

Johne's Disease in Cattle: An Overview and Update

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Paratuberculosis (Johne's disease) occurs worldwide and is widespread in the United States. A slaughter survey in cows from northeastern states estimated the prevalence of paratuberculosis in cull dairy cows at 7.3% and a similar survey in Wisconsin revealed a 10.8% prevalence.^{1,2}

Economic losses due to paratuberculosis may include decreased milk production, premature culling, and increased susceptibility to other diseases.^{3,4} In Pennsylvania alone, over 500 herds positive for Johne's disease have been identified with economic losses estimated at \$5.8 million annually. Of the 10.8 million dairy cows in the U.S., nearly 1 million are thought to be affected, and the total losses due to paratuberculosis are expected to exceed \$1.5 billion annually for the dairy industry in the United States.²

Diagnostic Tests

The most widely accepted diagnostic test for paratuberculosis is fecal culture for *Mycobacterium paratuberculosis*. The test is highly specific with no false-positive results if conducted properly. Disadvantages of this test include difficulty in specimen handling, 12 to 16 week incubation period, contamination of samples by molds and bacteria (especially in silage-fed animals) and lack of sensitivity (less than 50% of infected animals may be detected on a single test). Although the lack of sensitivity may be due to intermittent fecal shedding of *M. paratuberculosis* by infected animals, we believe that in most cases organisms are shed, but below the detection limit of the culture system, giving false-negative results. Improvements in the fecal culture technique have allowed improved detection of fewer organisms and thus, improved sensitivity. By centrifugation of the fecal suspension, organisms are concentrated into the pellet prior to re-suspension and inoculation onto Herrold's egg yolk medium slants. This technique alone has increased the sensitivity of the fecal culture test 200 to 300%.⁵ When routine fecal samples are collected (for herd testing for paratuberculosis), split, and

tested by both techniques (centrifugation versus traditional/sedimentation), there are two to three times as many positive cows detected with the centrifugation technique compared to the suspension method. These cows are most commonly asymptomatic, "light shedders" that will continue to shed few organisms but not be detected by the traditional fecal culture test for one to two years. Associated with the use of the centrifugation method is a slight increase in the bacterial and mold contamination rate (5 to 10%). A recent modification of the culture technique includes a double incubation step with antimicrobials added to culture medium which nearly eliminates contamination, permitting processing of a larger fecal sample (5 gm vs 2 gm) thus further enhancing sensitivity. Centrifugation with double incubation of a 5 gm sample should detect less than 10 CFU of organisms per gram of feces.

Despite the major improvements in the fecal culture testing technique, the major disadvantage remains the prolonged incubation period, and in some areas, limited laboratory capacity for handling a large number of samples. Therefore, more rapid serologic tests have been investigated. Currently, there is a commercially available agar gel immunodiffusion (AGID) test that is USDA licensed for use by veterinarians in some states (RJT®, Immunocell). The AGID test has very poor sensitivity when used to screen asymptomatic animals in herds where the disease is prevalent; as few as 10% of infected animals may be detected. However, the test has good sensitivity (70 to 90%) and specificity (80 to 90%) when used in cows with clinical signs of Johne's disease, i.e., diarrhea and weight loss. Therefore, the positive- and negative- predictive value for AGID test results is quite poor when the test is applied to the population at large, and it is inappropriate to use this test to screen herd replacements or to make culling decisions for asymptomatic animals on the basis of this test. However, when presented with an individual cow with weight loss and/or diarrhea, and faced with a decision to treat or cull, the AGID test can provide information that will lead to the more rapid culling of clinical Johne's suspect animals.

The ELISA test has been an attractive alternative to the AGID since it may be automated to accommodate a

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large number of samples, and improved sensitivity compared to the AGID test is expected. ELISA testing is currently available only through a limited number of commercial and research laboratories. Unfortunately, the improved sensitivity achieved with the ELISA is accompanied by a reduction in specificity. Whereas one study reported a sensitivity of 70% and a specificity of 90%,⁶ others report sensitivity of 40 to 60% and specificity of 50 to 80%.^{7,8} It is our opinion that, as is the case with the AGID test, ELISA testing as currently available should not be considered reliable for the screening of individual animals for paratuberculosis.

The most promising new development in the diagnosis of paratuberculosis is the DNA probe. Mycobacterial organisms in the test specimen are lysed to release DNA, and the double stranded DNA is cleaved. Enzyme- or radio-labeled DNA segments known to hybridize with *M. paratuberculosis* DNA are added, and the labeled, hybridized segments detected. Using Polymerase Chain Reaction technology, a small number of DNA segments in the test specimen can be reproduced many-fold, increasing the sensitivity of the test. Initial indications are that the DNA probe applied to fecal specimens will have sensitivity and specificity equal to the fecal culture without centrifugation. The major advantage of the DNA probe is the rapidity (36–48 hours), the elimination of the need for viable organisms that will grow in culture and the elimination of sample decontamination procedures. Due to equipment required to conduct the test (thermal cycler), the DNA probe in its current form will only be available in commercial or research laboratories.

