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Characterization of Johne's disease in Mississippi cattle

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CHARACTERIZATION OF JOHNE'S DISEASE IN MISSISSIPPI CATTLE

By

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The purpose of this study was to characterize Johne's disease in Mississippi cattle. Nine hundred eighteen animals from 23 sale barns in Mississippi were tested for *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Ten milliliters of blood and 4-10 grams of feces were collected from cattle over two years of age presented to the attending auction veterinarian. Information obtained at the time of collection included the animal's sex, type, and reproductive status. Serum samples were screened by an enzyme-linked immunosorbent assay (ELISA) for MAP. Shedding status was determined using polymerase chain reaction (PCR) on corresponding fecal samples. Overall, 17.3% (4/23) of sale barns had at least one animal sero-positive for MAP and 0.54% (5/918) were PCR positive. These results show a Johne's disease prevalence similar to the estimate of 0.4% of animals infected found by the USDA NAHMS Beef '97 study, emphasizing the need for continued prevention and control practices.

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CHAPTER I

LITERATURE REVIEW

Introduction

Johne's (yo –knees) disease is an infectious, granulomatous enteritis caused by the host immune response to an infection with the bacterium *Mycobacterium avium* ssp. *paratuberculosis* (Hill, West et al. 2003). The end result of Johne's disease in cattle is extreme diarrhea causing severe weight loss, eventually ending in death (Hill, West et al. 2003). It is a prevalent and economically important disease that affects cattle and other ruminants and economically impacts the cattle industry (Collins, Gardner et al. 2006). It is estimated that Johne's disease costs the dairy industry $200-250 million every year. It has been estimated that beef and dairy producer's annual loss from Johne's disease is $75-100 per adult animal (Chiodini et al. 1984). Because little research has been done in beef cattle, the current study is being conducted to help understand how Johne's disease affects beef production systems. Johne's causes significant death, culling, and production and reproduction losses due to clinical and subclinical disease (Radostits et al.; Stabel 1998; Manning and Collins 2001). Johne's is on the list of "multiple species diseases" notifiable to the World Organization for Animal Health (International Office of Epizootics. Biological Standards Commission. 2004). Johne's disease primarily occurs

in domestic and wild ruminant species. However, it has been reported in non-ruminant species (Anderson, Meece et al. 2007). Johne's is not a curable disease although treatments are available but considered unapproved, expensive, and long term. It is usually considered cost prohibitive and unpractical to treat this disease (Manning and Collins 2001).

History

J. McFadyean coined the term "Johne's disease" in the Annual Report for 1906 of the Principal of the Royal Veterinary College in London, England. Most publications since then have used either Johne's disease or paratuberculosis when referring to the disease. The term was coined for the enteric disease first named "pseudotuberculosis enteritis" by veterinary pathologist Dr. H.A. Johne, along with an American associate Dr. L. Frothingham in 1894. They discovered the organism causing this disease was *Mycobacterium avium*, similar to the bacterium that causes tuberculosis in birds. They isolated the organism from tissues of a cow that was purchased and had failed to produce milk or gain weight satisfactorily and eventually died. The veterinarian who had examined the animal and noted the diarrhea and weight loss submitted the tissues to the Veterinary Pathology Unit in Dresden where Johne and Frothingham examined them. Upon examination they noted thickened intestinal mucosa and enlarged mesenteric lymph nodes and observed many bacteria in the tissues using acid-fast stain. This type of chronic wasting enteritis had been described as far back as 1826 (Collins and Manning 2001).

Johne's disease was first described in the U.S. in 1908 by Dr. Leonard Pearson. It has since been diagnosed worldwide. Much has been learned about Johne's disease since its discovery. It is now widely accepted that the organism causing Johne's disease is *Mycobacterium avium* subspecies *paratuberculosis* (Hill, West et al. 2003). There is no cure for Johne's disease, no good treatment, and prevention can be difficult, but accomplished through biosecurity and good management. Although much knowledge has been gained through federal and private research endeavors, much more remains to be learned about this disease.

Biology

Mycobacterium avium ssp. *paratuberculosis* (MAP) is a relatively small, slow growing, mycobactin-dependent acid-fast bacterium (Sweeney 1996). MAP is a facultative intracellular bacterium that is an obligate parasite. The cell wall of mycobacteria is composed of a thick waxy mixture of lipids and polysaccharides. The cell wall of MAP is not very well studied, but seems similar in most respects to that of other mycobacterium. One feature is notable, however. While most strains of *M. avium* subsp. *avium* produce a surface glycolipid that allows strains to be serotyped (i.e., distinguished using antibodies specific for each glycolipid subtype), MAP strains lack such glycolipid antigens on their surface. On a genetic basis, *M. paratuberculosis* is virtually identical to *Mycobacterium avium*. Phenotypic characteristics of *M. paratuberculosis* are, however, different from those of *M. avium*: *M. paratuberculosis* grows much more slowly, requires an iron-transport chemical known as mycobactin for in vitro growth, forms rough colonies on solid agar media, and infects mammals instead

of birds (Collins and Manning 2001). Due to its fastidious nature this bacterium is very difficult to culture.

MAP typically affects ruminant species. Animals affected by Johne's include cattle, sheep, and deer, as well as many other ruminants. Studies have also demonstrated the presence of MAP in non-ruminant wildlife such as rabbits (*Oryctolagus cuniculus*) (Greig, Stevenson et al. 1999); a red fox (*Vulpes vulpes*), stoats (*Mustela erminea*), a weasel (*Mustela nivalis*), a vole (*Microtus agrestis* and *Clethrionomys glareolus*), a crow (*Corvus corone*) (Beard, Rhind et al. 2001); and feral cats (*Felis familiaris*) (Palmer, Stoffregen et al. 2005). MAP has also been cultured from the ileum of a coyote in Wisconsin (Anderson, Meece et al. 2007). The presence of MAP in such species offers the possibility of non-ruminant wildlife being able to spread the disease.

Pathology

Infection occurs from MAP when M cells in the intestinal lumen ingest the bacterium and take them to Peyer's patches to present them to the macrophages for phagocytosis. This concept was illustrated in a study that developed results suggesting calf ileal M cells take up the bacilli, and epithelial macrophages phagocytose the bacilli and bacterial debris expelled from these M cells (Momotani, Whipple et al. 1988). A contrasting study showed that it is not M cells that aid the invasion of intestinal mucosa with MAP, but actually enterocytes (Sangari, Goodman et al. 2001). Regardless, once inside the macrophages, MAP can survive and replicate, spreading to other macrophages and organs. More macrophages and lymphocytes are then recruited to fight the infection. Lymphocytes release cytokines to increase the killing power of the macrophages. Giant

cells are formed to help combat the infection. Invasion of all these defense mechanisms, into infected tissues, causes inflammation of the intestine. MAP typically inhabits the intestinal tract and mesenteric lymph nodes (Sweeney 1996) with the ileum being the predominant site of infection. The ileum contains the highest concentration of Peyer's patches making it the most prone location for lesions from MAP to occur. When these areas of the intestine become thickened nutrient absorption is inhibited. This causes diarrhea, which is the beginning sign of clinical Johne's disease in cattle. With Johne's disease the diarrhea may be intermittent at first and the animal's appetite may stay the same or possibly increase slightly. Eventually the diarrhea becomes persistent and severe, and body condition is gradually lost. When the intestine is unable to absorb protein correctly, protein-losing enteropathy occurs. "Bottle-jaw," edema in the submandibular region, may be seen as result of hypoalbuminemia resulting from the protein-losing enteropathy. In the terminal stage of the disease, the animal is in a wasting state, where all body condition has been lost. If the animal is not euthanized it will eventually perish due to malnourishment.

Because MAP is slow growing, it can take several years for clinical signs to develop in an infected animal. This causes much difficulty in diagnosing Johne's disease. Clinical signs typically do not occur in animals less than 2 years of age (Wu, Livesey et al. 2007).

Diagnosis

Currently there are several detection methods being used for Johne's disease. These detection methods are typically based on antigen or antibody detection. Tests for

Johne's include: serology, culture, polymerase chain reaction (PCR), and histology. There are different tests for each category. Serologic tests include enzyme linked immunosorbent assay (ELISA), agar-gel immunodiffusion (AGID), and complement fixation (CF). The ELISA may be the most widely used serologic test due to its rapid turn around time and relatively low cost (Collins, Wells et al. 2005). It has been reported that 2 widely used commercial ELISA kits have specificities reported at 95.3% and 99.7% when being used to test a large number of well characterized samples (Collins, Wells et al. 2005). However false positive rates can be higher than expected (Kalis, Barkema et al. 2002; Roussel, Libal et al. 2005; Roussel, Fosgate et al. 2007). One study found that some herds with other mycobacterium isolated from feces were more likely to be seropositive for MAP (Roussel, Fosgate et al. 2007). A second study showed environmental mycobacteria could cause false positives with MAP ELISA test kits (Osterstock, Fosgate et al. 2007). Results from a herd screening using a commercially available ELISA in beef cattle in Texas showed the proportions of false-positives were greater than expected based on the reported assay specificities (Roussel, Libal et al. 2005). A potential cause for these false-positive results in beef cattle is their exposure to *Mycobacterium* spp that may have antigenic similarity to MAP and hence induce production of serum antibodies that cross-react with antigens in conventional serologic tests (Osterstock, Fosgate et al. 2007). Testing with ELISAs is recommended for cattle herds for which the objective is to identify positive herds in an attempt to reduce economic impact (Collins, Gardner et al. 2006).

The AGID test is a simple two-day test to confirm a diagnosis of Johne's disease in cattle showing clinical signs of Johne's disease. AGID is typically used as a rule-in

test for Johne's in animals showing clinical signs. It is 100% specific, but the sensitivity is too low for this test to be used as a screening tool in cattle (Collins and Manning 2001).

Complement fixation (CF) is still used in some trade markets but for the most part is outdated. The CF test sensitivity has been reported at 10.8% (Sherman et al. 1990). This test may be more difficult to perform and interpret than the AGID (Sherman et al. 1990). This test can be used to find antigen or antibody in serum and is a delicate test with multiple steps (Barrett 1978). Published evaluations of the CF test for Johne's disease indicate that the sensitivity and specificity are less than those of the other commercially available tests for Johne's disease. Most countries are moving away from use of the CF test in favor of the ELISA (Collins and Manning 2001).

Fecal testing is performed via culture and polymerase chain reaction (PCR), which are recognized organism detection methods. Fecal culture is considered the gold standard because it is supposed to be 100% specific. Sensitivities of fecal culture have been reported to range from 38-55% (Sockett, Carr et al. 1992; Whitlock, Whitlock et al. 2000). It has been reported that test sensitivity is a direct function of the distribution of the infection stages in the test population (Collins and Sockett 1993). The MAP organism is dependent on mycobactin to survive and grow in culture. When culturing for MAP, a control culture is used that does not contain mycobactin while the other cultures contain mycobactin, providing the proper environment for MAP growth. If a sufficient amount of MAP is present in the sample being tested, fecal cultures can be used to generate reliable test results. This type of test is costly and time consuming, taking up to 16 weeks to complete, as well as space consuming. For this reason, the use of PCR has been gaining acceptability at a rapid pace and is used commonly now in diagnostic

laboratories. It has been determined that the insertion sequence 900 (IS900) is the key aspect of the genetic makeup of the MAP organism that allows it to be distinguished from other mycobacterium. Biopsies of the ileum and ileocecal lymph nodes can be tested in the same manner as fecal samples via PCR. Biopsies can also be examined histologically to detect the organism. The most definitive and sensitive method for use in confirming a diagnosis of paratuberculosis is a complete necropsy, which should include recording gross lesions and obtaining ileal and mesenteric lymph node tissues for bacterial culture and histological examination (Collins, Gardner et al. 2006). Because of the nature of this disease, these tests should be performed and interpreted by an experienced veterinarian who is qualified to make a proper diagnosis.

Sensitivity and specificity refer to characteristics of a diagnostic test. The higher these aspects of a test are the more reliable a test is in diagnosing a disease. Sensitivity is defined as how effective the test is at correctly identifying animals with the disease (Petrie et al. 2006). Specificity is defined as how effective the test is at correctly identifying animals without the disease (Petrie et al. 2006). Highly sensitive tests tend to produce fewer false negative results while highly specific tests have fewer false positive results (Smith 2005). Knowledge of test sensitivity and specificity can help determine which test is best suited for a particular situation. Typically highly sensitive tests are used to rule out a disease. Highly specific tests are generally used when a rule in diagnosis is desirable (Smith 2005). Often a combination of tests is used to properly diagnose a disease. As in the case with Johne's many times a herd is screened with an ELISA and any positives are then followed up with PCR or culture. The perfect test, a

gold standard, would be 100% sensitive and 100% specific and would give a definitive diagnosis. Unfortunately these tests do not exist for many diseases.

Predictive values are just as important as sensitivity and specificity, especially when evaluating test results between different populations. These numbers may be higher or lower depending on the disease status of the population being tested. Predictive values give an indication of the usefulness of the test in an animal population (Petrie et al. 2006). Predictive values are the probability that an individual test result reflects the true disease status of the individual (Smith 2005). A positive predictive value is the proportion of animals with a positive test result that are truly positive for the disease. A negative predictive value is the proportion of animals with a negative test result that are truly negative for the disease (Petrie et al. 2006).

Results from different tests are reported in different manners. Tests such as cultures, AGIDs, histological tests, and PCRs often report the result as positive or negative. ELISA tests, on the other hand, are not as simple. Their results are reported, as a numerical value, as sample to positive (S/P) ratios. S/P ratios are calculated from optical densities (OD) of the reactions (Collins 2002). Therefore it is important to understand how the ratios and cut off values are established for a particular laboratory.

