Introduction

Johne's is a chronic infectious disease of domestic and exotic ruminants. The causative agent is Mycobacterium *avium subsp. paratuberculosis* (Mptb), which induces a granulomatous degenerative enteritis that critically reduces the absorptive capacity of the small intestine. Efforts to effectively treat the disease or to develop a fully protective vaccine have yet to succeed. **Until an effective cure or preventative agent is found, disease detection and management remain the best weapons in the battle against this disease.**

Characterized in its clinical stages by persistent diarrhea, progressive weight loss, and eventually death, Johne's disease is a growing concern in the United States cattle industry. In 1997, the National Animal Health Monitoring Service (NAHMS) published an extensive study on Johne's disease in U.S. dairy operations. **The NAHMS study reported annual losses of $40 to $227 for every cow on dairy operations with a moderate incidence of clinical Johne's disease.** Losses are primarily attributed to reduced milk production and premature culling, but losses in milk component value and reproductive efficiency are also significant.

Infection primarily occurs in young animals by ingestion of contaminated feces or milk from infectious adults. Intrauterine transmission has also been documented. Risk factors for transmission include contaminated maternity areas, suckling, and proximity between replacements and infectious adults. Contaminated feed, including pooled colostrum, waste milk and contaminated water sources are also common points of infection in calves.

Johne's disease remains largely silent during its slow progression into the final clinical sages. **However, subclinical animals are often infectious and require testing for identification, management and removal.**

To date there is no single test sufficient for complete diagnosis of the disease; however, reasonably accurate and cost-effective tests are available for differing diagnostic and control needs. Like most diagnostic tests, those used in the detection of Johne's disease are evaluated in terms of sensitivity and specificity. Sensitivity is the percentage of diseased animals that have a positive test result. Specificity is the percentage of disease-free animals that have a negative test result. The probability that a given diagnostic test is correct is called the "predictive value" and is significantly affected by the prevalence of disease within the population being tested. **Therefore, the first priority in developing a Johne's control program is to establish its prevalence in the herd.**

The most cost-effective approach to determine the Johne's prevalence in a herd is by screening for potentially infected animals with enzyme-linked immunosorbant assays (ELISA). ELISA's are inexpensive, fast and easily automated. Lacking the sensitivity required of individual animal diagnostics, these antibody-based tests are very effective as herd screening and monitoring tools. Results are usually expressed on a continuous scale enabling users to change cutoffs. This allows users to define, to a limited extent, the level of sensitivity and specificity of the test. Most cutoff values selected for the analysis of ELISA scores result in sensitivities approaching 40 percent and specificities greater than 90 percent. **AntelBio recently developed an ELISA for milk samples, which is now available commercially.** The milk ELISA is equivalent to the serum ELISA in sensitivity and specificity*. In addition, the AntelBio Milk ELISA can be incorporated with the DHIA milk sample collection system, eliminating the labor required for collection and processing of blood samples. Thus, the AntelBio Milk ELISA is an excellent tool to incorporate into many Johne's control and management programs.

An alternative to the ELISA-based antibody tests are assays that detect the organism directly. Organism-based tests are considered more reliable than the antibody-based tests and are often used as follow-up tests to antibody-based assays. Sensitivities are usually reported in excess of 50 percent, with 99.9 percent specificity if done correctly. Traditional fecal culture on solid medium is considered the "gold standard" against which all other Johne's diagnostic tests are compared; however, traditional culture is slow (up to 16 weeks for test results) and relatively expensive. Several variations of traditional culture are available that have reduced reporting times to as little as six weeks. **AntelBio has recently developed a DNA probe test for feces that detects Mptb in as little as 72 hours.** With the AntelBio Rapid Fecal Test, it is not necessary to grow or culture the bacterium, thereby substantially reducing the time to achieve results with only minimal effects on sensitivity when compared to traditional fecal culture*.

Regardless of the assay, antibody-based or organism-based, their performance or sensitivity suffers because of the physiology of Johne's disease. During the early stages of Johne's disease, fecal shedding of the organism and seroconversion to antibody production have not yet occurred. **Therefore, Johne's management programs rely on controlling risk by quickly identifying test-positive animals and reducing the odds of transmission through selective management.** The case studies that follow have been derived from actual testing and management scenarios observed in the AntelBio Testing Center, and are presented as an exercise for the development of Johne's testing and management protocols in varying situations.

* Technical reviews are available upon request. Due to the proprietary nature of some information, a confidentiality agreement may be required to review some data sets.

Case Study Summary

Case Study A is a large corporate dairy that has a high incidence of Johne's disease. AntelBio has worked with their farm management team to develop a sound testing and management protocol through extensive confirmation experiments. This case study provides an insightful overview of the performance characteristics of Johne's diagnostic tests.

Case Study B markets specialty dairy products and seeks to quickly eradicate Johne's disease to assure their customers of their high quality dairy products. Animals are stratified into Johne's control management groups based on user-defined cutoffs applied to frequent whole-herd ELISA's. This operation has implemented a stringent set of animal management practices to reduce transmission. This case study provides an excellent example of Johne's risk management strategies.

