



LITERATURE REVIEW

OF

JOHNE'S DISEASE IN BEEF CATTLE

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INTRODUCTION:

Johne's disease, or paratuberculosis, is a chronic, granulomatous, bacterial enteritis that leads to cachexia and eventually death. While primarily a disease of ruminant livestock, it has also been found in many non-domestic ruminant and monogastric species. Although the disease was first described in 1826 (Chiodini et al., 1984), it continues to plague the cattle industry today. Johne and Frothingham first demonstrated the presence of acid-fast bacilli in affected intestines in 1895 (Chiodini et al., 1984). In 1912, Twort and Ingram successfully isolated the causative organism and named it *Mycobacterium enteriditis chronicae pseudotuberculosis bovis johne* (Chiodini et al., 1984). Despite its length, the original name described the disease quite well. Under the current nomenclature, the organism is now classified as *Mycobacterium avium* subspecies *paratuberculosis* (*M. paratuberculosis*). Over the last century, research studies have generated a vast amount of information that has helped improve current day knowledge of Johne's disease. Regardless of all the advances made, the disease probably remains as prevalent today, as it was when first described (Kreeger, 1991). Part of this reason lies in the fact that the organism is so fastidious, and results in an extremely long latency period between infection and clinical disease. This allows ample opportunity for horizontal and vertical spread of the disease, especially during the subclinical period. Our current understanding of the basic infective process remains limited by the time and money required to perform the necessary prospective studies. Complicating the issue further, is the lack of accurate tests to properly identify infections, particularly those in early development (Manning and Collins, 2001). Several therapies

have been investigated, but unfortunately no treatment has been found to be efficacious and cost-effective for paratuberculosis (Merkal and Larsen, 1973; Merkal and Richards, 1972; St-Jean and Jernigan, 1991). Johne's disease has distinguished itself as a disease of importance, not only due to the economic losses associated with limiting production on-farm and world trade, but as a potential zoonotic threat (Chiodini et al., 1984; Collins and Manning, 1995; Hermon-Taylor, 2000; Manning, 2001). Many researchers agree that there is an association between *M. paratuberculosis* and Crohn's disease, but whether or not it is causal remains to be proven (Bull et al., 2003; Chiodini and Rossiter, 1996; Van Kruiningen, 1999). All of the above concerns have provoked government attention, and several countries including Australia, Netherlands, and United States have developed national control programs for paratuberculosis in their cattle industries (Allworth and Kennedy, 2000; Benedictus et al., 2000; Bulaga, 1998; Groenendaal et al., 2003).

This review of the literature is not intended to be inclusive, as there are already several such reviews in existence (Chiodini et al., 1984; Collins, 1994; Kreeger, 1991; Larsen, 1972; Manning, 2001; Merkal, 1984; Olsen et al., 2002; Rideout et al., 2003; Simpson, 2002; Sweeney et al., 1996a; Thoen and Baum, 1988; Whipple et al., 1991) but will focus on the literature as it pertains to the beef industry. The objectives of this paper are to begin by summarizing what is known about the epidemiology of Johne's disease. A brief look at the pathogenesis of *M. paratuberculosis* will then be explored which will progress into a critical evaluation of commercially available diagnostic tests. This will lead to a discussion of prevention and control strategies and finally conclude with a look into future research areas that need to be considered for the beef industry.

EPIDEMIOLOGY

Understanding the epidemiology of Johne's disease has been very important in the development of prevention and control strategies. The epidemiology will be broken down into three key areas: host, agent and environment.

A. Host

Cattle are the host that will be discussed most thoroughly, although many other ruminant and non ruminant species play a role in the epidemiology of paratuberculosis (Chiodini et al., 1984; Corn et al., 2005; Daniels et al., 2003b). Infection with *M. paratuberculosis* is thought to occur predominately during the early post-natal period of life (<6 months) (Doyle, 1953; Hagan, 1938; Larsen et al., 1975). Animals in endemic herds probably receive more than a single dose of *M. paratuberculosis*, as demonstrated by the multifocal histopathologic lesions found in early infections (Chiodini, 1996). It has been estimated that only 1/3 of young animals with a single exposure to *M. paratuberculosis* will develop chronic infections (Chiodini et al., 1984). Experiments have also demonstrated that as cattle get older a larger dose of *M. paratuberculosis* is required to cause infection. This age-related infectivity has been explained by a more competent immune system as the cattle age (Manning and Collins, 2001; Rankin, 1961; Rankin, 1962; Sweeney et al., 1992a). Calves are commonly infected through ingestion of *M. paratuberculosis* in contaminated feces, milk or colostrum (Clarke, 1997; Streeter et al., 1995; Sweeney, 1996b). *M. paratuberculosis* may be shed directly into milk and colostrum. However, milking dirty udders can also cause fecal contamination of the milk

(Johnson-Ifearulundu and Kaneene, 1998). This would indicate that the dirty udder of a dam could potentially lead to bacterial exposure to the suckling beef calf.