The ELISA for Johne's disease detects antibody in the serum of cattle. The serum sample is placed in the wells of the plate and a conjugate is added to it. If Johne's antibody is present when the conjugate is added, the liquid in the well changes color. The higher the antibody concentration is in the sample the stronger the reaction to the conjugate and the more intense the color change. The samples are then placed on a plate reader where light is passed through each sample on the plate. The more light that passes

through the sample the lower the optical density is. Conversely the less light that passes through the sample the higher the optical density is. Every time a plate of samples is run a positive and negative control are included in the same plate. After obtaining the ODs from all samples the test samples can be compared to the control samples. The computer software then calculates the S/P ratios. The cutoff S/P ratio for a Johne's ELISA positive result is typically .25 (IDEXX Laboratories 2007).

Epidemiology

In most cases animals become infected with MAP soon after birth (Sweeney 1996) but do not typically show signs of disease until 2-5 years of age (Garry, Wells et al. 1999). The long latency period of the disease contributes to the difficulty of identifying and controlling it. Transmission of the organism causing Johne's disease is fecal-oral (Sweeney 1996). The most probable source of infection with MAP is contaminated feed and water (Garry, Wells et al. 1999). Feed can become contaminated by different means. Hay or feed dropped on the ground can become contaminated with MAP from feces and then infect an animal when it is ingested. Contamination of feed can occur if manure gets into a feed trough by any means. Natural water supplies can become contaminated from runoff of pastures or if contaminated manure gets into the water source. One study found 38% of runoff samples collected were culture positive for MAP (Raizman, Wells et al. 2004) Artificial water sources can be contaminated by an animal defecating in the water source. While not as common, a subclinically infected animal that is purchased and brought onto the farm can spread the disease by shedding MAP in manure (Sweeney 1996).

The amount of MAP an animal is exposed to contributes to whether or not that animal becomes infected. One study showed that a large inoculum resulted in a higher level of colonization of the lymph nodes than did a smaller inoculum, implying that the intestinal invasion and/or movement of the organisms to the lymph nodes is a dose-dependent process (Wu, Livesey et al. 2007). Calves are the most susceptible to infection with MAP (Garry, Wells et al. 1999) and a small dose of MAP may be all that is needed to infect a newborn calf (Sweeney 1996). Calves can become infected by being born in a contaminated environment and by nursing a teat contaminated with MAP (Sweeney 1996). For these reasons it is a good management practice to provide a clean environment for calving. Calves can become infected if the dam is infected and shedding the bacterium into colostrum and milk (Garry, Wells et al. 1999). It may also be possible for calves to become infected in utero (Sweeney et al. 1992). The older an animal becomes, the less likely it is to become infected (Larsen et al. 1975).

Other methods of transmitting Johne's disease have been suggested, including in semen, in sex organs of bulls and embryo transfer. One study isolated MAP from semen, seminal vesicles, and prostate gland of a bull (Larsen et al. 1981). Inoculation of the uterus with MAP can result in infection of the cow (Sweeney 1996). Uterine flush fluids have been found positive for MAP (Rohde et al. 1990a). MAP has also been isolated from washed bovine ova after in vitro exposure (Rohde et al. 1990b). Therefore it is theoretically possible for embryo transfer to result in an infected fetus (Sweeney 1996). However, one study concluded that MAP is unlikely to be transmitted by embryo transfer when the embryo has been washed as recommended by the International Embryo Transfer Society (Bielanski et al. 2006).

It has been suggested that soil type and pH where cattle are raised may influence the survival of MAP in the environment and therefore influence transmission of the disease. It has been shown that survival of MAP may be enhanced by silt or sand content in loamy soils (Ward and Perez 2004). A study done in Michigan found that the prevalence of MAP positive dairy herds was positively associated with acidic soil and increased iron content. The same study found that application of lime to pastures was associated with reduced risk of MAP (Johnson-Ifearulundu and Kaneene 1999). Another study gathered information that was valuable in showing the strength of association between entisol soil types and herd prevalence of MAP in ovine and caprine flocks. This study suggests that the data gathered are highly indicative of the role of the soil type, as an important part of environmental conditions, in the epidemiology of MAP (Reviriego, Moreno et al. 2000). These findings may be applicable for cattle herds as well. Additionally, a study aimed at defining the role of earthworms in the survival of mycobacteria found that earthworms may become vectors for mycobacteria (Fischer, Matlova et al. 2003). In epidemiology, criteria for determining causal associations have been established. The basic elements include: strength of association, consistency, specificity, temporality, biological gradient, plausibility, coherence, experimental evidence, and analogy (Hill 1965). A literature review was conducted and the findings for each element were published to define the relationship between soil type and MAP (Johnson-Ifearulundu and Kaneene 1997). One study did appear to indicate a biological gradient, but lacked the information needed to support the fact that increased exposure caused increased cases of disease. Information in the literature showed that

environmental pH and iron availability are crucial factors influencing growth of MAP (Johnson-Ifearulundu and Kaneene 1997).

The National Animal Health Monitoring System (NAHMS) Beef '97 study indicated that approximately 0.4% of adult beef animals are infected with Johne's disease and approximately 8% of beef herds in the US are infected (United States. Animal and Plant Health Inspection Service. Veterinary Services. Centers for Epidemiology and Animal Health. and N309.899 1999). In that study 10,372 cows in 380 herds from 21 states were tested using an ELISA. Forty of the 10,372 samples were positive for antibodies to MAP, giving 0.4% of the animals positive. Those 40 positive animals came from 30 (7.9%) of the herds tested. The estimate of 7.9% herd prevalence should be considered a conservative estimate because of the testing protocol set for the study (United States. Animal and Plant Health Inspection Service. Veterinary Services. Centers for Epidemiology and Animal Health. and N309.899 1999). The testing protocol was designed to identify herds that had at least 10% prevalence. Using this type of testing protocol it is possible to misclassify a herd that has disease prevalence less than 10%. The NAHMS Dairy '96 (National Animal Health Monitoring System (U.S.), National Animal Health Monitoring System (U.S.) et al. 1996) study showed approximately 21.6% of dairies in the U.S. are infected, with a prevalence rate of at least 10%, and that 3.4% of dairy cows are infected with Johne's. One very important fact discovered in the Beef '97 study was that knowledge of Johne's disease among beef producers is very limited. It showed that 92.2% of beef producers were either unaware of Johne's or recognized the name but knew very little about the disease. This fact highlighted the need for education on Johne's and gave a reason for the lack of adoption of Johne's prevention efforts. The

NAHMS Dairy '96 study was able to estimate the loss due to Johne's disease for the dairy industry is approximately $200-250 million annually. The Beef '97 study was unable to estimate the annual cost to the beef industry because it was not designed to be analytical of the economics of Johne's disease.

A regional study was done in 2003 to try to determine the overall seroprevalence of animals infected with Johne's disease in Alabama beef cattle. Samples were obtained from the C.S. Roberts Alabama Veterinary Diagnostic Laboratory through the Alabama Brucellosis Certification program (Hill, West et al. 2003). For the study the samples were randomly selected from 79 herds but the herds were not randomly selected from the population of interest, Alabama beef cattle. The study was conducted on serum samples taken from the brucellosis program from October through November 1998 and April through May 1999. A possible bias exists with this study because herds in the Brucellosis Certification program may be better managed herds or possibly herds that are involved in other health programs such as Johne's. Eight percent (166/2,073) of the total number of animals tested was ELISA positive. The study calculated the true prevalence for Johne's disease in Alabama to be 8.75% ± 1.5%, after adjustments were made for test sensitivity and specificity and the proportion of animals sampled per herd. Herds identified as Johne's positive herds were calculated to be minimally 53.5% of the herds in the state of Alabama (Hill, West et al. 2003).

A regional study done in Florida in 1990 estimated the seroprevalence of Johne's to be 17.1% in dairy cattle and 8.6% in beef cattle (Braun et al. 1990). In this study 3,874 beef cattle from 392 and 617 dairy cattle from 60 herds were obtained from February 1986 to February 1987. Approximately 10 samples were taken from each herd. These

samples were not obtained by a randomized plan but were obtained as they came available through the Brucellosis Testing Program in Jacksonville, FL.

In Georgia, a survey was conducted in 2000 to determine the seroprevalence of Johne's disease in the cattle population. Data was gathered from the random sampling of 5,307 serum samples collected for brucellosis testing. The samples had been taken from sale barns across the state of Georgia from June 1999 to June 2000; 251 (4.73%) of the samples tested positive for antibodies against the Johne's organism. When the data was broken into cattle type, 3.95% of beef cattle were positive, 9.58% of dairy cattle were positive, and 4.72% of unknown type cattle were positive (Pence, Baldwin et al.).

A survey done in the fall of 1999 in Saskatchewan on herds using community (shared) pastures showed an apparent prevalence of 0.8% (0.4% to 1.5%). Sixty-six herds, in their entirety, from 4 community pastures were sampled during routine pregnancy checking. All 1799 cows from these pastures were sampled with 15 (0.8%) positive. After adjusting for test sensitivity and specificity, the true prevalence of Johne's was not significantly different from 0.0%. However, it is unlikely that all of the samples with high S/P ratios were false positives. It was noted that 3 of the samples with high S/P ratios came from herds with no previous signs of Johne's. This fact emphasizes the potential for infection in herds with no previous history of clinical Johne's disease (Waldner, Cunningham et al. 2002).

A small scale serologic study was conducted in 2000 by the Mississippi Board of Animal Health on auction cattle in Mississippi. In that study 815 animals were tested with 38 found to be positive, resulting in a 4.75% seroprevalence (Watson 2000).

Clearly, variations exist in the estimates of Johne's disease in cattle. It is widely accepted many environmental and management factors influence the presence of the disease. The purpose of this study was to further characterized Johne's disease in Mississippi cattle.

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CHAPTER II

PREVALENCE OF JOHNE'S DISEASE IN MISSISSIPPI AUCTION CATTLE

Carter, JL; Huston, CL; Zhang, S; Hostetler, DE; Warren, RV

Abstract

Johne's (yo –knees) disease is an infectious, granulomatous enteritis caused by the host immune response to an infection with the bacterium *Mycobacterium avium* ssp. *paratuberculosis* (Hill, West et al. 2003). Johne's disease affects domestic and wild ruminants (Manning and Collins 2001) worldwide and causes much economic loss to the cattle industry. The disease is reported to cost beef and dairy producers $75-100 per animal, annually (Chiodini, Chiodini et al. 1984). The purpose of the present study was to characterize Johne's disease in Mississippi cattle. Nine hundred eighteen animals from 23 sale barns in Mississippi were tested for *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Overall, 17.3% (4/23) of sale barns had at least one animal positive for MAP. Of all animal samples, 0.54% (5/918) were PCR positive. These results show a Johne's disease prevalence similar to the estimate of 0.4% of animals infected found by the USDA NAHMS Beef '97 study, emphasizing the need for continued prevention and control practices.

Introduction

Johne's disease is a worldwide problem affecting cattle and other ruminants. In the U.S., Johne's cost the dairy industry $200-250 million annually (United States. Animal and Plant Health Inspection Service. Veterinary Services. and N245.1097 1997) Johne's causes significant death, culling, and production and reproduction losses due to clinical and subclinical disease (Radostits et al.; Stabel 1998; Manning and Collins 2001). To date the research that has been done to determine how much Johne's affects the beef industry has been somewhat inconclusive. Johne's can cause losses to beef cattle much the same as dairy cattle (United States. Animal and Plant Health Inspection Service. Veterinary Services. Centers for Epidemiology and Animal Health. and N309.899 1999). The current estimate for beef herd prevalence of Johne's disease is 8% (United States. Animal and Plant Health Inspection Service. Veterinary Services. Centers for Epidemiology and Animal Health. and N309.899 1999). It is known that Johne's disease is present in Mississippi cattle through a study done by the Mississippi Board of Animal Health that showed an approximate 4.75% seroprevalence (Watson 2000). Although the presence of Johne's is known, the true prevalence of Johne's in Mississippi cattle has not been determined.

The present study was concerned with characterizing Johne's disease in Mississippi cattle, with an emphasis on beef cattle. There has been little research done in the state to determine the prevalence of Johne's, and subsequently little research has been done in beef cattle. This was an auction market study in which 23 livestock auction barns across the state of Mississippi were chosen as collection sites. The samples were tested at the Mississippi Veterinary Research and Diagnostic Laboratory in Pearl, MS. This is the

official statewide diagnostic laboratory that performs all testing for the state funded program. After all testing was complete the data were analyzed to characterize prevalence of Johne's disease in the state of Mississippi.

Materials and Methods

All auctions and attending veterinarians were contacted prior to sample collection. At each barn samples were collected from every other animal that was presented to the attending veterinarian that was at least 2 years of age. A minimum of 700 animals needed to be tested for this study. Due to fluctuations in markets and varying availability of animals in the free market place, a maximum of 1000 could be tested. Samples were collected from September to December 2006 in conjunction with the Mississippi Cooperative State-Federal Brucellosis testing program. At the time of sampling 9-10 mLs of blood was collected from the jugular vein or the caudal tail vein. Using plastic disposable sleeves 4-10 grams of feces were also collected from the rectum of each animal tested. Blood samples were centrifuged at 2500 rpm for 10 minutes and serum was separated. Serum samples were frozen at -20°C until they were packaged and submitted for testing at the Mississippi Veterinary Research and Diagnostic Laboratory in Pearl, MS. Serum samples were tested using enzyme-linked immunosorbent assay (ELISA) and were considered positive if they had a sample to positive ratio (S/P) of .25 or greater[1]. Feces were collected in 50 mL centrifuge tubes and frozen at -80°C and submitted for real time polymerase chain reaction (RT-PCR) only if the corresponding serum sample tested positive.

[1] S/P cut off value was determined by IDEXX Laboratories Validation Report 2007.