Case Study C is a purebred beef operation that seeks official Johne's certification. An initial whole-herd ELISA screen was promising, but results from two positive tests are currently being appealed by traditional fecal culture. This case study summarizes the procedures to achieve and maintain official certification while demonstrating the risks associated with animal management practices in beef operations.

Case Study A

History

Case Study A is a 4,000 cow dairy operation managed by a team of veterinarians, nutritionists, and economists. The operation originated in 1990 as a division of a corporate farm, and was populated by the purchase of small to medium sized herds from a number of Midwest and Eastern states. The operation utilizes freestall housing and a double 30 parlor to milk twice a day. Cows are managed in eight groups based on age, production level and stage of lactation. Milk production is currently at 70 pounds per head per day. Among the facilities located on the operation is a separate barn, which contains the dry cow and maternity area, as well as the hospital area. Calves are removed from the premises within approximately one week of birth. Heifers are contract raised and re-enter the herd when bred. While calves are still on-farm, they are group housed in a single pen and are fed pooled colostrum. This operation suspects a high incidence of Johne's disease may be responsible for high culling rates. Based on the observation of two to three clinical cases of Johne's per month, it was determined that Johne's disease was costing this dairy between $250,00 and $500,000 annually.

Goals

The farm management team has determined that its priorities are to 1) establish a plan for Johne's reduction, 2) eliminate clinical cases of Johne's and 3) to reduce the test prevalence of Johne's below 2 percent, a level at which the negative financial impacts of Johne's have been deemed minimal for this herd. To accomplish these goals, this operation initially decided to identify "high risk" animals by serologic testing at dry-off and isolating them on a provisional dairy for the remainder of their productive lifespan.

Analysis of Preliminary Testing Results

The operation initiated dry-off testing with a commercial serum ELISA in 2000 and continued for approximately three months. This testing, while it does not provide sufficient data to establish true herd prevalence, indicates that Johne's is present at a very high level in the herd. Figure 1 shows a representative set of test results of serum ELISA testing during this three month period. Using a minimum threshold of 0.1 for an ELISA positive score, the test prevalence for Johne's in the herd was approximately 30 percent. Since 30 percent prevalence was an unmanageable proportion of the herd, the farm management team requested fecal confirmations on a subset of the seropositive animals. Figure 2 shows only eight of the 26 seropositive animals were confirmed fecal positive by the AntelBio Rapid Fecal Test (RFT). The management team concluded that single point ELISA testing did not adequately define "high risk" animals in this population. Explanations for the apparent discrepancy between ELISA and RFT results include other transient infections, previous Johne's vaccinations, dry-cow treatments, etc.; all of which can potentially increase the level of false-positive results in the serum ELISA.

Figure 1

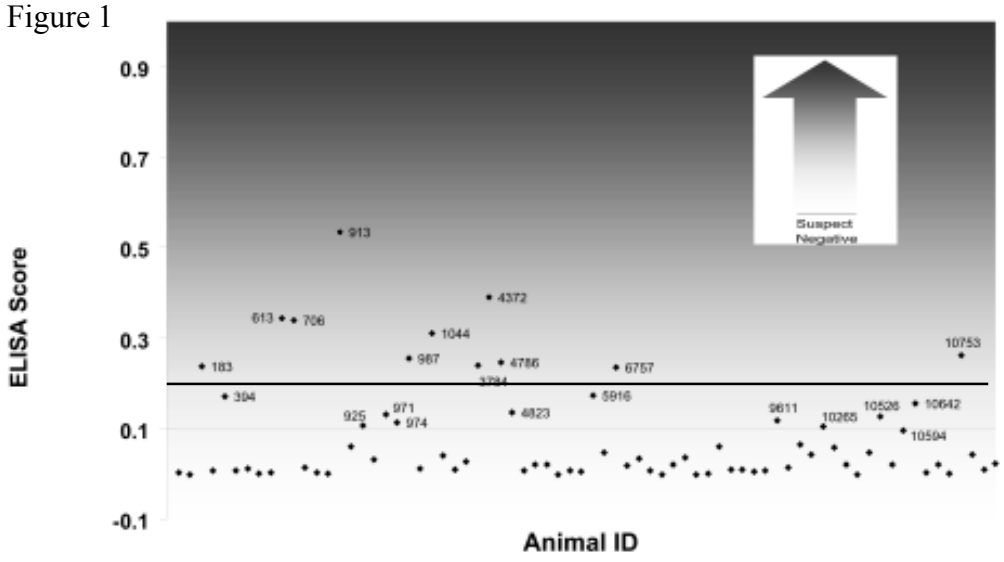

Figure 1. Serological ELISA results of animals entering the dry lot on Case Study A.

