PIs out of the

herd, IF a good biosecurity program is in place to keep it out.

In working with producers to eradicate BVD, the first question is almost always, "Do I have it so that I need to get rid of it?" This appears to be an excellent tool to help answer that question very economically. BVD was eradicated from both the beef and dairy herds at USU over three years ago and it has made a major reduction in some other health problems, especially calf scours and some reproductive problems.

To learn the details of the testing procedure and costs, go to:

http://www.dlab.colostate.edu/bvdcontrolprogram/bvdcontrolprog_main.cfm or contact

Dr. Jim Kennedy or a staff member at:

Veterinary Diagnostic Lab
College of Veterinary Medicine and
Biomedical Sciences
Colorado State University
Rocky Ford, CO 81067
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(CVB)

JOHNE'S DISEASE – POTENTIAL NEW CONTROLS AND POTENTIAL INCREASED CONCERN

Following are four abstracts of articles on Johne's Disease. The first two offer some new potentially useful methods for control. The second two focus on the relation of JD to Crohn's Disease of man and the potential for increased concern related to it.

(CVB)

JOHNE'S DISEASE: THE EFFECT OF FEEDING MONENSIN TO REDUCE THE BIOBURDEN OF *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* IN NEONATAL CALVES

Johne's disease (paratuberculosis) is a chronic, granulomatous infection of the intestinal tract of ruminants caused by *Mycobacterium avium* subspecies paratuberculosis (MAP). There is no approved treatment, no known way to eliminate the infection once established, nor is there an effective vaccine for the disease. Johne's disease (JD) has emerged as an important disease of cattle due to the economic impact and the potential link to human Crohn's disease.

Methods to control the spread and to reduce within herd transmission of Johne's disease (JD) are being adopted by both dairy and beef herds in many states through implementation of the national Johne's disease program. Implementation of best management practices (BMP) following a herd risk assessment (RA) designed to reduce the risk of transmission of JD remains the focal point of the national effort to reduce the prevalence of JD in cattle herds today.

Initial infection with MAP is generally considered to occur in the neonatal calves. Our laboratory at the University of Pennsylvania, New Bolton Center has successfully induced experimental MAP infection in neonatal calves via oral gavage of MAP on two consecutive days. The current experiment was designed to assess the efficacy of monensin to reduce pass-through fecal shedding and to reduce tissue bacterial load (bioburden) of MAP in calves.

Twelve neonatal Holstein heifer calves (one to three days of age) were purchased from a local dairy. The herd had no evidence of clinical Johne's disease and was considered a low-risk herd with excellent biosecurity measures for newborn calves. Calves were randomly assigned to receive a carrier containing 35 mg monensin (n=6) or placebo (n=6) added to the milk replacer at each twice-a-day feeding upon arrival at the research facility. The trial was conducted as a randomized double blind trial. Both groups of calves were administered two oral doses of viable *Mycobacterium avium* subspecies paratuberculosis (MAP) on two consecutive days

between days seven and nine of the trial or days eight to 11 of age.

Results: There were no significant differences in feed consumption or weight gain between the two groups. Calves fed monensin had fewer culture-positive (55%) fecal samples, fewer total HEYM positive tubes (63%) and less MAP cfu (72%) detected in the manure compared to controls. Furthermore, monensin fed calves had fewer culture-positive tissues (66%), fewer total culture positive HEYM tissue tubes (68%) and lower MAP cfu (87%) in the tissues compared to controls. All MAP isolates from both groups were from tissues within the abdominal cavity. No isolations of MAP were made from liver, hepatic lymph node or kidney tissues of either group of calves.

Significance: Results of this study suggest that monensin effectively reduced tissue colonization with MAP following oral challenge, and also reduced fecal pass-through shedding of the organism. The MAP detected in fecal samples was clearly the result of pass-through and not active shedding from infected mucosal epithelial cells. Presumably, reduced tissue colonization in the short-term model would translate to lower mycobacterial burden and likelihood of shedding MAP in manure and clinical disease in adulthood.