All data were entered in spreadsheet format. All statistical analyses were done using SAS software (SAS 2002; SAS 2006) and all statistical data were analyzed at the 0.05 significance level. Sample size was determined using the appropriate proportional data calculation (Smith 2005).

Results

Mississippi has 24 cattle auctions across the state, (Figure 1). Twenty-three of these auctions were used as test sites. Figure 1 shows the location of the positive and negative test sites. A site was considered positive if at least one animal tested positive for MAP on ELISA. Six of the 23 (26%) test sites had at least one animal positive for Johne's disease at the time of testing. Of all animals tested, 43% came from these 6 auctions. Four of the 23 (17%) test sites had at least one animal that was ELISA and fecal PCR positive for Johne's at the time testing.

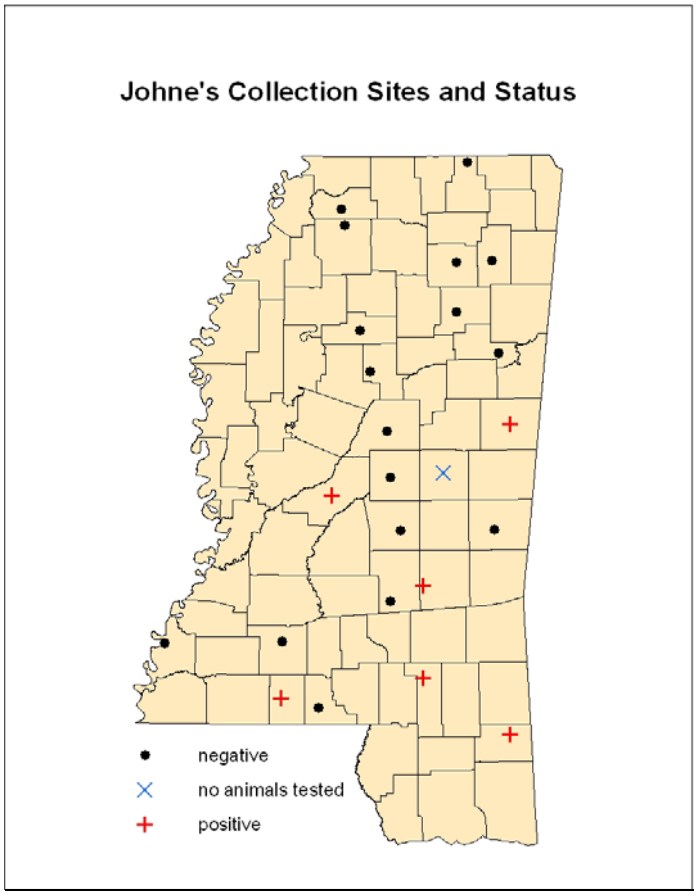

Figure 1

Johne's Collection Sites and Status

A total of 918 samples were collected from cows and bulls randomly selected from the target group of sale cattle. From the samples taken, 909 were from beef animals and 9 were from dairy animals. The 909 beef animals were comprised of 891 cows (474 pregnant, 279 open, 138 unknown) and 18 bulls. The 9 dairy animals were all cows. Of all 918 samples, 9 beef cows were seropositive resulting in a 0.98% (0.3-1.6%) overall seroprevalence of auction cattle. Additionally, any cattle that were found seropositive

were then tested by fecal PCR. Overall, 5 (0.54%) of the animals tested were confirmed positive (PCR) for the organism causing Johne's disease.

The overall seroprevalence and the confirmed (PCR) prevalence and associated confidence intervals were calculated using the means procedure. The data was collected in a binomial form and analyzed with a chi-square test. The overall seroprevalence was 0.98% with a confidence interval of 0.3-1.6%. The confirmed (PCR) prevalence was 0.54% with a confidence interval of 0.06-1.02%. Chi-square tests were performed on the associations with reproductive status, sex, and type (dairy vs. beef) for the seropositive samples. Data were entered and analyzed using Fisher's exact tests. The p-values for each of the associations examined were ($p > 0.5$), indicating no significant differences. The same tests were performed for the groups of confirmed (PCR) positive samples. The p-values for each of those groups were ($p > 0.5$), again indicating no significant differences.

A map was produced to show where the auction markets were located in relation to 9 different soil associations found within the state. It was found that the six positive test sites were located in only three soil associations. Figure 2 shows the location of the auction markets and the soil associations for the state. Although not enough information was gathered during the present study to determine statistical significance of these locations it is interesting to see where these positive test sites lie, and may provide for additional research direction. As stated earlier, it has been suggested that soil type and pH (Johnson-Ifearulundu and Kaneene 1999) may play a role in the epidemiology of Johne's disease (Reviriego, Moreno et al. 2000).

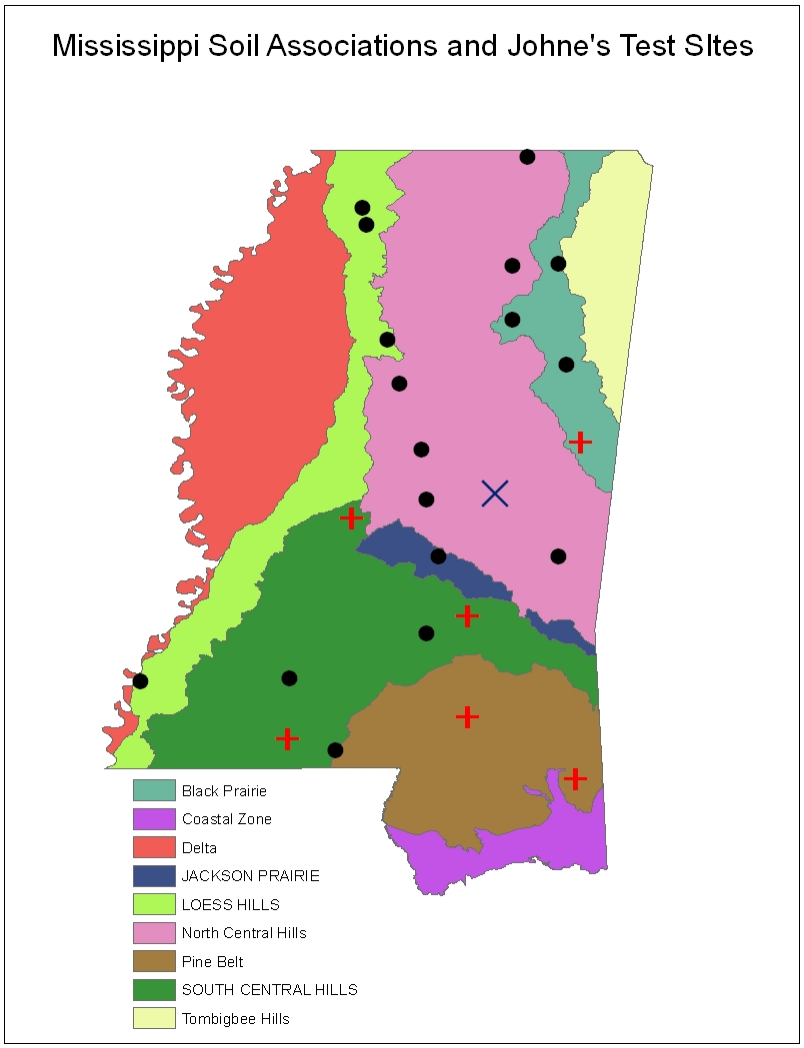

Figure 2

Mississippi Soil Associations and Johne's Test Sites

Discussion

The target population of this study was sale cattle and was assumed to have a higher percentage of cull cattle, and therefore, a higher prevalence of disease was expected. Upon completion of the study very few positives were found. The 0.54% of animals found positive for MAP is very close to the 0.4% of animals infected nationally,

as suggested by the NAHMS '97 beef study. One theory pointed to drought that had been impacting the state. It is possible the markets were flooded with healthy animals that simply could not be supported on the farms from which they came. During periods of drought, forage quality can be poor and maintaining a herd can be difficult (Parish, et al. 2007). If there were more animals in the markets this could dilute the population that was being targeted. In addition, not all the cattle that come to the auction are seen by the veterinarian. This fact could create a sample selection bias that favored healthy animals presented for sale. Many cattle that are going for slaughter and animals that are sick are segregated from the other animals and thus may not be seen by the attending veterinarian. These animals could have quite possibly added significant numbers of positive samples to the data. All of the animals that were tested were sold at the auction and many returned to a farm. Therefore the population of animals tested could be indicative of either healthy culls or possibly on-farm subclinical infections. Another theory for the low number of positive samples could be the biosecurity measures practiced by Mississippi cattlemen and the sale barns. No evidence was found to indicate that Mississippi was any different than other states in the practice of biosecurity measures. Attention was also brought to animal densities. It has been shown that dairies typically have higher rates of Johne's (United States. Animal and Plant Health Inspection Service. Veterinary Services. and N245.1097 1997) than beef operations (United States. Animal and Plant Health Inspection Service. Veterinary Services. Centers for Epidemiology and Animal Health. and (U.S.). 1997). This may be due to the more intensive management practices of dairy operations. Intensity of management on beef operations may also have a role in Johne's disease prevalence. This aspect would be a good topic for further research but was

unable to be addressed in the current study. One interesting bit of knowledge gained was insight in cow trading. Several times throughout the course of data collection for this study, animals could be seen at different auctions within the same week (J. Carter, personal observation). It is possible this practice could increase the risk of exposure to uninfected animals and that "pass through" transmission could play a role in the spread of the disease (Whitlock et al. 2000). Intuitively one would think this practice of cow trading could facilitate the spread of Johne's through cattle auctions, although adult animals are less susceptible. Why these results are lower than the previous study done on the same population of cattle was the overall question that arose. It could easily be any combination of the things already mentioned. The one aspect that seemed to be plausible was that the test kit for Johne's had been updated (IDEXX Laboratories 2006). The new kit had improved specificity which should decrease the number of false positives and therefore decrease the apparent prevalence of Johne's disease. This could influence the outcome of the study and account for at least some of the difference.

Currently, biosecurity, along with good management practices, are the only measures available in Mississippi to control Johne's. There are several ways biosecurity measures can be instituted on a farm. These include: testing and culling animals with the disease, purchasing animals only from herds that are low risk herds, and implementing biosecurity practices on farm. Additional practices that can be implemented are isolating herd additions, ensure visitors wear boot covers, and cleaning and disinfecting equipment (Wolfgang).

Conclusion

Although the prevalence estimates from the present study were lower than expected, it is still apparent that Johne's disease is a problem in Mississippi cattle. If 1 clinical case is found in an animal that was born on the farm, a minimum of 25 other animals are probably infected (Whitlock and Buergelt 1996). Further investigation into the effects of Johne's in beef herds is undoubtedly warranted. For example, collecting and testing various environmental samples may be useful in learning about the disposition of the Johne's organism. Continued study of Johne's in beef cattle is needed to determine the effects the disease has on production. With more information on the effects of the disease, emphasis on eradication efforts will be greatly improved and economic losses from the disease could be minimized. Although improvement has been made in Johne's testing, better tests are still needed to be able to diagnose the disease at earlier stages to enhance control of Johne's disease. Until science can develop a way to eliminate or eradicate Johne's disease, control of the disease will be left up to producer's management practices. The continued involvement of the state's cattle producers in national prevention and control programs will be critical to the control of Johne's disease in Mississippi cattle.

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CHAPTER III

MISSISSIPPI DEMONSTRATION HERD PROJECT

Carter, JL; Huston, CL

Abstract

The National Johne's Disease Demonstration Project was proposed in 2002 by an Ad Hoc Steering Committee of the U.S. Animal Health Association (USAHA) Committee on Johne's Disease (USDA-APHIS-Animal Health Monitoring & Surveillance). The purpose and objectives of the project were "to develop and validate model strategies for control of Johne's disease". Data collection in Mississippi began in May 2004. Annual risk assessment and management plans (RAMP) were conducted along with annual herd testing. Blood was collected and tested by enzyme-linked immunosorbent assay (ELISA) and positives were followed up with fecal culture. Currently 6 herds are participating in the Demonstration Herd Project in Mississippi. The apparent prevalence of Johne's disease has decreased for most of the herds participating in this project.

Introduction

The National Johne's Disease Demonstration Herd Project (NJDDHP) was proposed in 2002 by an Ad Hoc Steering Committee of the U.S. Animal Health Association (USAHA) Committee on Johne's Disease (USDA-APHIS-Animal Health Monitoring & Surveillance) . In fiscal year 2003, $1.5 million was allocated to develop and support the project by funding it for at least 5 years.

The purpose and objectives of the project were "to develop and validate model strategies for control of Johne's disease". The main objective is to evaluate the long-term effectiveness and feasibility of management-related disease control measures on Johne's disease and infection on dairy and beef cattle operations. Secondary objectives were to educate and train veterinarians and producers; find strategies to control Johne's in cattle herds; and to create opportunities for related research.

Data collection in Mississippi began in May 2004. Herd selection and testing was performed and the herds were enrolled in the Mississippi Voluntary Johne's Disease Control Program. Herds with a history of Johne's disease were chosen with the assistance of their regular veterinarian. Additional herds were chosen throughout the study to replace herds that had been removed from the study for various reasons. New educational materials were developed and delivered throughout the state during the project period.

The Mississippi Voluntary Johne's Disease Control Program is cooperative effort between the Mississippi Board of Animal Health (MBAH) and USDA APHIS Veterinary Services. The program consists of a test-negative "Status Program" for herds wishing to

certify “test-negative” and a “Management Plan” for herds that are infected. It also follows guidelines from the National Johne’s Disease Working Group of USAHA.