Figure 2

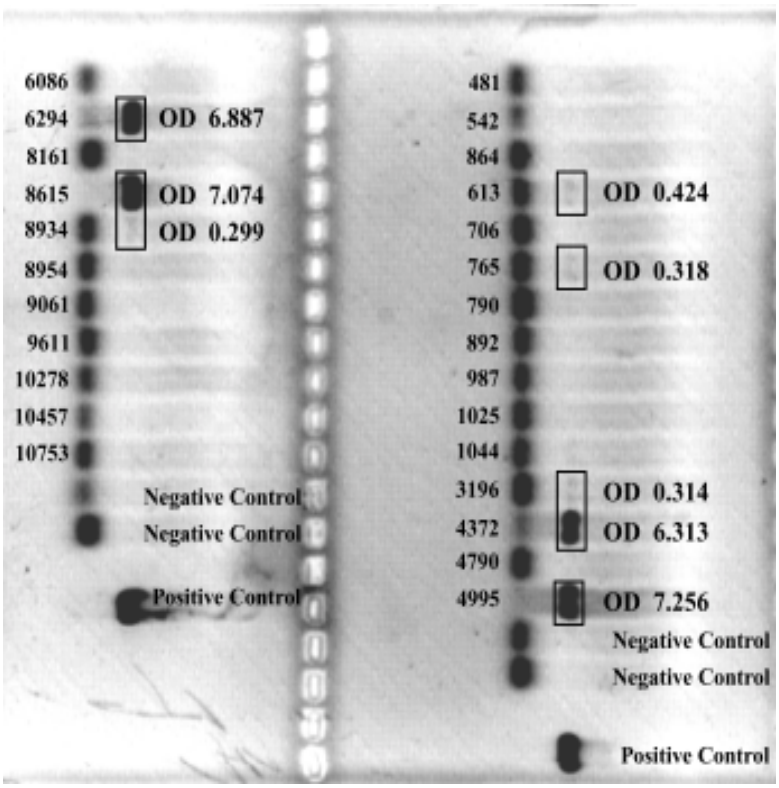

Figure 2. AntelBio's Rapid Fecal Test on seropositive dry cows.

Testing Scenario

Four testing options were discussed to more accurately define the Johne's high-risk population; 1) continue serum testing at dry-off and raise the threshold for the determination of the "high risk" population, 2) conduct an annual whole-herd ELISA with the AntelBio Milk ELISA and retest milk positive animals at dry-off with the serum ELISA, 3) conduct an annual whole-herd ELISA with the AntelBio Milk ELISA and retest milk positive animals at dry-off with the AntelBio Rapid Fecal Test and 4) test all animals at dry-off with the Rapid Fecal Test. Table 1 describes the advantages, disadvantages and costs of each option.

Scenario 2 was determined to be the most cost effective approach for the identification of animals at the highest risk of carrying and shedding Mptb. This testing approach involves an annual whole-herd AntelBio Milk ELISA and retesting only the positive animals with a serum ELISA during the subsequent dry-off period. Since the milk ELISA would be conducted through routine DHIA testing, the labor required for this option would be substantially reduced, requiring blood sampling from only 1,200 animals annually compared to 4,000 in Scenario 1. Any animals that were reported positive on both the AntelBio Milk ELISA and the serum ELISA would be classified as "high risk" and segregated to the provisional dairy. Removal of these "high risk" animals to an independent facility will enable development of specific management practices to optimize disease reduction. In addition, data from the annual whole-herd milk ELISA would be useful in monitoring Johne's prevalence and the effectiveness of this testing and management scenario.

Table 1

Scenario Number	Scenario	Advantages	Disadvantages	Annual Cost
1	• **Continue serological testing at dry-off** • Raise threshold to 0.3 for determination of "high risk" population	• Low cost • Improved specificity • Reduces "high risk" population to manageable number	• Reduced sensitivity • Seronegative, fecal positive infectious animals are missed • Previous vaccination and transient conditions interfere ELISA • Prolongs Johne's reduction efforts • Progress monitoring difficult	**$19,000** (4,000 animals; $4.75/ELISA)
2	• Annual whole-herd ELISA with AntelBio Milk ELISA • Serum ELISA on milk positive animals at dry-off (30%)	• Reduced labor via DHIA collection system • Initial sensitivity maintained while reducing "high risk" population to manageable number • Removes confounding from transient conditions • Progress easily monitored	• Seronegative, fecal positive animals missed • Results confounded by previous vaccination	**$29,700** (4,000 animals; $6/ELISA) (1,200 animals; $4.75/ELISA)
3	• Annual whole-herd ELISA with AntelBio Milk ELISA • AntelBio Rapid Fecal Test on milk-positive animals (30%)	• Initial sensitivity maintained while reducing "high risk" population to manageable number • High specificity, identifies truly infectious fecal positive animals	• Increased labor for fecal sample collection • Seronegative, fecal positive animals missed	**$48,000** (4,000 animals; $6/ELISA) (1,200 animals; $20/fecal test)
4	• AntelBio Rapid Fecal Test at dry-off	• High sensitivity • High specificity • Hasten reduction of Johne's	• High cost • Increased labor for fecal sample collection	**$80,000** (4,000 animals; $20/fecal test)

Table 1. Testing scenarios discussed for Case Study A.