In a prior study, monensin was shown to either halt the progression of lesions or reverse the lesions in cattle, with clinical signs of Johne's disease. Taken together, the results of these two studies **suggest that monensin may play a useful role both in the prevention of MAP infection in young cattle, and in the treatment of established infection in adults.** The amount of monensin (70 mg) administered per day to calves in this study is higher than the amount that would normally be consumed by a neonatal calf in a calf starter. This study was a proof-of-concept study to determine the efficacy of monensin in controlling infection with MAP in the neonatal calf. Additional work to determine efficacy for controlling infection with MAP with normal inclusion rates in a calf starter are indicated. **Monensin added to cattle rations at all phases of their life, coupled with stringent implementation of biosecurity management practices at the farm level, offers new hope to**

help reduce the unyielding spread of this disease among the nation's cattle herds. The costs are modest compared to many other management tools designed to reduce MAP bioburden within cattle herds. No other management technique evaluated to date has been shown to reduce MAP shed in manure of cattle and to reduce the tissue uptake of MAP to this extent. In these experiment calves, **monensin greatly reduced (>60%) both the pass-through fecal shedding and systemic tissue uptake.**

AABP Proceedings 38:191-192 (Sep 2005)

MAP SUPER-SHEDDERS: ANOTHER FACTOR IN THE CONTROL OF JOHNE'S DISEASE

Traditionally, fecal cultures were either positive or negative for growth of *Mycobacterium paratuberculosis* (MAP). Nearly all culture-positive cattle were judged to be infected, shedding MAP, thus a threat to spread the disease to susceptible cattle and were culled from the herd as quickly as possible. Over time it was recognized that differences in MAP shedding existed among culture-positive cattle. Cattle were classified as low, moderate or high shedders based on the visible colonies of MAP on the surface of solid media. Rarely did authors provide the estimated MAP colony forming units (cfu) per gram of manure. Additionally, culture methods were not standardized among diagnostic laboratories in the US, which made it difficult to compare results from one laboratory to another.

Over the past five years most laboratories in the US report the number of visible MAP colonies on each tube of HEYM, but rarely enumerate above 50 to 70 colonies per tube. Accounting for sample preparation, 50 colonies on each of four tubes represents an estimated 1,050 colony forming units of MAP per gram of manure. The objective of this study was to determine the range of MAP cfu/gram of manure in cattle classified as heavy shedders.

Fecal samples from cattle classified as high shedders were serially diluted: 1:5, 1:10, 1:50; 1:100, 1:500, 1:1,000, 1:5,000, 1:10,000 and 1:50,000. This initial pilot serial dilution series helped define a closer range to process a larger number of heavy shedders. Fecal samples from

more than 200 cattle classified as heavy shedders were cultured with the routine culture methods and serially diluted at 1:100 and 1:1,000. Super-shedders are defined as cattle with fecal samples having more than 10,000 cfu MAP per gram of manure.

Results: The vast majority of heavy shedders would be classified as super-shedders with more than 10,000 cfu MAP per gram of manure. Based on serial dilution of fecal samples, we have demonstrated that some infected cattle not showing clinical signs of Johne's disease (JD) shed more than 1,000,000 cfu of MAP per gram of manure. The typical range of MAP cfu for cattle showing clinical signs of JD is from 50,000 to 250,000 cfu of MAP per gram of manure.

Significance: Based on this commonly accepted numerical assessment of MAP cfu, high shedders represent the greatest threat to spread the disease, but rarely did anyone appreciate that one heavy shedder could excrete adequate MAP in the environment to be equivalent to a high shedder in composite manure samples, or that one heavy shedder could excrete more MAP cfu than 5,000 low shedder cattle. **Super-shedders represent the greatest risk to spread Johne's disease among herd mates. Some super-shedders could contaminate the environment with more MAP than 160 heavy shedders, more than 2,000 moderate shedders and more than 20,000 low shedders.** The frequency of super-shedders among culture positive cattle in infected herds is being investigated at this time. Based on this new dimension of super-shedders, **a significant proportion of low shedders are likely to represent "pass-through" and not active true infections.** As little as 10 ml of manure (1 x 10⁷ cfu of MAP) from a super-shedder could both infect another calf or heifer and result in the manure of the heifer being culture-positive with several colonies per tube. Previous research from this laboratory has shown that "pass-through" can result when cattle consume manure from cattle with clinical Johne's disease. The challenge to the Johne's academic community will be to develop diagnostic methods to detect these super-shedders in a cost efficient manner and eliminate them from the herd prior to massive environmental contamination.

AABP Proceedings 38:193-194 (Sep 2005)

JOHNE'S-CROHN'S LINK GAINS GROUND

More 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.

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 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.

For the first time, the newly reported genome map identifies gene sequences unique to

MAP, a breakthrough that allow more specific tests for the disease... The work, led by Dr. Vivek Kapur ... was completed through a USDA grant... The team already has developed a commercial test that will identify MAP in both fecal samples and milk.

...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." ... 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.

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.

(Feedstuffs, September 19, 2005; pg1 &32)

MARSHFIELD STUDY STARTLES VET COMMUNITY

The Marshfield Clinic retail milk study published this spring in the *Journal of Food Protection* startled the veterinary community when preliminary findings were first reported last fall at the U.S. Animal Health Assn.'s annual meeting.