Materials and Methods

Mississippi began data collection for the Demonstration Herd project in May 2004. In the initial selection process 5 herds were selected (Demo 1-5), three beef herds and two dairy herds. Four additional herds were enrolled in the program to account for original herds that were found to contain no positive animals. Herd selection was done by contacting private veterinarians and asking them to enroll herds with prior history of Johne’s disease. These herds were enrolled in the Mississippi Voluntary Johne’s Disease Control Program as well. The criteria used for herd selection was that one or more animals in the herd had previously tested positive for Johne’s disease. A risk-assessment and management plan (RAMP) was conducted for each herd. The herds then underwent whole-herd ELISA testing with fecal culture follow-up on any seropositive animals. Environmental samples and animal information was collected at the same time. At the time of sampling 9-10 mLs of blood was collected from the jugular vein or the caudal tail vein. Blood samples were centrifuged at 2500 rpm for 10 minutes. Serum samples were frozen at -20°C until they were packaged and submitted for testing at the Mississippi Veterinary Research and Diagnostic Laboratory in Pearl, MS. Serum samples were tested using an enzyme-linked immunosorbent assay (ELISA) and were considered positive if they had a sample to positive ratio (S/P) of .25 or greater (IDEXX Laboratories 2007). Upon finding a seropositive animal, feces were obtained from the animal and

placed in a 50 mL centrifuge tube and submitted for culture. The risk-assessments and management plans were conducted each year and the herds were tested annually.

Results

In the second year (2005) of the study, most of the herds that participated in the project saw a decreased prevalence of Johne's disease. Table 1 shows the total number of animals tested, total number of ELISA positive animals, total number of culture positive animals, total number of environmental samples taken and total number of positive environmental samples for the 5 herds enrolled in the project in 2004. In 2004, 1098 animals were tested, with 58 (5.2%) testing ELISA positive.

Table 1

Summary of Demonstration Herds 2004

		2004				
		Animal tests	**ELISA+**	**culture+**	**EV tests**	**EV tests +**
Dairy	DEMO1	121	1	1	4	lost
	DEMO2	370	16	0	5	0
Total Dairy		491	17	1	9	0
Beef	DEMO3	110	3	0	5	0
	DEMO4	171	24	0	8	1
	DEMO5	326	14	0	0	0
Total Beef		607	41	0	13	1
TOTAL _ALL HERDS		1098	58	1	22	1

One dairy and 3 beef herds were added to the project in 2005 and underwent the same protocols as the initial herds. Table 2 shows the total number of animals tested, total number of ELISA positive animals, total number of culture positive animals, total number of environmental (EV) samples taken and total number of positive environmental samples for the 10 herds enrolled in the project in 2005. With the new herds, 1626 animals were tested in 2005. Fifty-two (3.1%) were ELISA positive.

Table 2

Summary of Demonstration Herds 2005

		2005				
		Animal tests	**ELISA+**	**culture+**	**EV tests**	**EV tests +**
Dairy	DEMO1	191	2	1 PCR	8	1
	DEMO2	468	13	3	6	1
	DEMO10	164	2	0		
Total Dairy		823	17	3	14	2
Beef	DEMO3	110	1	1	5	0
	DEMO4	192	13	0	5	0
	DEMO5	285	7	0	8	0
	DEMO7	46	0	0	6	0
	DEMO8	28	1	0	6	0
	DEMO9	142	13	missing	5	0
Total Beef		803	35	1	35	0
TOTAL _ALL HERDS		1626	52	4	49	2

In 2006, 1461 animals were tested, with 24 (1.6%) testing ELISA positive. Twenty-six environmental samples were taken for culture with 1 testing positive for the Johne's organism. Table 3 shows the total number of animals tested, total number of ELISA positive animals, total number of culture positive animals, total number of environmental samples taken and total number of positive environmental samples for the 7 herds enrolled in the project in 2006.

Table 3

Summary of Demonstration Herds 2006

		2006				
		Animal tests	**ELISA+**	**culture+**	**EV tests**	**EV tests +**
Dairy	DEMO2	562	14	4	8	0
Total Dairy		562	14	4	8	0
Beef	DEMO3	154	0	0	6	0
	DEMO4	158	5	3	10	0
	DEMO5	370	3	0	7	0
	DEMO7	28	0	0	7	0
	DEMO8	36	0	0	6	1
	DEMO9	153	2	0	6	0
Total Beef		899	10	3	42	1
TOTAL _ALL HERDS		1461	24	7	50	1

Testing for the 2007 study year resulted in 1177 animals being tested with 8 (0.6%) found seropositive. Due to some herds being tested late in the year, not all fecal cultures and environmental cultures have been finalized to date. Table 4 shows the total number of animals tested, total number of ELISA positive animals, total number of culture positive animals, total number of environmental samples taken and total number of positive environmental samples for the 6 herds enrolled in the project in 2007.

Table 4

Summary of Demonstration Herds 2007

		2007				
		Animal tests	**ELISA+**	**culture+**	**EV tests**	**EV tests +**
Dairy	DEMO2	471	4	Pending	8	0
Total Dairy		471	4	Pending	8	0
Beef	DEMO3	143	0		6	Pending
	DEMO5	325	0		7	Pending
	DEMO7	43	3	Pending	7	Pending
	DEMO8	36	0		7	Pending
	DEMO9	159	1	Pending	7	1
Total Beef		706	4	Pending	34	1
TOTAL _ALL HERDS		1177	8	pending	42	1

Figure 3 shows the prevalence rates from year to year for each herd in the program. It is a good representation of the decrease in disease prevalence for herds enrolled in the Demonstration Herd Project. Most of the herds showed a steady decrease in the prevalence of Johne's. However, there were two herds that did show an increase. Demo 1 had an increase from 0.8% to 1.04%. Demo 7 had a spike to 6.97% prevalence after having tested negative for two years.

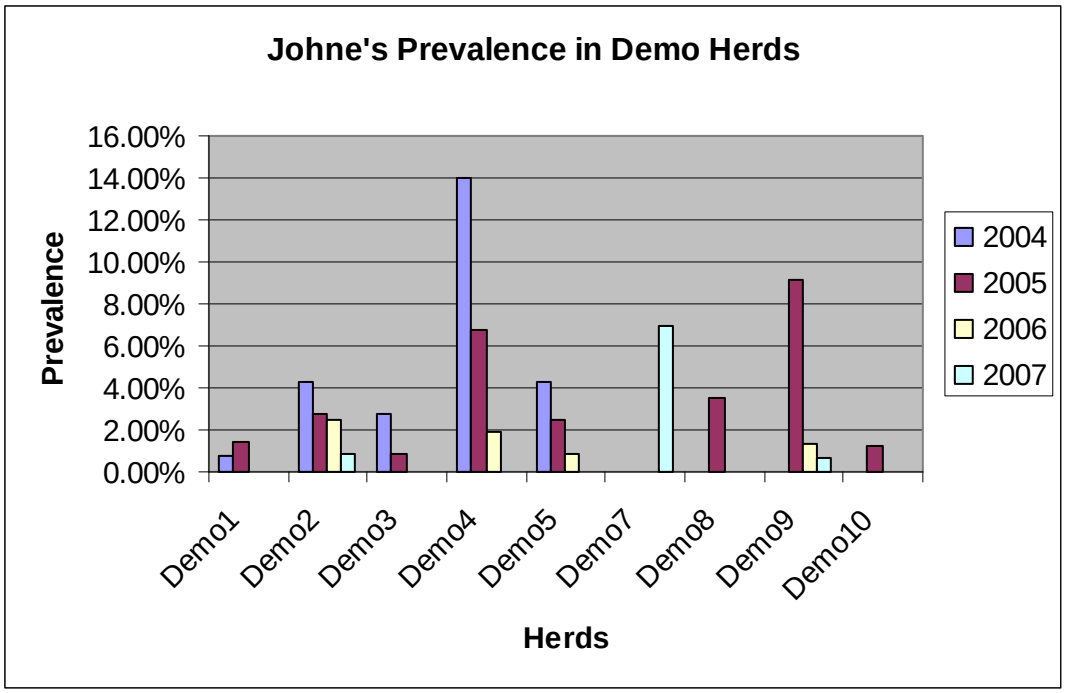

Figure 3

Johne's Prevalence in Demo Herds

Three of the herds participating in the project have been enrolled since the beginning of the study, Demo2, Demo3 and Demo5. These herds have seen a steady decrease in the

prevalence of Johne's with each year of participation in the Demonstration Herd Project. Figure 4 shows the decreasing prevalence for these herds throughout the project.

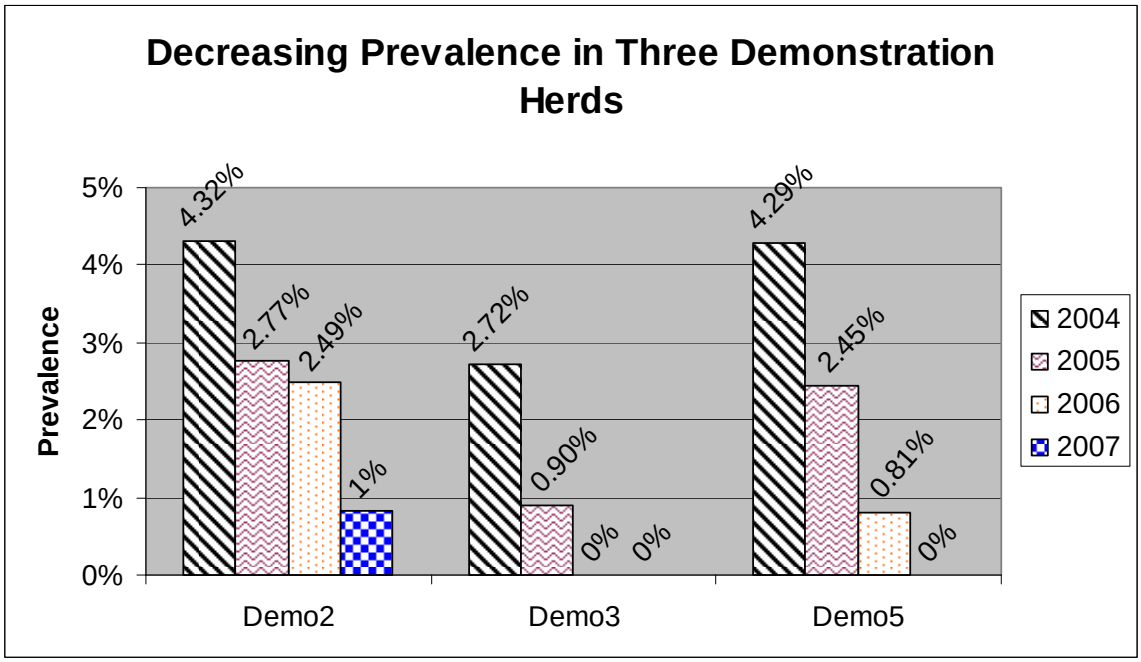

Figure 4

Decreasing Prevalence in Three Demonstration Herds

Demo1 was a small, 26 year old dairy herd that completed the first testing in 2004. The herd sold out in 2006 after suffering great damage from a tornado. This herd had an increase in prevalence from the first year and after the second year of testing the herd was sold out.

Demo2 is a larger dairy herd that was 26 years old when the project began. The herd was enrolled in the program and began testing in 2004. On the initial herd test 370 animals were tested with 16 (4.32%) being seropositive. None of the seropositive

animals were found positive for the Johne's organism on culture. Five environmental samples were taken and none tested positive. The herd expanded in 2005 and tested 468 animals with 13 (2.77%) testing seropositive. Three of the seropositive animals were confirmed positive for the Johne's organism. Six environmental samples were taken with 1 testing positive for the Johne's organism. In 2006 the herd was again expanded and tested 562 animals. Fourteen (2.49%) of the animals were seropositive and 4 confirmed positive for the Johne's organism. Eight environmental samples were taken and none were positive. Fewer animals were tested in 2007. Only 471 animals were tested with 4 (.84%) of those being seropositive. Organism detection testing is not yet complete. Environmental samples have been collected and results are pending. Figure 5 shows the decrease in prevalence for Demo2.

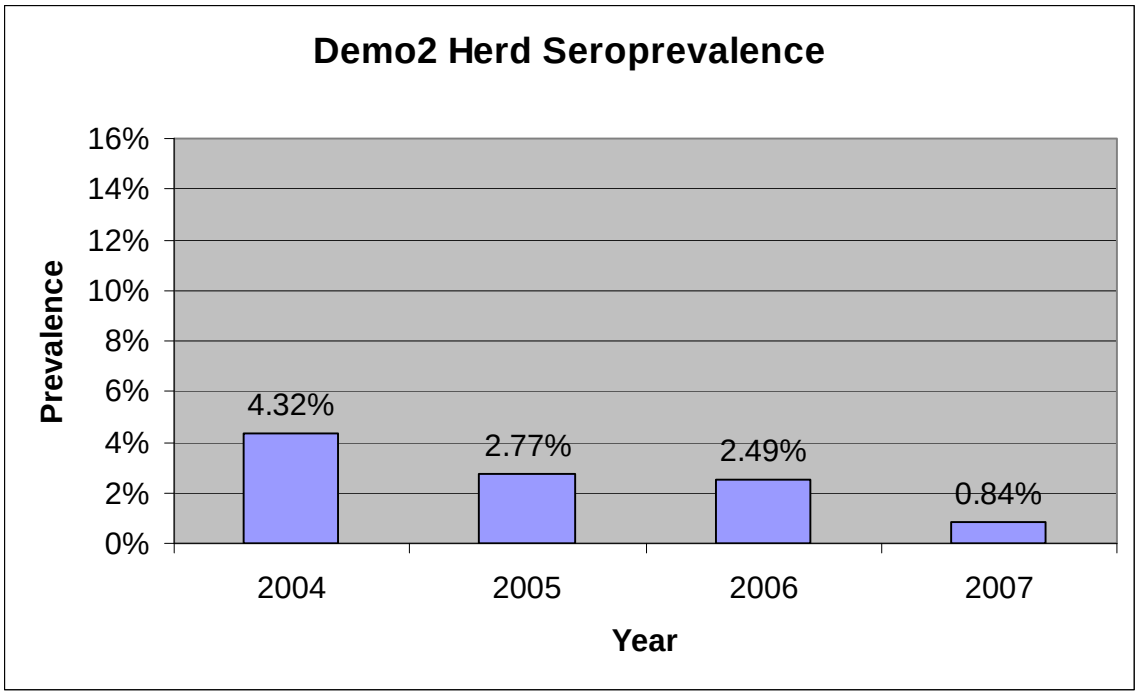

Figure 5

Demo2 Herd Seroprevalence

Demo3 is a beef herd that was 10 years old when the project began. In 2004, 110 animals were tested with 3 (2.72%) being seropositive. None of the seropositive animals were confirmed positive for the Johne's organism. Five environmental samples were also collected. None of the environmental samples tested positive. One hundred and ten animals were tested again in 2005. One animal was seropositive and confirmed by organism detection. Five environmental samples were taken and none were found positive. The number of animals tested in 2006 increased slightly to 154. None tested positive. Six environmental samples were taken and all were negative. In 2007, 143 animals were tested and again none were found positive. Six environmental samples were taken again, results are pending. Figure 6 shows the decrease for Demo3.