M. paratuberculosis has also been found in the reproductive tract of infected animals (Ayele et al., 2004; Kopecky et al., 1967; Larsen and Kopecky, 1970; Larsen et al., 1981). However, the risk of sexual transmission is believed to be extremely low compared to those previously mentioned (Manning and Collins, 2001). Vertical transmission will be discussed at more length later in this review.

Although infection usually occurs during early life, clinical signs do not appear until much later. The onset of clinical signs most commonly occurs between 2 to 6 years of age. The dose of organisms and age at infection are considered to be the two main factors that determine when clinical signs become apparent (Rankin, 1961; Sockett et al., 1992a). It has been suggested that if mature cattle were to become infected, the chances of developing clinical signs would be unlikely due to a long pre-patent period and a high risk of being culled for other reasons (Whitlock and Buergelt, 1996). For chronic paratuberculosis infections it is widely accepted that there are several stages of disease that an animal goes through (Whitlock and Buergelt, 1996). Although these stages are arbitrary, they do help to understand the disease and many of the diagnostic complications that go with it.

Stage one typically includes young calves and heifers that have been infected with *M. paratuberculosis* and extends until two years of age (Whitlock and Buergelt, 1996). During this time period infections remain silent as no overt evidence of infection is manifested through clinical signs. The growth, weight gain, and general appearance are the same as non-infected herd mates. A small percentage of infections may be

demonstrated through culture or histologic evaluation of intestine or lymph nodes.

M. paratuberculosis may also be shed into the environment, but below the threshold of detection for current diagnostics (de Lisle et al., 1980).

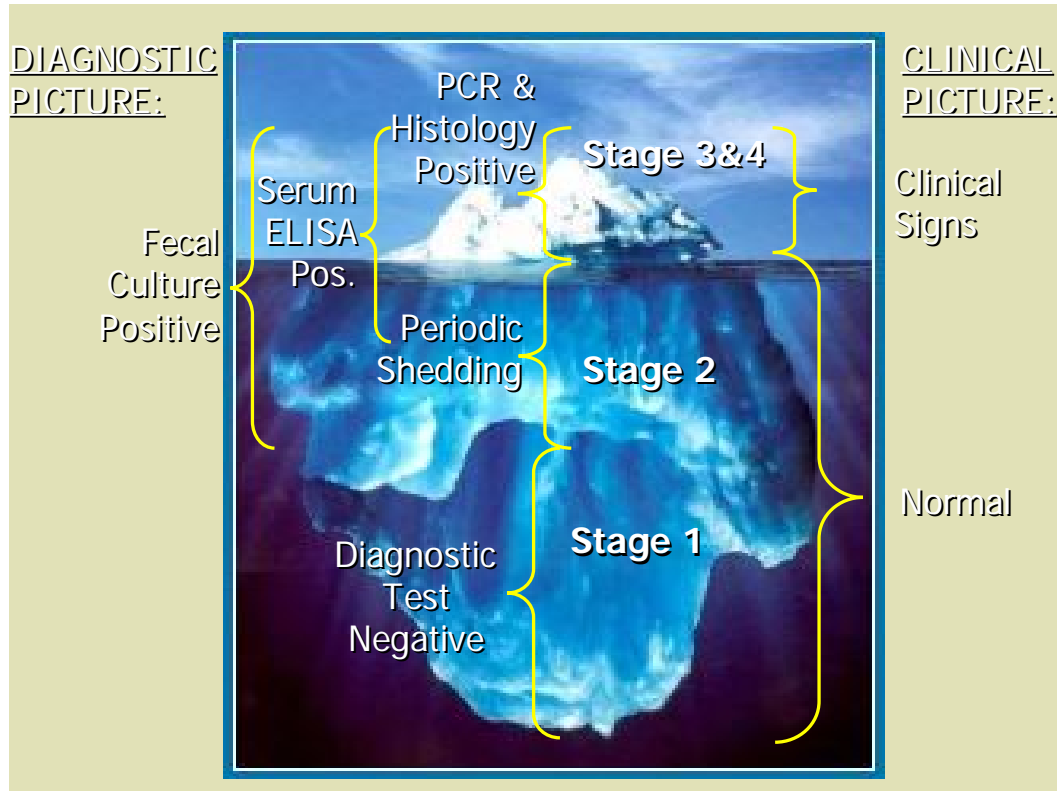
Infected cattle in stage two do not exhibit clinical signs (i.e. subclinical infection), although many will be shedding *M. paratuberculosis* in low numbers in their feces (Sherman, 1985). Since only 15-25% of these individuals can be identified with current diagnostic tests, the risk that these subclinical cattle present to non-infected herd mates is large (Dargatz et al., 2001a; Sweeney et al., 1995). Some subclinical cattle classified as super-shedders can shed more *M. paratuberculosis* than 160 heavy shedding cattle or 20,000 low shedding cattle (Whitlock et al., 2005). Excretion of *M. paratuberculosis* in the feces often occurs between 1 and 2.5 years before onset of clinical disease (Larsen and Merkal, 1968; Whitlock et al., 1991). Studies have also shown that cattle are capable of shedding *M. paratuberculosis* prior to two years of age and one reported fecal shedding in a calf less than 6 months of age (Bolton et al, 2005; Weber et al., 2005). Many of the economic losses associated with paratuberculosis occur at this stage of disease (Nordlund et al., 1996). By the end of this subclinical stage, many of the cattle will have mounted a detectable humeral immune response (van Schaik et al., 2003a).