Interpretation

The first annual whole-herd milk ELISA was conducted in the spring of 2001; results from Group 7 (late lactation, low production) are shown in Figure 3. As with serological testing, the AntelBio Milk ELISA found approximately 30 percent of the animals with an ELISA score above the 0.1 positive threshold.

When the first group of animals reached the dry lot, serum and feces were obtained from 54 animals with positive milk ELISA scores. The fecal samples were analyzed with the AntelBio Rapid Fecal Test and by traditional culture. Serum ELISA and fecal results are shown in Table 2. Forty-six percent (25/54) of the animals with positive milk ELISA scores tested positive by the serum ELISA. Of these milk ELISA positive/serum ELISA positive animals, 80 percent (20/25)

were shedding Mptb as determined by the Rapid Fecal Test. Alternatively, only nine of 29 milk ELISA positive/serum ELISA negative animals were found positive by the Rapid Fecal Test. This demonstrates that the "double ELISA positive" approach for identifying "high risk" animals is highly specific for detecting fecal shedders when compared to the single-point serological testing performed at dry-off (see Figure 2). Although some fecal positive animals will be missed by the "double ELISA positive" approach, the advantage of this approach is identifying and removing **only** those animals at highest risk of shedding Mptb. This eliminates the need to micro-manage low risk animals that pose little threat to spreading Johne's disease during their next reproductive cycle, which is a costly exercise.

Figure 3

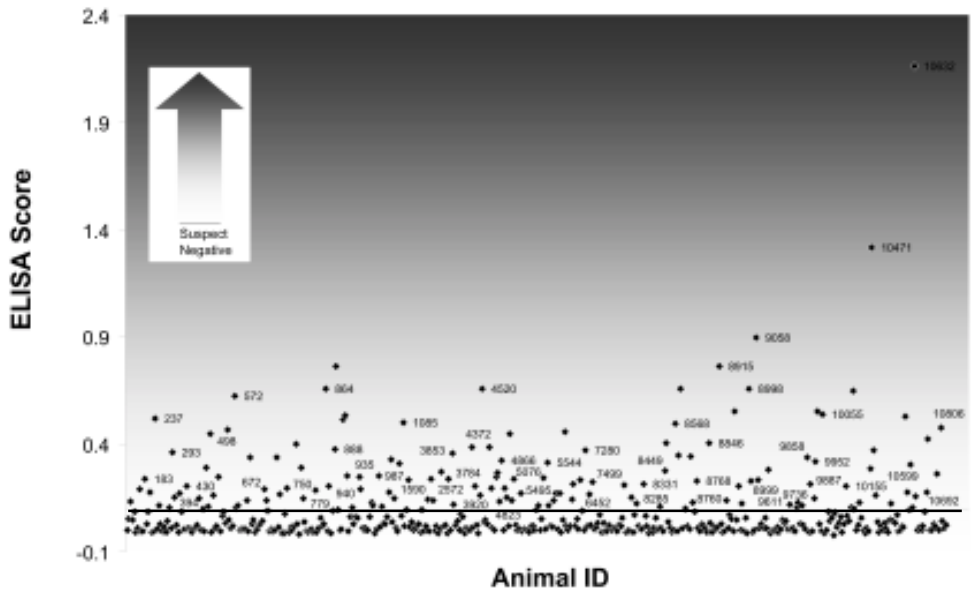

Figure 3. AntelBio Milk ELISA results for Group 7 on Case Study A.

Table 2

Serum Positive Animals

Animal ID	ELISA Score	ELISA Result	RFT Score	RFT Result	Culture, 12-wk cfu*
10632	1.87	Positive	1.25	Positive	24
8568	1.21	Positive	7.31	Positive	tntc
572	1.12	Positive	5.85	Positive	tntc
8998	1.10	Positive	7.84	Positive	tntc
4372	0.99	Positive	5.17	Positive	tntc
1085	0.97	Positive	5.21	Positive	tntc
5076	0.86	Positive	5.27	Positive	tntc
10471	0.86	Positive	6.62	Positive	tntc
9858	0.78	Positive	7.19	Positive	tntc
888	0.77	Positive	6.65	Positive	tntc
4823	0.66	Positive	5.08	Positive	tntc
9058	0.65	Positive	4.89	Positive	tntc
394	0.56	Positive	0.13	N	0
10055	0.55	Positive	0.95	Positive	20
8915	0.52	Positive	6.74	Positive	tntc
3784	0.44	Positive	0.13	N	0
4520	0.37	Positive	7.52	Positive	tntc
5495	0.37	Positive	3.34	Positive	30
8846	0.36	Positive	7.33	Positive	tntc
430	0.33	Positive	0.09	N	0
237	0.23	Positive	5.72	Positive	tntc
7499	0.22	Positive	7.08	Positive	tntc
10806	0.13	Positive	0.11	N	1
750	0.11	Positive	0.06	N	3
987	0.11	Positive	0.38	Positive	0