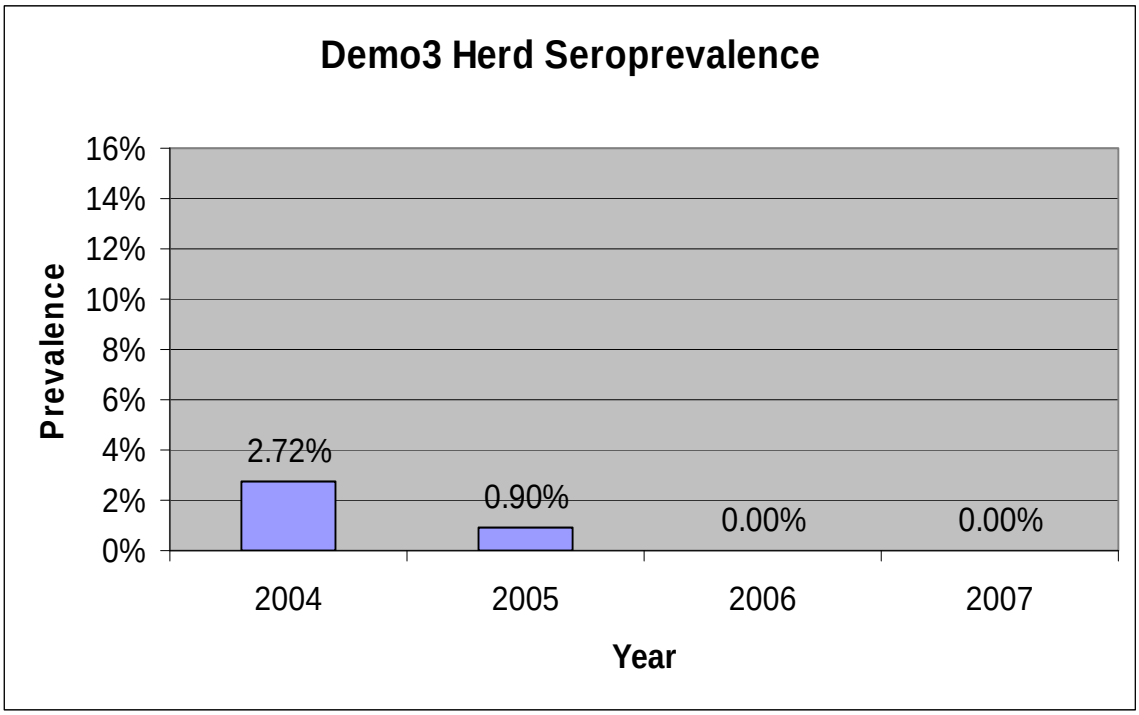

Figure 6

Demo3 Herd Seroprevalence

Demo4 was a 9 year old beef herd initially enrolled in 2004 with 171 animals tested for Johne's disease. Twenty-four (14%) animals were seropositive but none were positive on culture for the Johne's organism. Interestingly eight environmental samples were taken and 1 was positive. One hundred ninety-two animals were tested in 2005. Of those, 13 (6.77%) were seropositive. Again, none were positive for the Johne's organism. Only 5 environmental samples were taken and none were positive. In 2006, 158 animals were tested with 3 (1.89%) being seropositive. The number of environmental samples collected was increased to 10. None of which were positive. This

herd was removed due to insufficient data for the 2007 test year. Figure 7 shows the decrease in prevalence.

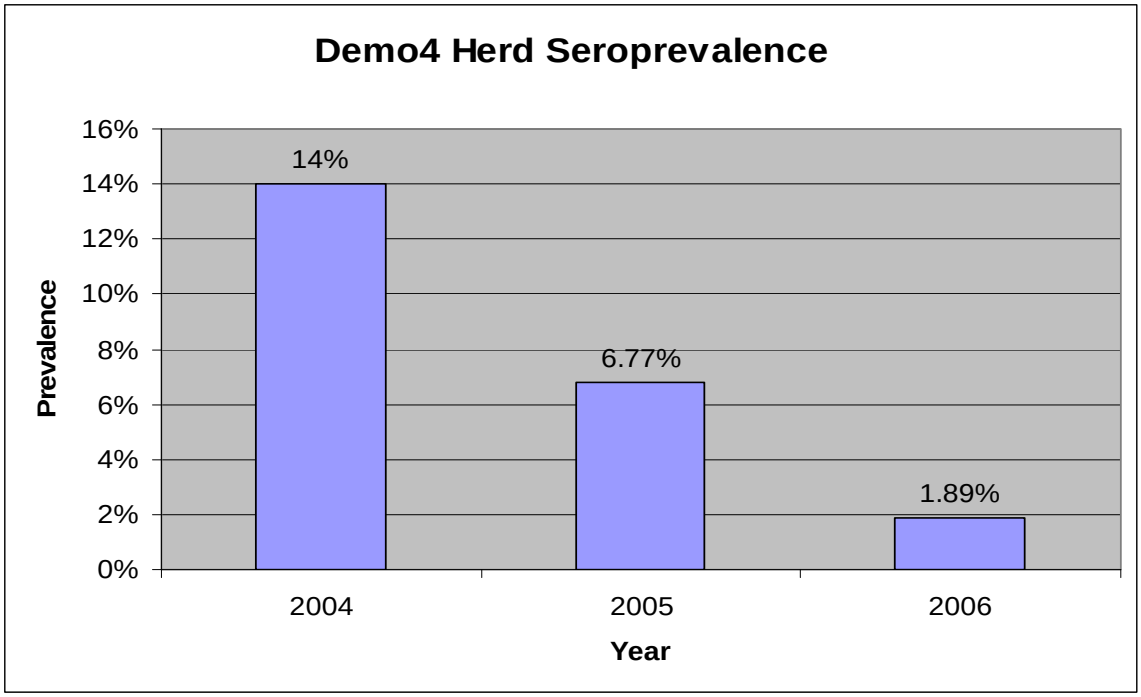

Figure 7

Demo4 Herd Seroprevalence

Demo5 is a larger beef herd that was 25 years old when the project began. In 2004, 326 animals were tested, and 14 (4.29%) were seropositive. None were positive for the Johne's organism on culture. Environmental samples were not collected for this year. Two hundred eighty-five animals were tested in 2005. Seven (2.45%) of them were seropositive. Again, none were positive for the Johne's organism. Eight environmental samples were collected and none were positive. In 2006, 370 animals were tested with 3 (.81%) being seropositive. None tested positive for the Johne's organism. Only 7

environmental samples were collected this year and none were positive. For the study year 2007, 325 animals were tested and none were positive. Seven environmental samples were collected and results are pending. Figure 8 shows the decrease in prevalence.

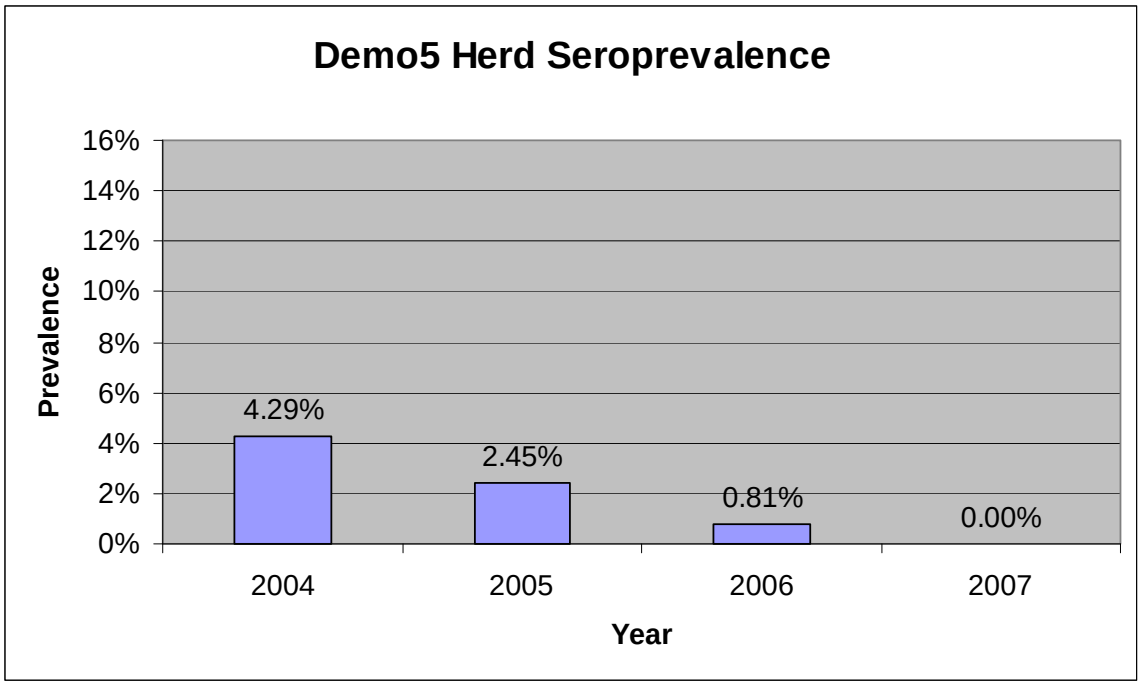

Figure 8

Demo5 Herd Seroprevalence

Demo7 is a small beef herd that was 15 years old when enrolled in the project. In 2005, 46 animals were tested. None tested positive. Six environmental samples were taken and none were positive. Twenty-eight animals were tested in 2006, again none were positive. Seven environmental samples were taken this year and none were positive. In 2007, 43 animals were tested with 3 (6.97%) being seropositive. Organism

detection testing has not been completed to date. Seven environmental samples were collected and results are pending. Figure 9 shows the increase in prevalence for this herd.

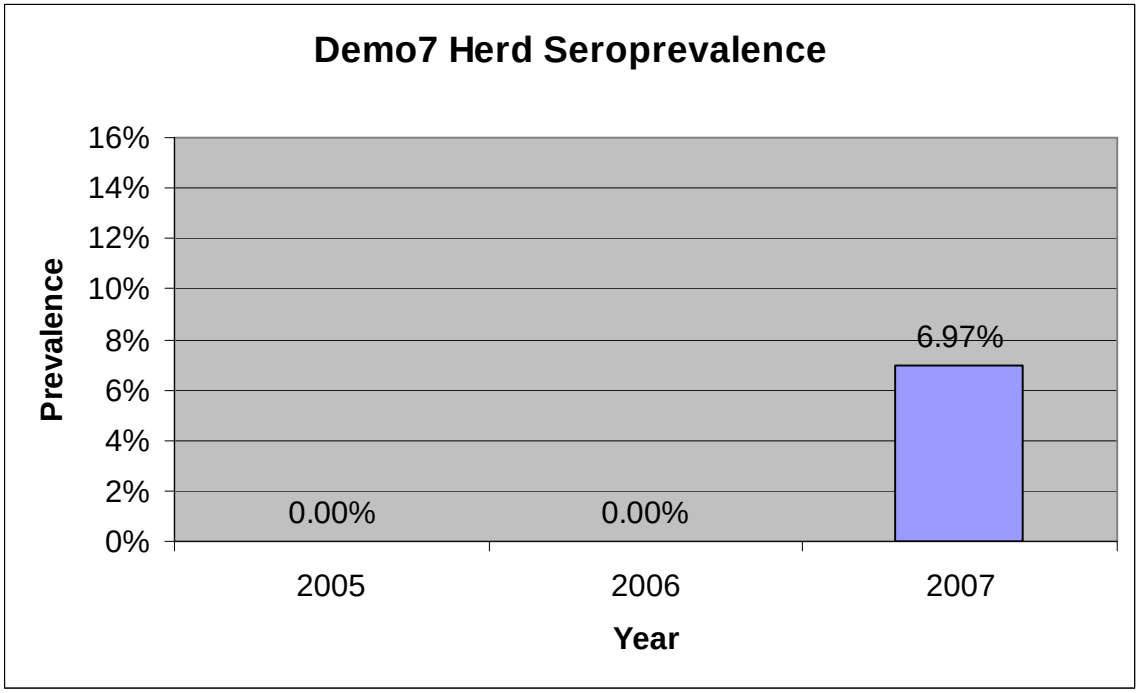

Figure 9

Demo7 Herd Seroprevalence

Demo8 is another small beef herd that was 8 years old when enrolled in the project. Twenty-eight animals were tested in 2005. Of those, 1 was seropositive. It was not positive for the Johne's organism on culture. Six environmental samples were taken and none were positive. In 2006, 36 animals were tested and none were positive. Six environmental samples were taken with 1 testing positive for the Johne's organism. In 2007, 36 animals were tested with none being seropositive. This herd was consolidated

with Demo5 onto one premise and additional environmental samples were not taken. Figure 10 shows the decrease in prevalence.