Clinical disease becomes apparent during stage three and four. Manure consistency becomes periodically to persistently diarrheic and corresponds with progressive weight loss, both of which are non-responsive to therapy (Chiodini et al., 1984). An increased appetite may occur initially, but usually progresses to anorexia in the very terminal part of stage four. Another distinguishing feature of paratuberculosis is that cattle remain non-febrile during the clinical stages of disease (Sherman, 1985).

Cattle in stage four, with advanced clinical disease, typically present with intermandibular edema (bottlejaw), cachexia, and “pipe-stream” diarrhea. Often these animals are very weak and lethargic, and if not culled they will become recumbent and die. Clinical disease often corresponds to an increase in fecal shedding of *M. paratuberculosis*, as well as serum antibodies becoming more readily detectable. It has been suggested, that animals in the very terminal part of disease become anergic, such that antibodies are no longer measurable (Bendixen, 1978).

The proportions of cattle in each stage of disease are not equal. It has been proposed that for every clinical case of paratuberculosis, another 15 to 25 infected animals exist in the herd (Whitlock and Buergelt, 1996). It is for this reason that Johne’s disease is commonly compared to an iceberg. That is, only a small percentage of an iceberg’s mass is visible above the water surface, which is similar to the cattle with clinical disease in stages three and four. The larger and more dangerous portion of the iceberg is submerged below the surface of the water, and is comparable to all the clinical cattle in stages one and two.

Figure 1: The classical “iceberg” description of the diagnostic and clinical picture of Johne’s disease in cattle.



The prevalence of Johne’s disease in the cattle population has been evaluated in many countries and regions of the world (Kennedy and Benedictus, 2001). The majority of these studies have focused on cattle in the dairy industry. The dairy cow level prevalence estimates vary by geography, but range globally from as low as 0.8% to as high as 18% (Adaska and Anderson, 2003; Chiodini and van Kruiningen, 1986; Dargatz et al., 2001b; Doyle, 1956; Hill et al., 2003; McKenna et al., 2004; McNab et al., 1991a; Merkal et al., 1987; Stephan et al., 2002; VanLeeuwen et al., 2001). Dairy herd level prevalences have been reported to range from 8% to 33% in North America (Chiodini et al., 1984).

The USDA National Animal Health Monitoring System (NAHMS) 1997 National Beef Survey suggested that the US beef industry has a cow level seroprevalence of 0.4%, with 7.9% of beef herds having at least one seropositive animal (Dargatz et al., 2001a). Louisiana beef herds were found to have a herd seroprevalence of 30% and a cow level seroprevalence of 4.4% (Turnquist et al., 1991). Beef cattle in Florida had a cow level seroprevalence of 8.8% (Braun et al., 1990). When adjusted for test inaccuracies it was estimated that at least 50% of Alabama beef herds are infected with *M. paratuberculosis* and this had a cow level prevalence of approximately 8% (Hill et al., 2003). A recent study reported 44% of Texas beef herds had at least one seropositive animal and a cow level seroprevalence of 3% (Roussel et al., 2005).