Serum Negative Animals

Animal ID	ELISA Score	ELISA Result	RFT Score	RFT Result	Culture, 12-wk cfu*
10599	0.087	N	0.10	N	0
940	0.067	N	0.50	Positive	1
5544	0.055	N	0.09	N	0
4866	0.044	N	0.07	N	3
8768	0.035	N	0.06	N	0
10692	0.034	N	0.26	N	0
3853	0.032	N	0.13	N	0
8449	0.032	N	0.87	Positive	6
935	0.023	N	1.64	Positive	17
2572	0.023	N	0.09	N	0
9887	0.021	N	0.04	N	0
9952	0.016	N	0.11	N	0
779	0.011	N	0.12	N	1
8999	0.004	N	0.32	Positive	0
293	0.001	N	1.23	Positive	40
7280	0.001	N	0.44	Positive	0
498	-0.002	N	0.19	N	0
864	-0.003	N	0.04	N	0
8760	-0.003	N	0.20	N	0
9736	-0.003	N	0.05	N	0
3920	-0.005	N	0.37	Positive	0
10155	-0.005	N	0.46	Positive	0
8285	-0.009	N	0.06	N	0
8331	-0.010	N	0.69	Positive	2
183	-0.015	N	0.09	N	1
6452	-0.015	N	0.10	N	0
672	-0.023	N	0.17	N	0
1590	-0.025	N	0.10	N	0
9611	-0.035	N	0.10	N	0

* Colony forming units, tntc are too numerous to count

Table 2. Serum and fecal test results for dry cows that previously tested positive with the AntelBio Milk ELISA.

Risk Factors

In establishing an effective Johne's management program, identifying additional risk factors that may contribute to the spread of Johne's disease is critical. Specific to this case study, the following factors were identified as potentially contributing to the Johne's problem in the herd:

- The feeding of colostrum pooled from all cows is undoubtedly contributing to the transmission of Johne's to potential replacement heifers. At a minimum, colostrum from any "high risk" animal should be discarded. The elimination of pooling, so colostrum from one cow is fed to only one calf, would reduce transmission to calves born free of Johne's disease. In a more aggressive management strategy, a colostrum replacer could be used to completely avoid transmission in this manner.
- The close proximity of the calving area to the hospital area also increases risk of disease transmission. Moving the hospital area away from the calving area would limit exposure of non-clinical animals to the contaminated feces of Johne's clinical cows.
- Replacement heifers from this herd are all strongly suspect of Johne's disease due to the high Johne's prevalence in the herd. Special attention must be given to the selection and raising of replacements. Environmental and management conditions at the contract facility should be investigated. The purchase of replacements from herds with low prevalence will hasten the reduction of Johne's prevalence in this herd.

Next Step

- It is recommended that annual monitoring of herd Johne's prevalence via the AntelBio Milk ELISA is conducted to evaluate the success of this program. It is expected that a quick reduction in prevalence over the first five years (30 percent to 10 percent) will be observed and then smaller increments thereafter. Changes in testing strategy (e.g., addition of fecal testing) may be required to fully achieve goals during the waning years of this program.
- The expectation is to reduce Johne's disease in this herd to the desired levels in approximately 20 years.

Case Study B

History

Case Study B originated in 1962 as a 50 cow dairy and has since expanded into a complete 600 cow dairy product operation, including a milk processing and marketing facility that offers a variety of specialty dairy products. The animal facilities consist of recently converted freestall barns and a new double 20 parlor to milk three times per day. Cows are grouped by age, production level and stage of lactation. Milk production currently averages 85 pounds per head per day. Replacements are initially raised individually at a geographically separated site, and fed pooled colostrum and waste milk. They are subsequently transferred to isolated transition pens and receive a TMR until introduction into the milking herd. The culling rate in this operation has been low to moderate to accommodate expansion, and Johne's disease has been diagnosed by fecal culture in two recent culls. Quality control procedures (HACCP) have been adopted in the processing plant, however biosecurity within the animal facilities has only recently become a major issue with the diagnosis of Johne's disease.

Goals

The transmission route of Johne's disease and its potential association with milk quality have highlighted the necessity of biosecurity programs in the management of animals in this dairy operation. Until its diagnosis, Johne's disease was not suspected and its prevalence was unknown. The owners and their veterinarian have expressed a desire to quickly eradicate this disease from this population, and are devising operational procedures to avoid similar occurrences in the future. State certification of Johne's-free status for the marketing of dairy products and replacement heifers is desired within five to seven years.