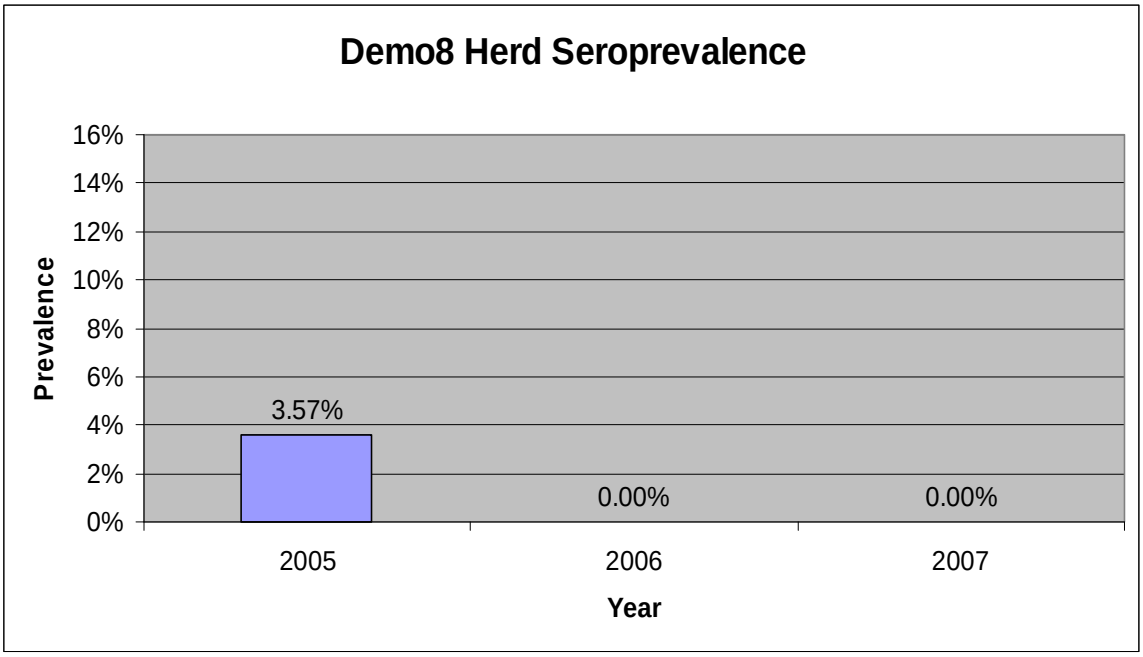

Figure 10

Demo8 Herd Seroprevalence

Demo9 is a beef herd that was 4 years old when enrolled in the project. In 2005, 142 animals were tested with 13 (9.15%) being seropositive. The organism detection results were not available due to reporting errors. Five environmental samples were collected and none were positive. The 2006 study saw the testing of 153 animals, 2 (1.3%) of which were positive. In 2007, 159 animals were tested and 1 (.62%) was seropositive. Organism detection results are pending. Seven environmental samples

were collected and those results are also pending. Figure 11 shows the decrease in prevalence.

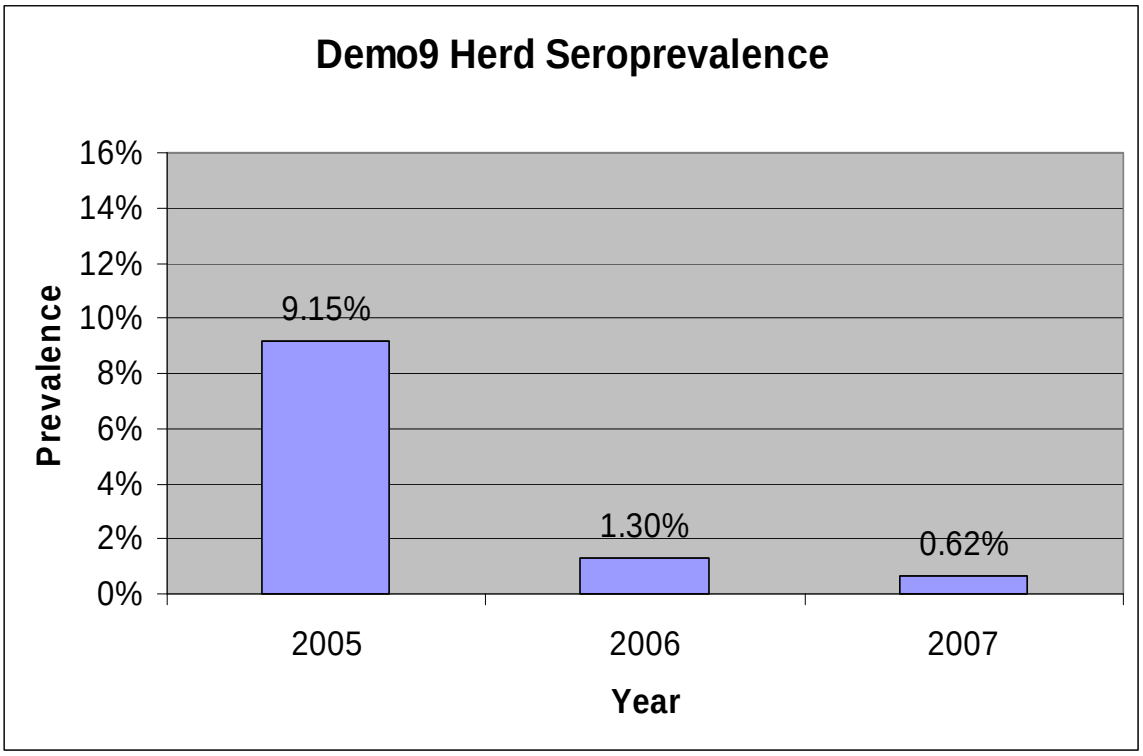

Figure 11

Demo9 Herd Seroprevalence

Demo10 was a dairy herd. Study year 2005 was the first and only year the herd was tested. One hundred sixty-four animals were tested with 2 (1.21%) being seropositive. Neither of them tested positive for the Johne's organism on culture. This herd was liquidated in 2006.

Discussion

The initial herds selected for the project turned out to have few confirmed Johne's positive animals. Because of a lack of positive animals in these herds it would be hard to determine if the goals of the program were being achieved. It will be difficult to know if the management strategies had any significant effects, and there would be no way to demonstrate effective ways to control the disease.

The data from this study showed an overall decreasing trend of apparent prevalence of Johne's disease the longer a herd participated in the project. Except for the spike in seroprevalence in 2007 for Demo7 and slight increase for Demo1, all herds showed a decrease. By performing a risk analysis and management plan and using it to identify areas of concern and addressing those concerns, most of the herds were able to decrease the prevalence of disease.

Over the course of the study environmental samples were taken. A small number of these samples were positive. Interestingly, a positive environmental sample was found on a farm in which none of the animals tested positive for Johne's disease on culture. It may be possible an animal was brought into the herd and culled before developing signs of Johne's disease. Also it is a possibility that a neighboring herd was infected because the sample was taken from a stream flowing through the property. This could provide evidence of the longevity of MAP in the environment. Two positive samples were taken from streams running through the farms and the other positive samples were soil samples taken from congregating areas.

Risk assessments were completed annually along with testing. These risk assessments were performed to help producers identify problem areas on the operation

and to assist with how to reconcile those problems. All risk assessments were performed by Johne's certified veterinarians. The risk assessments were divided into several different categories and the scores were weighted for each category. The scoring for the risk assessment and the weight given to each category is discussed in the Appendix. The score for each category is a numerical value given that represents the level of threat of the transmission of Johne's. By plotting the scores of the risk assessments a visual representation was made. An association was examined with how long a herd participated in the program and several risk factors for disease. This information gave the appearance that the longer a herd participated in the program, the risk factors for disease decreased, much like the prevalence. Some of the information also showed that in the event a risk factor score increased disease prevalence could be influenced, as with Demo7 in the 3rd year of participation. Five of the herds had complete risk assessments that could be used for analysis.

Figure 12 shows the numbers for Demo2. This herd was enrolled in the program for the duration of the project and had exceptional records. All but one of the risk factors declined after the first year of the project. This decline can possibly be attributed to the increased knowledge of the producer after enrolling in the program. The implementation of management practices found lacking by the risk assessment could also be a factor. It is apparent that changes were made after the first risk assessment was performed, by the decline of the score given to those risk factors in subsequent years. It is interesting to note that the risk assessment for "Additions" increased. While this operation was able to fix some of the problems it had, others arose. The increase in this risk factor could be reflective of management issues, some type of reproductive problem, or other problems.

A likely problem that arose was the availability of replacement animals. There are few herds enrolled in any type of Johne's disease control program and finding the replacement animals suitable to a particular operation is difficult. For this reason the producer may have had to explore alternative methods for securing replacements.

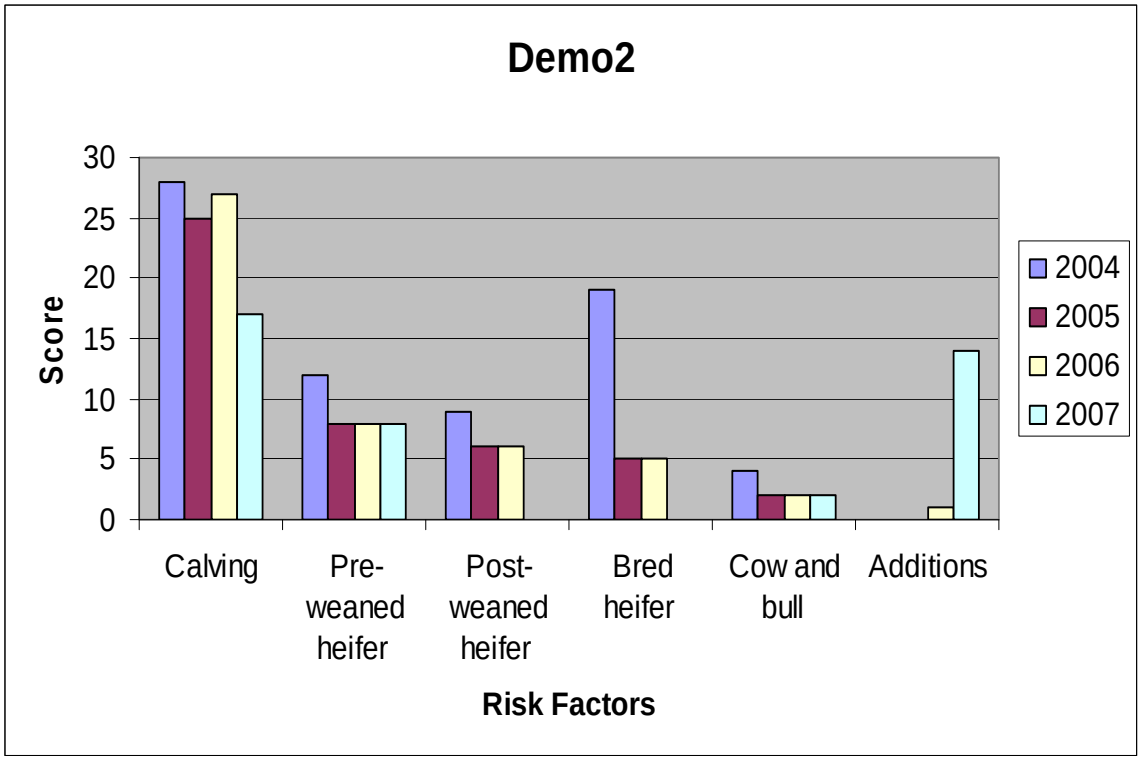

Figure 12

Demo2 Risk Factors

The numbers for Demo3 in Figure 13 look somewhat different from Demo2 in that most of the risk factors increased as time went by. The only factor that decreased was the Additions factor, possibly because of a change in the source of replacement

cattle. This information is interesting because although the risk increased for disease, the prevalence of Johne's disease decreased to 0.0% the third year of the project.