Transmission

It is commonly recognized that *M. paratuberculosis* is transmitted to young calves by oral ingestion of organisms from feces of infected cows, often by suckling a manure-contaminated udder. However, alternative mechanisms have been recognized: we have investigated the trans-placental transmission of paratuberculosis and the possible direct shedding of *M. paratuberculosis* into the milk of infected cows.

The possibility of transplacental transmission of paratuberculosis has been recognized since 1929, when Alexejff-Goloff reported isolation of *M. paratuberculosis* from the fetus of a cow with clinical signs of Johne's disease. More recent reports have confirmed that 25 to 30% of fetuses from cows with symptomatic Johne's disease are infected *in utero*.^{9,10} However, our studies involving asymptomatic cows (which comprise the majority of infected cattle) showed a much lower prevalence (5%) of uterine infection in this group. Transplacental transmission in asymptomatic cows would appear to be restricted to the "heavy shedders."

Alexejff-Goloff reported the isolation of *M. paratu-*

erculosis from milk in three of four cows symptomatic with Johne's disease in 1935, and reports since then have shown up to 35% of symptomatic cows had organism detected in the milk.^{11,12} However, our work shows that 10% of asymptomatic cows have the organism detected in milk, with the likelihood of detection greater in the "heavy shedders." We do not currently recommend the pasteurization of milk or colostrum from all cows on endemic farms, but milk from known culture-positive cows (especially clinically affected cows) should not be used to feed calves.

Eradication—Case Experience

A heavy infected Guernsey herd was identified in 1983. Of 42 adult cows sampled on the initial test, 16 were positive (36%). An additional 13 of the original 42 were identified as culture-positive on subsequent tests over the next five years. Strict control procedures were instituted including immediate removal of newborn calves from dam and bottle feeding of colostrum, housing of heifers in a newly-constructed barn physically separated from the adults, and immediate culling of fecal culture-positive animals. Of 159 home raised replacements cultured repeatedly for paratuberculosis from 1983 to 1989, 45 (28%) were positive but with a decreasing prevalence over time. All animals were culture negative on the most recent test. Of the calves born to positive cows before the management changes, 65% were subsequently positive, whereas only 17% of the calves born to positive cows after the management changes were subsequently positive. Of the calves born to negative cows, 54% eventually were positive if born before the changes, 4% after the changes. Seemingly, these management changes were effective in limited transmission. This case history emphasizes that even with stringent control procedures, a minimum of five to eight years will be required to eliminate the disease from a herd.

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Abstracts

Fertility indices for beef bulls

VEA PERRY, PJ CHENOWETH, TB POST and RK MUNRO

Summary:

Young (16- to 30- month-old) beef bulls of 6 different genotypes were assessed for production and reproduction traits at different ages and intervals from single-sire mating. Fertility indices, in the form of multiple regression equations using pregnancy rate as the dependent variable, were derived from these assessments using non-orthogonal analyses of variance and covariance.

"Among" and "within" genotype fertility indices showed significant correlations with pregnancy rate. "Within" genotype fertility indices showing significant multiple correlations ($p < 0.01$) at 11 ($r=0.75$), 8 ($r=0.89$), 6 ($r=0.86$) and 2 ($r=0.80$) months prior to mating. It was found that the most important traits to include in the fertility indices were peripheral LH levels following GnRH stimulation, testicular volume, libido and body weight. In general, the fertility indices showed good correlations with bull reproductive performance and were not significantly affected by bull genotype.

Aust Vet J 67: 13-16

Inherited epidermal dysplasia in Holstein-Friesian calves

TF JUBB, J and MALMO, JM MORTON, C BUTTON and IV JERRETT

Summary

Inherited epidermal dysplasia (IED), formerly called baldy calf syndrome, is a lethal disease of calves of Holstein-Friesian ancestry. The disease causes progressive illthrift and skin, horn hoof lesions, which can be confused with inherited zinc deficiency. The clinicopathological features and ancestry of 10 affected calves in Gippsland, Victoria are described.

Aust Vet J 67: 16-18

A comparison of interactions between vaccination against *Dictyocaulus viviparus* and anthelmintic suppression in immunised and unimmunised yearling cattle

S. M. Taylor, T. R. Mallon, W. P. Green

Veterinary Record (1990) 126, 185-189

Twenty parasite-naive calves aged approximately four months were divided randomly into four groups of five. Two groups were treated with oral lungworm vaccine. One immunised group plus another non-immunised group were put out to graze on May 1 on a pasture known to be contaminated with *Dictyocaulus viviparus* infective larvae during the previous autumn. All the calves both indoor and outdoor were treated with ivermectin at three, eight and 13 weeks after the first groups started to graze and again at housing at the end of September. After the winter housing period on April 27 of the following year all the calves were given an artificial challenge of *D Viviparus* infective larvae at the rate of 15 larvae per kg bodyweight, and the clinical and parasitological reactions monitored. All the calves which had been vaccinated or exposed to field infection during year 1 reacted strongly in ELISAs using antigen prepared from fourth stage *D viviparus* larvae but much less strongly in similar tests using adult derived antigen. Clinically those calves exposed to previous field infections were less severely affected than housed calves, although parasitologically all three groups with prior exposure to *D viviparus* appeared to have a similar functional level of immunity to the challenge infection in comparison to the unexposed calves of the same age.