Figure 4

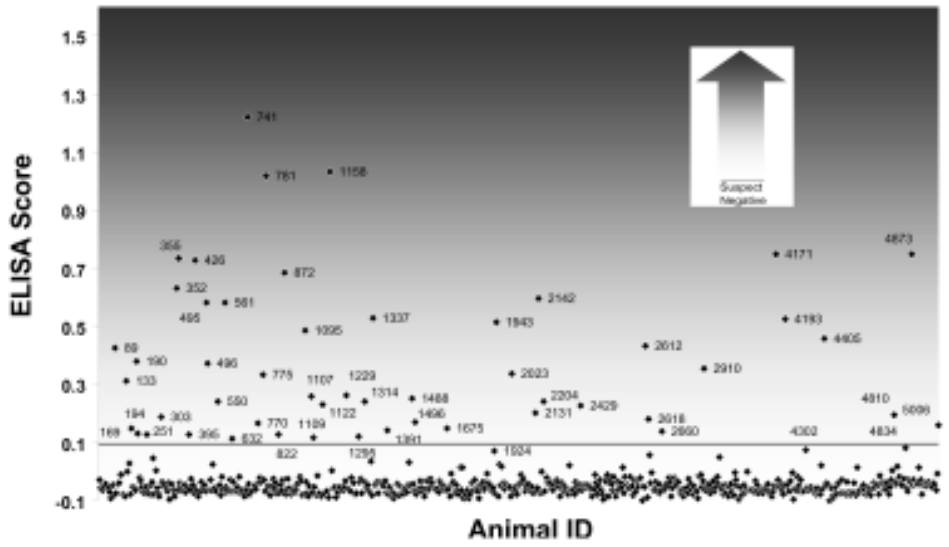

Figure 4. Whole herd AntelBio Milk ELISA on all lactating animals on Case B.

Testing Scenario

To initiate a program for the management of Johne's disease, the owners and their veterinarian decided to conduct a whole-herd test with the AntelBio Milk ELISA. Results from the initial screen are depicted in Figure 4 and shows an 8 percent test prevalence of Johne's disease based on a minimal positive threshold of 0.1 for the ELISA score. Opting to delay participation in the state certification program, the owners decided to schedule semi-annual whole-herd milk ELISA and stratify animals into management scenarios based on the magnitude of their ELISA score. Research has indicated that the risk of transmission increases with increasing ELISA score. Figure 5 shows AntelBio's Johne's Watch List that prescribes separate precautionary actions on animals stratified into the 0.50 and higher group (tagged red), the 0.15 to 0.50 group (tagged yellow) and the 0.07 to 0.15 group (tagged green). The Johne's Watch List offers the veterinarian a wide range of options to assist the herd manager in the development of a Johne's control program. Case Study B has budgeted $7,200 per year (600 animals x $6 per test x 2 tests per year) to accommodate this Johne's testing strategy.

Interpretation

The owners of Case Study B and their veterinarian have decided to lower the threshold of the ELISA score to 0.07 to improve the sensitivity of the milk ELISA. The sacrifice in specificity will be offset by implementing less aggressive management actions in the low risk group. Considering that animals with ELISA scores between 0.07 and 0.15 generally have a low risk of intrauterine transmission of Johne's to their offspring, the veterinarian has suggested that keeping replacements from these animals is an acceptable practice. However, use of a separate calving area and discarding the

dam's colostrum in favor of colostrum from animals three years and older with ELISA scores below 0.07 is advised. Assuming the risk of transmission is higher in animals with higher ELISA scores, another calving area will be utilized for moderate risk animals, their calves sold and the cows will not be bred back for a subsequent lactation. Cows with ELISA scores above 0.5 are considered major threats for transmission of *M. paratuberculosis* on this dairy and will be immediately culled. In addition, any of their daughters that are still in the herd will be automatically considered low risk and tagged accordingly. This testing and management scenario will be utilized until no further reductions in milk ELISA test prevalence are observed over two successive years. Annual whole-herd fecal analysis using the AntelBio Rapid Fecal Test ($12,000 per year = 600 animals x $20 per test) will follow and fecal positives eliminated until zero test prevalence is achieved for two successive years. Application for state Johne's certification will be evaluated on a continual basis.