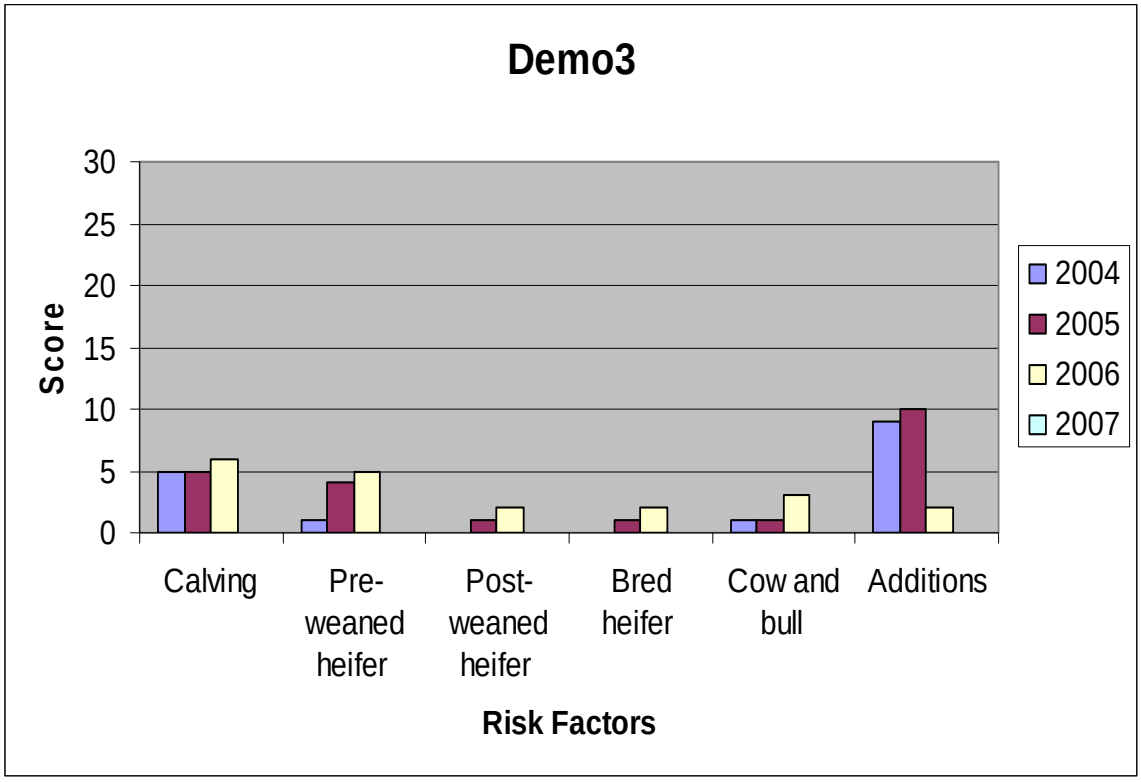

Figure 13

Demo3 Risk Factors

Demo5 was enrolled in the program for the duration of the project as well. The data for this herd show a decrease in some risk factors along with an increase in other risk factors the longer the herd was enrolled, Figure 14. The largest decrease for this herd was the Additions factor. Such a sharp change could have come from implementing a management practice changing the way replacement animals were entered into the herd.

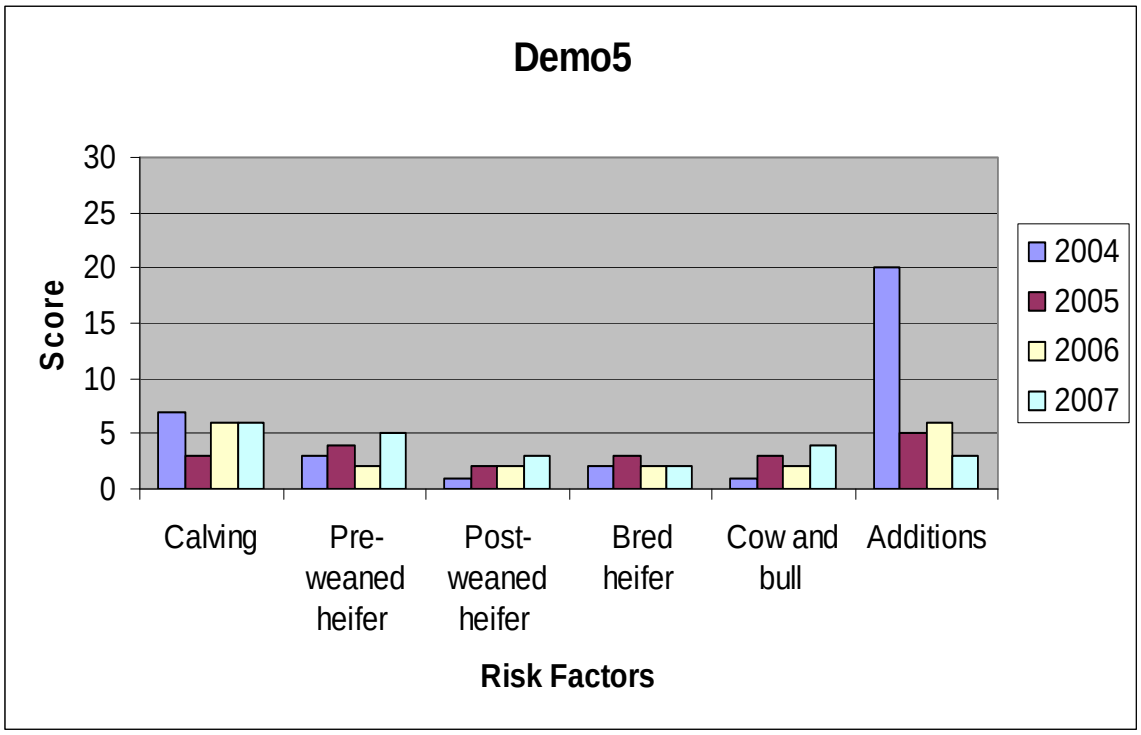

Figure 14

Demo5 Risk Factors

Figure 15 shows how management can affect an operation over time. Each year the herd participated in the program all risk factors declined. In the 3rd year of participation, 2007, the risk factor for Additions spiked with a value of 5 from the two previous years of 0, with a maximum score of 60 possible. The reason for this spike in seropositive animals has not yet been determined. This herd went from a closed herd to purchasing some animals that increased the risk for disease. Although this increased the risk score, the herd maintained a very low rating for susceptibility to Johne's. When examining this information alongside the prevalence of Johne's for Demo7, there seems

to be an association with increasing risk factors and increasing prevalence. Figure 9 shows the increase in prevalence in 2007.

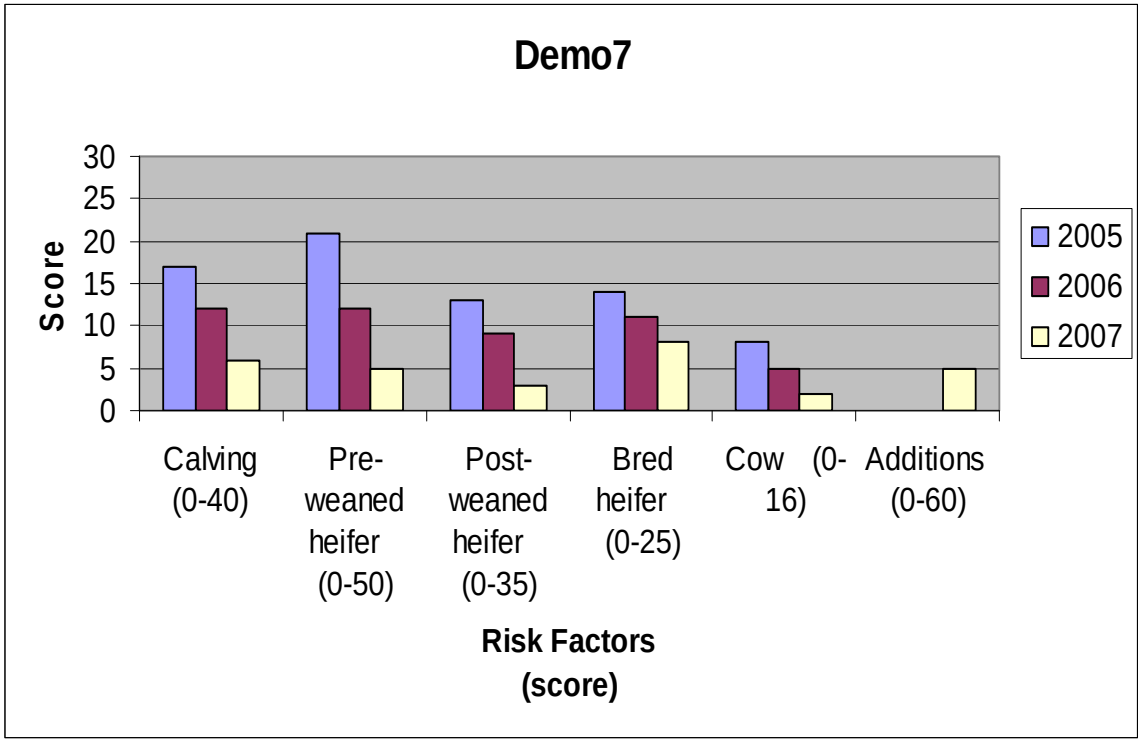

Figure 15

Demo7 Risk Factors

Demo8 risk factors were not very inconsistent. Some risk factors declined, some did not change, and some increased. However, this herd did see an overall decrease in disease prevalence. The scores for this herd are shown in Figure 16.

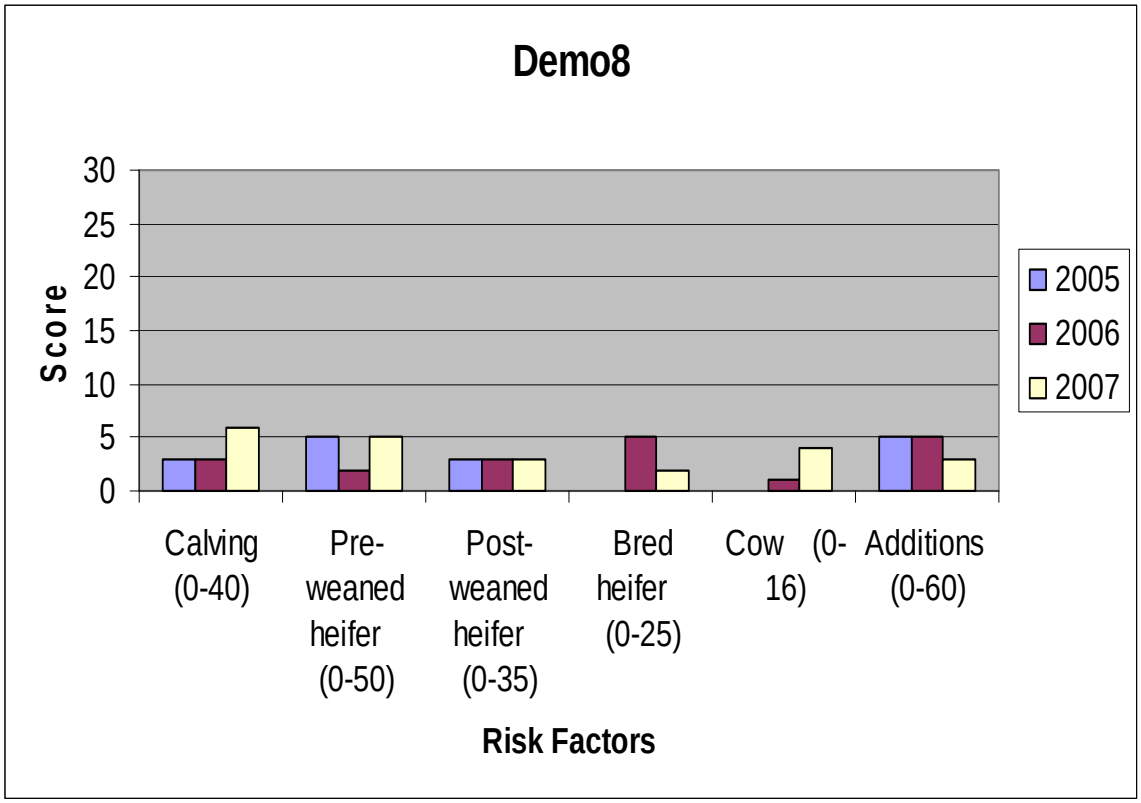

Figure 16

Demo8 Risk Factors

Due to the length of the study and timing of some aspects of the study it is possible the risk assessments were not performed by the same investigator each year. This could be a reason for some of the variance in the risk scores.

Conclusion

Most of the herds that participated in the Demonstration Herd Project saw a decrease in Johne's prevalence. This may also be the case in the Mississippi Voluntary Johne's Disease Control Program if a producer follows the guidelines of the program.

Evidence has been found that voluntary Johne's control programs provide economic value to participants and is a valuable source for replacement cattle with low infection risk for MAP (Kovich 2006). Overall the Demonstration Herd Project seems to have been a success. Many of the herds have seen decreases in prevalence of Johne's disease. Demo8 went from 3.57% to 0% prevalence and has maintained that level thus far. By implementing guidelines set forth by the Johne's Working Group, prevalence of Johne's in the Demonstration Herds has been reduced. The Demonstration Herd Project seems to have accomplished some of its initial goals. It appears that control of Johne's disease can be accomplished through management strategies and with the funding provided for the program, education on Johne's disease has reached many people in the livestock industry.

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CHAPTER IV

SUMMARY

Johne's disease is a disease that affects several species of animals and is costly to the cattle industry. While it has been studied for many years very little is actually known about the disease. To date no effective vaccine has been manufactured to prevent MAP infection and no cure has been found. Progress has been made in understanding the organism that causes Johne's disease, diagnostic testing for Johne's has improved, and a growing number of people have been educated about Johne's. Much of the research done on Johne's disease has been with dairy cattle and the effects on those animals and the dairy industry. It is known that Johne's disease costs the dairy industry $200-250 million annually. Johne's disease affects beef cattle as well and has implications on the beef industry. There are limited estimates on the cost of Johne's for the beef industry due to the lack of research in this field. For this reason more research is needed to understand the effects of Johne's on the beef industry. The NAHMS Beef '97 study estimated the herd prevalence of Johne's for beef operations to be 7.9% with 0.4% of animals infected. Several regional studies have been performed to determine the prevalence of Johne's in beef herds in the U.S. and in other countries. The prevalence of Johne's disease in those studies ranged from 0.8% to 8.75%.

The current study being presented was aimed at characterizing the prevalence of Johne's in Mississippi and resulted in an overall prevalence of 0.98% of Mississippi auction cattle. This study found that 26% of the cattle auctions in the state of Mississippi had at least one animal seropositive for Johne's present at the time of sample collection. The number of animals found to be both seropositive and fecal culture positive (0.54%) was close to the 0.4% of animals infected found by the NAHMS Beef '97 study. The six positive auction locations were found in only three soil associations suggesting that soil type may play a part in the prevalence of Johne's in Mississippi. Additionally, 43% of all animals tested came from the six auctions that were designated as positive. Johne's disease remains a problem in Mississippi and control of the disease at this point lies with the producer. Voluntary disease control programs have been established by federal and state authorities to assist producers in the fight against Johne's.