In the study, a research team from the Marshfield Clinic in Wisconsin, led by Dr. Jay Ellingson, reported finding viable *Mycobacterium avium subspecies paratuberculosis* (MAP) present in 2.8% of milk samples taken directly from retail grocery stores in three of the nation's five largest dairy states.

The study also found remnant DNA from MAP in 64% of the 702 pints of retail milk sampled from California, Minnesota and Wisconsin. That DNA was not viable but indicated that the milk had been exposed to MAP. The results revealed that viable MAP, which causes Johne's was present in almost three out of every 100 bottles of milk.

However, Ellingson pointed out, pasteurization is not intended to kill every microorganism. "Pasteurization is not a lethality step, meaning it sterilizes milk." Rather, he explained, pasteurization aims at reducing

microorganisms through a “log reduction kill” that prevents them from reaching risky levels.

MAP is enormously tough. “It knows how to survive in multiple ranges of temperature and multiple ranges of acidity,” he said. “The organism is a survivor.”

That retail milk study “was developed to help farmers,” Ellingson reported, “to try to get the organism reduced on the farm and improve farmer’s economics. We know Johne’s is a big economic issue.”

Other researchers had tested milk pasteurized in laboratories to simulate commercial conditions. However, Ellingson said, “The real scientific laboratory is on the shelf of all of the grocery stores.” In the testing, “Every month, we took the same amount of samples from the same locations in the same states.” The samples were “double-blinded” and tested under the Marshfield protocol and by an independent lab.

(Feedstuffs, September 19, 2005; pg 33)

A FIRST-HAND LOOK AT RUMEN DEVELOPMENT

Calf raisers attending the Professional Dairy Producers of Wisconsin’s Calf Care Connection seminars this week saw proof that weaning can begin as early as four weeks of age. The seminars, held at three locations across Wisconsin, showcased real calf rumens at different stages of development. Interestingly, the rumen of a four-week-old calf fed milk and starter was nearly identical in development to that of an eight-week-old calf fed milk and starter. “The added effect of going from four to eight weeks was virtually nothing,” says Jud Heinrichs, professor of dairy science at Penn State University. Early weaning isn’t for everyone, he cautions, but with proper management, some calves can be ready before eight weeks.

Dairy Alert, Wednesday, November 30, 2005,
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NEW ADVICE FOR DISTILLERS GRAINS

As ethanol production increases around the country, so does the availability of distillers grains. The National Corn Growers Association offers a new publication for livestock producers

interested in learning more about using this feed. Based on research conducted at several leading universities, it contains feeding recommendations for beef, dairy, swine and poultry. You can download the publication (138 pages) at: <http://www.ncga.com/ethanol/pdfs/111005DGFeedingRecommendations.pdf>

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ORGANIC GRAZING RULES CLARIFIED

In the coming weeks, the USDA is expected to clarify the amount of grazing time required for organically managed cows. According to the Nov. 25 issue of Capital Press Agriculture Weekly, this clarification, as recommended to the USDA by the National Organic Standards Board, would mandate that cows have access to pasture during the growing season of the area where they’re being raised and milked.

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RELATIONSHIP BETWEEN ANTIMICROBIAL SUSCEPTIBILITY OF CLINICAL MASTITIS PATHOGENS AND TREATMENT OUTCOME IN COWS

One hundred thirty-three cows with mild or moderate mastitis in a single quarter were enrolled in a study to determine whether there was any association between results of in vitro antimicrobial susceptibility testing of the causative organism and outcome of treatment. All cows were treated by means of intramammary infusion of pirlimycin (50 mg) in the affected quarter once daily for 2 days; additional intramammary treatments with the same product were administered if the milk continued to appear abnormal after 2 days of treatment. Duration of treatment and days until clinical cure were recorded. Bacterial isolates were tested for antimicrobial susceptibility by means of a broth microdilution technique.

Environmental *streptococci*, *coliforms*, and coagulase-negative *Staphylococcus* spp were the most commonly isolated pathogens. Duration of treatment and days until clinical cure were not significantly different for cows from which

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pathogens that were susceptible or resistant to pirlimycin were isolated. Bacteriologic cure rates 14 and 21 days after treatment were not significantly different for cows with mastitis caused by susceptible or resistant bacteria. Similar results were found when data from cows with mastitis

caused by gram-positive isolates were analyzed. The use of in vitro susceptibility testing to guide intramammary mastitis treatment cannot be recommended on the basis of results of this study.

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