Figure 5

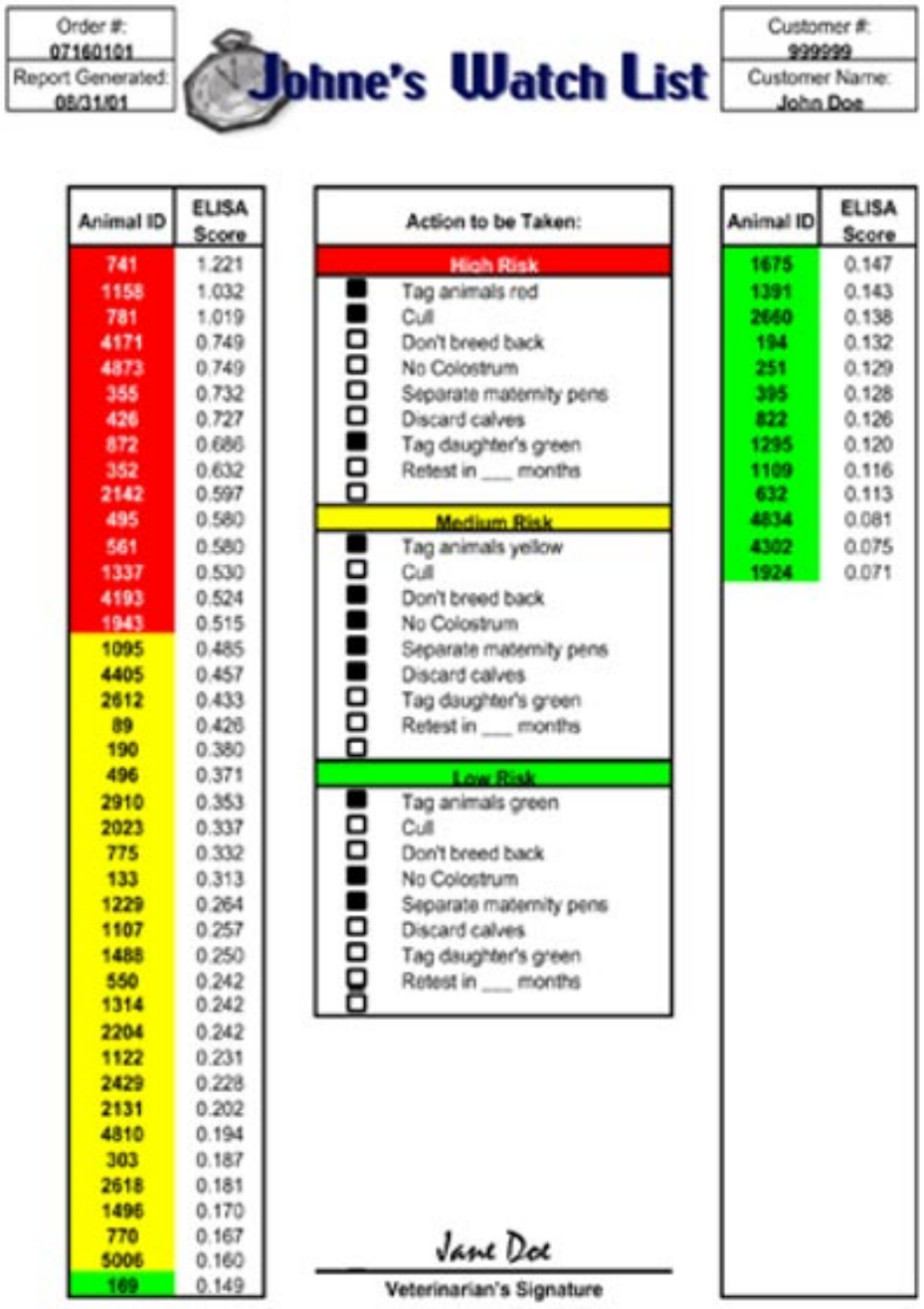

Figure 5. Johne's Watch List for Case Study B.

Risk Factors

Several risk factors were identified in the evaluation of animal management practices utilized by Case Study B. Specific to this case study, the following factors were identified as potentially contributing to the Johne's problem in the herd.

- The feeding of pooled colostrum and specifically waste milk to potential replacement heifers was identified as the greatest risk factor for the transmission of Johne's disease. Banked colostrum from ELISA negative cows and milk replacer were adopted as alternatives. Procedures for the pasteurization of waste milk are being considered.
- Another risk factor was the common calving area, which has since been separated into three maternity pens, one for negative cows, one for low risk cows and one for moderate risk cows.
- The Johne's testing and management system adopted by Case Study B will require the purchase of replacement heifers to maintain animal numbers. Purchasing should be limited to herds with an established record of zero test prevalence.

Next Step

Entrance into the state Johne's certification program will be evaluated on an ongoing basis. Once certified as a status herd, they will continue testing according to the certified protocol in order to achieve and maintain the most advanced level (Level 4) in the status program.

Case Study C

History

Case Study C is a closed purebred Angus operation that began with the purchase of 20 bred heifers in 1978 and has since expanded to 120 cows. The animals are rotationally grazed on improved pastures with a common watering hole. Cows are housed in a large dry lot over the winter months and supplemented with corn and hay. Bulls are available for natural breeding in the summer. Born in late spring (April-May), the calves are weaned in September and heifers and select bull calves are sold as seed stock to commercial cow/calf producers. Johne's disease has never been observed in this herd. After noticing the sales advantage of test negative animals in recent sales, the owners inquired about testing bulls and heifers for Johne's disease. Explaining that 1) Johne's tests are not meant to be used as individual animal tests, 2) the performance of Johne's tests are poor on young animals and 3) official programs are available for the certification of herd-level Johne's status, AntelBio suggested a consultation with the herds' veterinarian regarding inclusion in the State Voluntary Johne's Program.

Goals

The goal of this operation is to achieve and maintain official certification of Johne's-free status. Given the absence of any Johne's diagnosis in the past five years, the herd veterinarian has opted for the fast track to achieve Status Level 2 the first year and Level 4 after the second year.