In Canada, a serological study done on beef herds on community pastures in Saskatchewan found a cow level prevalence of 0.8%. When they classified positive herds as needing only one positive serological test the herd prevalence was 15.2% as opposed to only 3.0% when requiring 2 positive serological tests for a herd to classify as infected (Waldner et al., 2002). The province of Alberta has reported a cow level seroprevalence of 1.5% which was estimated to equal a true prevalence of 1.2% of the provincial adult beef herd. 28.5% of herds had at least one positive serological test and 7.9% had two or more positive serological tests (Scott, 2004).

A study looking at thin market cows at slaughter in the United States was able to culture *M. paratuberculosis* from the tissue of 15/189 (7.9%) dairy cattle and 1/350 (0.3%) beef cattle (Rossiter et al., 2005). This research supports the findings of other studies suggesting that the prevalence of *M. paratuberculosis* is relatively low in the national herd and the potential food safety risk is also minimal.

Direct comparisons between these various studies are difficult because of the different sampling strategies and diagnostic tests used. However, they do indicate that while the national seroprevalence may be relatively low certain regions may experience a much more significant problem. Overall, the cow level prevalence on dairy herds is typically low, and on beef herds it is even lower. The prevalence within a herd varies considerably depending on the management of the herd.

It has been reported in the literature there may be breed differences that affect the level of *M. paratuberculosis* infection in cattle. In the dairy industry, it has been found that a higher prevalence of Johne's disease occurs in Shorthorn and Channel Island breeds of cattle, such as Jersey and Guernsey (Whithers, 1959). One of the reasons for a higher prevalence of paratuberculosis in these breeds is that there are fewer of these herds in North America; so many animals are traded between purebred breeders to maintain genetic diversity. It has, however, also been argued that the most predominate breed in the population will experience the highest frequency of disease (Chiodini et al., 1984). In fact, probably both the management and abundance of a particular breed, influence the perceived incidence of disease. In regards to a genetic link to Johne's disease, the heritability in Dutch Holstein cattle has been estimated to be approximately 0.06, which is relatively low as compared to other traits (Koets et al., 2000). No similar study has been completed for the Shorthorn, Channel Island or any of the beef breeds. Different incidences of paratuberculosis have also been observed between beef and dairy cattle (Merkal et al., 1984). Alberta dairy cattle and herds tend to have a 4-5 times greater prevalence than their beef counterparts (Scott, 2004). Beef cattle commonly have a lower incidence of Johne's disease, as they generally range over larger areas and have less

exposure to other cattle and their feces (Chiodini et al., 1984; Dargatz et al., 2001b; Thoen and Baum, 1988). One beef study from Texas has shown that cattle from *Bos indicus*-based herds were more than 17 times as likely to be seropositive as were cattle from *Bos taurus*-based herds, and cattle from interspecies-based herds were 3.6 times more likely to be seropositive as were cattle from *Bos taurus*-based herds. It was suggested that this increased seropositivity may have been due to breed differences in immune response to a different organism, as multiple seropositive herds showed no clinical or microbiological evidence of disease. Other potential causes included a higher prevalence of infection in *Bos indicus*-based herds, higher susceptibility to infection although clinical and microbiological evidence did not support this, or increased resistance to disease with the serological effect resulting from a successful immunologic response to infection (Roussel et al., 2005).

As suggested earlier, many other species have also become infected with *M. paratuberculosis* under natural or experimental conditions (Chiodini et al., 1984; Clarke, 1997; Hines et al., 1995). *M. paratuberculosis* has even been isolated from diptera (Fischer et al., 2001) and an earthworm (Fischer et al., 2003). The role that these species play in the epidemiology still needs to be better elucidated. Several epidemiological studies have evaluated wildlife in the United Kingdom, Norway, and the Czech Republic in regards to paratuberculosis (Beard et al., 2001; Daniels et al., 2003a; Fredriksen et al., 2004; Greig et al., 1999; Machackova et al., 2004). Researchers in Scotland have suggested that various wild species, especially rabbits, may actually represent a significant concern to their livestock industries due to the level of *M. paratuberculosis* they are able to shed into the environment and the lack of fecal pellet avoidance

behaviour evident in the cattle (Daniels et al., 2003a). In North America, a number of free ranging ruminants as well as various other wild mammals and birds have been identified with *M. paratuberculosis* (Corn et al., 2005; Temple et al., 1979). However, the epidemiologic role that they play in Johne's disease of livestock has not been described. These species are likely of greatest importance to pasture based management systems, such as beef cow-calf herds, as compared to dairy cattle in confinement.