The National Johne's Demonstration Herd Project was established in 2002 with the objectives of evaluating the effectiveness and feasibility of management-related disease control measures, providing information and materials for the education and training of veterinarians and cattle producers, finding strategies useful in controlling Johne's, and creating additional opportunities for related projects. Mississippi is one of 18 states that participated in the project. Data collection in Mississippi began in 2004 and the last year of testing will be 2008. Herds chosen for the project were herds that had been previously diagnosed with Johne's infection. Five herds were chosen in the first year and 4 herds were added in the second year of the project. The overall seroprevalence of Johne's disease at the beginning of the study for the demo herds was 5.2%. Risk assessments were part of the program used to identify areas of concern for

the spread of disease. Upon completing these risk assessments producers could make changes in their management practices that could potentially decrease the chance for disease transmission. Each year the entire herd was tested for Johne's disease and the herd prevalence was determined. From year to year many changes were implemented in management practices and as a result the prevalence among the demonstration herds decreased annually. The fourth year of the project overall Johne's seroprevalence for the demonstration herds was 0.6%. The Johne's Demonstration Herd Project achieved its goals by showing that management-related control for Johne's is feasible. Many people, veterinarians and cattle producers have been educated and trained about Johne's. Through the various management practices used strategies have been developed to use in controlling disease. Areas of needed research have been identified for further progression of the knowledge base for Johne's disease.

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APPENDIX

MISSISSIPPI DEMONSTRATION HERD PROJECT RISK ASSESSMENT SCORES

The risk assessment scores are weighted for each category of the assessment. The assessment is divided into the following risk factor categories: Calving Area Risk Factors, Pre-Weaned Heifer Risk Factors, Post-Weaned Heifer Risk Factors, Bred Heifer Risk Factors, Cow and Bull Risk Factors, and Sources of Addition and Replacement. Several items were scored to compile a total score for each risk factor. The areas that are of highest risk of Johne's transmission are more heavily weighted and the areas of lower risk are less heavily weighted. The official risk assessment used by the National Johne's Disease Demonstration Herd Project (NJDDHP) has been provided.

JOHNE'S DISEASE RISK ASSESSMENT - BEEF

Premises Unique ID (Begin with 2 letter State abbreviation): _____

Data Collection Date: _____

Study Year: _____

Today's Date: _____

Investigators Name: _____

All three parts of the Risk Assessment Form must be completed (Herd Information, History and Prevalence of Johne's, and Risk Assessment Scores). Most fields in this survey are required. If you fail to complete a required field you will be prompted to do so before submitting.

General Herd Information / Inventory

Cows _____

1st Calf Heifers _____

Total Cows _____

Bred Heifers _____

Unbred Heifers _____

Bulls _____

Yearling Bulls _____

Total Head _____

In addition to beef cattle, what other animals do you raise or do your animals have contact with?

☐ Dairy Cattle ☐ Poultry

☐ Sheep/Goats ☐ Cervids

☐ Swine ☐ Horses

Other _____

Farm Goals and Biosecurity

Do you plan to be raising beef cattle in five years? ○ Yes ○ No

Describe short and longer-term goals or priorities for the farm.

Short-term goals / priorities (this year)

_____

Long-term goals / priorities (3-5 years)

_____

	Current	Goal
Weaning Weight	_____	_____
Pregnancy Rate	_____	_____
Calving percent	_____	_____
Calf death loss (1st 21 days)	_____	_____
Cow death loss	_____	_____
Cow culling percentage	_____	_____

JOHNE'S DISEASE RISK ASSESSMENT - BEEF

What are your top four overall concerns for your operation?

First

Second

Third

Fourth

What herd health improvements are you making or plan to make?

What management concerns and/or facility issues are you addressing or do you plan to address?

Imagine that you are given $10,000 (in cash, resources, time) over the next year to diagnose, address or prevent the following "diseases" in your herd. How would you allocate (percentage of funds) these resources to each area this year? (This is in addition to what you are already doing!)

___ Calf Scours / Pneumonia
___ Bovine Viral Diarrhea (BVD)
___ Removals/Death loss
___ Foot Health
___ Johne's Disease
___ Periparturient Diseases (Dystocia, RP, etc.)
___ Salmonella
___ Weaning Weight / Percent
___ Reproduction
___ Other

Replacements (Cattle purchased to replace current members of the herd)

Where do you obtain replacements? (Mark all that apply.)

☐ Home Raised
☐ Dealer
☐ Auction Market
☐ Video Market
☐ Single Owner
☐ Multiple Owners
☐ Born on farm - raised elsewhere

Other

List planned changes for obtaining replacements.

Additions (Cattle purchased to increase herd size)

Where do you obtain additions? (Mark all that apply.)

☐ Home Raised
☐ Dealer
☐ Auction Market
☐ Multiple Owners
☐ Single Owners
☐ Video Market

Other

Are health prerequisites required for herd additions? ○ Yes ○ No

If Yes, please specify

JOHNE'S DISEASE RISK ASSESSMENT - BEEF

Other Herd Information

How are cows identified: (Mark all that apply.)

☐ Ear Tags ☐ Branding
☐ Collars ☐ Electronic ID
☐ Photos or Sketches ☐ Tattoos (not Brucella)
Other _____

What percent of calves are identified as belonging to their dam? _____

Herd Risk Assessment, History and Prevalence of Johne's Disease

How long has the herd been here (years)? _____

How was the herd initially assembled?

○ Purchased whole herd ○ Purchased from multiple sources ○ Unknown
Other _____

What percent of the current herd was born on the premises? _____

What percent was purchased? _____

What percent of the herd was born here, but raised elsewhere? _____

Were those animals commingled with animals from other farms? ○ Yes ○ No

When was the first clinical case of Johne's diagnosed or suspected (year)? _____

What was the age of the first case (months)? _____

When was the youngest case diagnosed or suspected (year)? _____

What was the age of the youngest case (months)? _____

What was the source of the youngest case? ○ Home Raised ○ Purchased

Were any clinical cases observed in the last 12 months? If no, skip next question. ○ Yes ○ No

List clinical cases in the last 12 months:

ID	Date	Age (mo)	Source	ID of offspring still in herd
_____	_____	_____	○ Home Raised ○ Purchase	_____
_____	_____	_____	○ Home Raised ○ Purchase	_____
_____	_____	_____	○ Home Raised ○ Purchase	_____
_____	_____	_____	○ Home Raised ○ Purchase	_____
_____	_____	_____	○ Home Raised ○ Purchase	_____

JOHNE'S DISEASE RISK ASSESSMENT - BEEF

Record information from the last 12 months:

	1st Calf	2nd Calf	3+ Calf	Total Head	% of herd
Clinical Johne's cases					
Cattle culled last 12 months					
Johne's cases as % of cows culled					
# fecal culture positive animals					
# ELISA positive animals					

Introduction of New Cattle

Number of cows purchased last *12 months* _____

JD Status of selling herd ○ test neg ○ test pos ○ unknown

Number of heifers purchased last *12 months* _____

JD Status of selling herd ○ test neg ○ test pos ○ unknown

Number of other cattle purchased last *12 months* _____

JD Status of selling herd ○ test neg ○ test pos ○ unknown

Number of cows purchased *2-5 years ago* _____

JD Status of selling herd ○ test neg ○ test pos ○ unknown

Number of heifers purchased *2-5 years ago* _____

JD Status of selling herd ○ test neg ○ test pos ○ unknown

Number of other cattle purchased *2-5 years ago* _____

JD Status of selling herd ○ test neg ○ test pos ○ unknown

Estimate the prevalence of Johne's disease in the herd:

○ Low ○ Moderately low ○ Moderate ○ Moderately high ○ High

A. Calving Area Risk Factors

Score each item from 0 to 10 where 0=none, 1=very low, 5=moderate and 10=very high

1. Multiple animal use (single pen to dense group) _____
2. Manure build up risk for calf ingestion _____
3. Manure soiled udders/legs _____
4. Presence of JD clinicals/suspects _____

Maximum score is 40. Your score is _____

Are separate pastures used to calve first calf heifers versus older cows? ○ Yes ○ No

Are multiple pastures used as calving areas for first calf heifers in order to reduce calves exposure to pathogens? ○ Yes ○ No

Are multiple pastures used as calving areas for mature cows in order to reduce calves exposure to pathogens? ○ Yes ○ No

Estimated risk of spreading MAP in calving area

○ Very low ○ Low ○ Moderate ○ High ○ Very high

JOHNE'S DISEASE RISK ASSESSMENT - BEEF

B. Nursing Calf Risk Factors

Score each item from 0 to 10 where 0=none, 1=very low, 5=moderate and 10=very high

1. Cow/calf pairs kept with JD clinical or suspect animals _____
2. Manure build up risk for calf ingestion _____
3. Possible manure contamination of water: (By cows, traffic splatter, equipment or people.) _____
4. Possible manure contamination of feed: (By cows, traffic, equipment or people.) _____
5. Sick calves exposed to sick cows _____

Maximum score is 50. Your score is _____

B. Nursing Calf Risk Factors (continued)

How many calves are purchased from outside sources to nurse cows that lost their calf? _____

On average, calves are weaned at what age? (months) _____

Estimated risk of spreading MAP in pre-weaned calves

O Very low O Low O Moderate O High O Very high

C. Weaned Heifers and Bull Calves Risk Factors

Score each item from 0 to 7 where 0=none, 1=very low, 4=moderate and 7=very high

1. Direct contact with cows or their manure _____
2. Possible manure contamination of feed _____
3. Potential water contamination _____
4. Share pasture with cows/bulls _____
5. Manure spread on forage grazed/harvested same season _____

Maximum score is 35. Your score is _____

Estimated risk of spreading MAP in post-weaned calves

O Very low O Low O Moderate O High O Very high

D. Bred Heifer and Yearling Bull Risk Factors

Score each item from 0 to 5 where 0=none, 1=very low, 3=moderate and 5=very high

1. Direct contact with cows or their manure _____
2. Possible manure contamination of feed _____
3. Potential water contamination _____
4. Share pasture with cows/bulls _____
5. Manure spread on forage grazed/harvested same season _____

Maximum score is 25. Your score is _____

Do bred heifers or yearling bulls graze pastures *with* older animals? O Yes O No

Do bred heifers or yearling bulls graze pastures *after* older animals? O Yes O No

Estimated risk of spreading MAP in bred heifers and yearling bulls

O Very low O Low O Moderate O High O Very high

JOHNE'S DISEASE RISK ASSESSMENT - BEEF

E. Cow Risk Factors

Score each item from 0 to 4 where 0=none, 1= low, 4=high

1. Possible manure contamination of feed _____
2. Potential water contamination _____
3. Direct access to accumulated or stored manure _____
4. Manure spread on forage grazed/harvested same season _____

Maximum score is 16. Your score is _____

Estimated risk of spreading MAP among cows

O Very low O Low O Moderate O High O Very high

F. Sources of Additions and Replacements

See Handbook for scores

1. Use quality heifer raiser or get additions from Level 2-4 herds _____
2. From Low Risk herds, Level 1 or pretested herds _____
3. From Single source non-tested or non-program herds _____
4. From Multiple sources non-tested or non-program herds _____
5 Additions/replacements from market or unknown source _____

Maximum score is 80. Your score is _____

Estimated risk of spreading MAP from additions/replacements

O Very low O Low O Moderate O High O Very high

Comments _____

JOHNE'S DISEASE MANAGEMENT PLAN - BEEF

List the risk factors of most importance identified by assessment:

Risk factor 1:

Risk factor 2:

Risk factor 3:

Risk factor 4:

Risk factor 5:

Building the elements of the testing strategy for the Johne's Disease Management Plan

1. What is the testing scheme expected to accomplish? (Mark all that apply.)

- ☐ Determine changes in prevalence
- ☐ Prevent further disease introduction
- ☐ Prevent further disease spread within the herd
- ☐ Identify test-positive cattle for removal
- ☐ Identify test-positive cattle for management

Other

2. What test(s) will be used? (Mark all that apply.)

- ☐ serum ELISA
- ☐ Johnin skin test
- ☐ fecal culture
- ☐ Fecal PCR
- ☐ serum ELISA with fecal culture of ELISA positives
- ☐ Pooled fecal cultures

Other

3. Who will be tested? (Mark all that apply.)

- ☐ Herd additions
- ☐ All adult cattle (whole herd)
- ☐ Cattle with clinical signs of JD
- ☐ All cull cattle

Other

4. When will they be tested? (Mark all that apply.)

- ☐ At the time of introduction
- ☐ 2 times annually
- ☐ When clinical signs appear
- ☐ At the time of removal
- ☐ Annually

Other

5. What decision(s) will be made based on test results? (Mark all that apply.)

- ☐ Remove test-positives from herd
- ☐ Monitor for clinical signs
- ☐ Segregate test-positive cows at calving
- ☐ Retest
- ☐ Use colostrum from test-negative cows
- ☐ None
- ☐ Remove last calf from herd

Other

JOHNE'S DISEASE MANAGMENT PLAN - BEEF

Johne's Management Plan

What are the objectives of the herd plan? (Mark all that apply.)

☐ Determine herd status
☐ Establish test-negative status
☐ Prevent JD introduction into herd
☐ Reduce the infection in herd
☐ Prevent further spread

Other _____

Management practices to reduce identified risks for JD in this herd

_____

_____

How does this practice benefit and/or integrate with existing health / management objectives?

_____

Priority

○ Low ○ Medium ○ High

Management practices to reduce identified risks for JD in this herd

_____

_____

How does this practice benefit and/or integrate with existing health / management objectives?

_____

Priority

○ Low ○ Medium ○ High

Management practices to reduce identified risks for JD in this herd

_____

_____

How does this practice benefit and/or integrate with existing health / management objectives?

_____

Priority

○ Low ○ Medium ○ High

Comments

_____

_____