Testing Scenario

Status Level 2 requires a negative screening test on a statistical subset (all eligible animals for Case Study C) of second or greater lactation animals. Serological ELISA results of 103 second or greater lactation animals sampled by an accredited veterinarian are presented in Figure 6. Two animals tested positive and fecal samples from these two animals were collected the following week to appeal the initial screening results. Both the AntelBio Rapid Fecal Test and traditional culture were requested on these samples. Results from the AntelBio Rapid Fecal Test were requested in 72 hours to expedite the removal of these two animals and their calves from the herd in the event they were indeed positive. Traditional culture was requested for official appeal of the initial screening to achieve Status Level 2 after a 16-week waiting period. The AntelBio Rapid Fecal Test did not identify Mptb in either of the fecal samples taken from the two seropositive animals. Culture results are pending. The total cost for this round of testing was $739 [(103 animals x $4.75 per serum ELISA) + (2 animals x $100 Rapid Fecal Test) + (2 animals x $25 per traditional culture)].

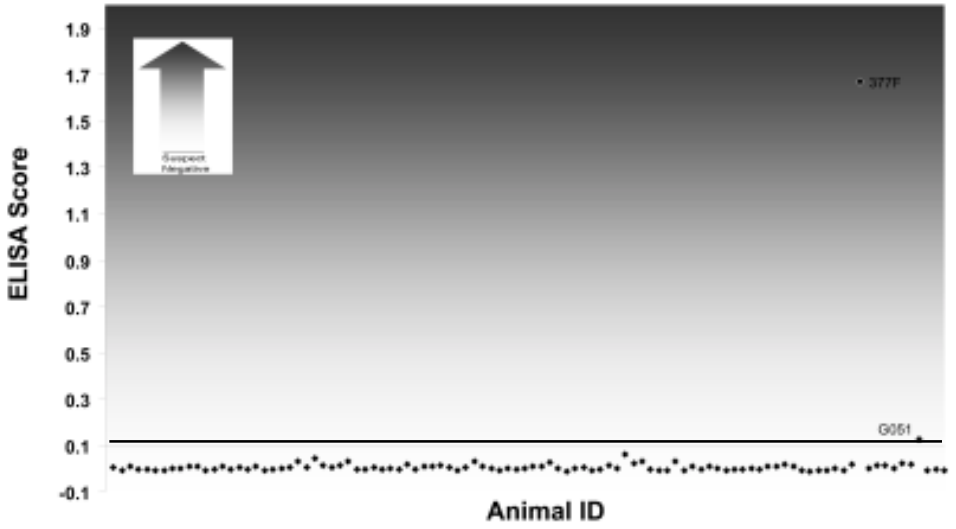

Figure 6. Serum ELISA results on second or higher lactation animals.

Figure 6

Interpretation

Although the stated specificity of commercial serum ELISA tests suggests false positives are unlikely, the positive predictive value (PPV) of any single test is dependent on herd-level Johne's prevalence. The low estimate of prevalence in the initial serological screening of this herd (2 percent test prevalence, 5 percent true prevalence) yields a PPV value below 60 percent for these two test results. Certainly, fecal confirmation and appeals to ELISA screening results are warranted in this case and are corroborated by the negative results returned by the AntelBio Rapid Fecal Test. This example highlights the value of prevalence estimates from whole-herd tests for the interpretation and use of individual test results.

Risk Factors

While purebred beef operations lack most of the common risk factors found in dairy operations, such as high animal density, excessive manure buildup, pooling colostrum, and feeding waste milk, significant risk factors specific to beef operations do exist.

Risk Factors

While purebred beef operations lack most of the common risk factors found in dairy operations, such as high animal density, excessive manure buildup, pooling colostrum, and feeding waste milk, significant risk factors specific to beef operations do exist.

- Use of a pond for watering purposes is common practice, but these sources of water are often fed by the runoff from rainwater and easily contaminated by animals entering the pooled water. Especially in late spring during calving, rainwater can carry Mtpb from contaminated feces into these water sources providing regular, concentrated doses of the organism while watering. Access to these areas should be restricted in favor of "cleaner" water sources.
- The maintenance of cow/calf pairs until weaning significantly increases the probability of transmission from mother to daughter through repeated suckling and proximity. Replacements from even suspect dams should be avoided.
- Grazing allows continual access to contaminated feed sources. Avoid manure buildup on pastures to minimize contamination of these feed sources, especially on pastures where calving occurs.

Next Step

- Negative results from traditional culture will confirm a negative screening test and Case Study C will be certified Status Level 2.
- Status Level 3 can be achieved with negative fecal culture results on 30 second or higher lactation cows and all bulls two years and older. Both seropositive cows from the initial screen should be included in this sampling. Fecal samples must be collected between 10 and 14 months from the initial herd screen. The cost for this round of testing is $700 (35 animals x $20 per traditional fecal culture).
- Status Level 4 can be achieved with a negative whole-herd serum ELISA (statistical subset) within 10-14 months of achieving Status Level 3. The cost for this round of testing is $475 (100 animals x $4.75 per serum ELISA).
- Status Level 4 is maintained with annual negative serum ELISA on 30 second lactation or higher cows. The annual cost to maintain Status Level 4 is $180 (30 animals x $6 per serum ELISA).
- It is important to review the guidelines dictating entrance and participation in certified voluntary Johne's control programs. Deviation from these guidelines could mean loss of status level.