Paratuberculosis has generally been regarded as an enteric infection. Several reports now exist demonstrating the dissemination of *M. paratuberculosis* to extra intestinal sites such as the uterus, supramammary lymph nodes, udder, reproductive organs of bulls, and may be excreted directly in milk or semen (Ayele et al., 2004; Giese and Ahrens, 2000; Koenig et al., 1993; Kopecky et al., 1967; Larsen and Kopecky, 1970; Larsen et al., 1981; Streeter et al., 1995; Sweeney et al., 1992a; Taylor et al., 1981). The dissemination of *M. paratuberculosis* is believed to correlate to the stage of infection, meaning that it is the clinical cases that are most likely to have infections spread beyond the gastrointestinal system.

Several papers have discussed the transplacental or vertical transmission of *M. paratuberculosis* (Aly and Thurmond, 2005; Doyle, 1958; Lawrence, 1956; McQueen and Russell, 1979; Seitz et al., 1989; Sweeney et al., 1992b). It has been estimated from these studies that 18% to 37% of clinically effected and 9% of asymptomatic cows will result in an infected calf upon parturition. It has been speculated, but not confirmed, that calves born with infections may be more likely to progress to clinical disease at any earlier age as compared to animals exposed post-natally (Sweeney, 1996). In one large Holstein dairy herd, daughters born to seropositive dams were calculated to be 3.6 to 6.6

times as likely to be seropositive as were those born to seronegative dams. In fact, it was estimated that 1/3 of the herd prevalence of infection was due to transmission from seropositive dams to their daughters (Aly and Thurmond, 2005). The issue of vertical transmission has obvious potential implications for management on both dairy and beef operations.

M. paratuberculosis has been cultured from the uterine washings of infected cows and semen of infected bulls (Ayele et al., 2004; Larsen et al., 1981; Rohde and Shulaw, 1990). However, infection of a cow or embryo from artificial insemination or embryo transfer is still regarded as relatively low (Kruip et al., 2003; Sweeney, 1996b).

Quite simply stated, paratuberculosis is a production limiting disease of cattle. Several studies have evaluated the association between Johne's disease test status and various production outcomes related to milk production, milk quality, reproduction and culling in dairy cattle. Unfortunately the estimates provided from these studies are quite variable due to differences in the parity, days in milk, and stage of disease for cattle included in the investigations (Johnson et al., 2001). The control group and diagnostic test chosen for the evaluation, combined with herd level productivity, management practices, and prevalence are also important factors affecting the impact of subclinical *M. paratuberculosis* infection on production indexes (Johnson et al., 2001). Milk production losses have been reported to range from 2.2 to 25%, although two studies have even documented an increase in milk production in subclinically infected cattle (Benedictus et al., 1987; McNab et al., 1991b; Nordlund et al., 1996). Daily milk fat and protein were significantly reduced in culture positive cows when compared with culture negative cows (Sweeney et al., 1994a). Conversely, two other studies have found no

significant differences in lactation average percentages of fat or protein (Nordlund et al., 1996), or mature equivalent fat and protein production (Johnson et al., 2001).

Paratuberculosis has also been associated with increased mastitis culling (Merkal et al., 1975), decreased clinical mastitis (Wilson et al., 1993), increased individual and herd SCC (McNab et al., 1991b), or unaffected SCC (Spangler et al., 1992). While milk production and quality tends not be a common concern in the beef industry, it is possible that this could lead to reduced growth in calves of infected cows.

Johne's disease is commonly associated with premature culling of infected cattle, however, only two studies have actually evaluated this (Wilson et al., 1993; Hendrick et al. 2005a). The results of these studies suggest that test positive animals are 2 to 6 times as likely to be culled as compared to their test negative herd mates. However, the herd manager was not blinded to the fecal culture status of the cows when culling decisions were made in the study by Wilson et al. (1993). It is presumed that effect of *M. paratuberculosis* on culling in beef cattle is similar to that in dairy cattle, although this has never been investigated.

The current literature also fails to demonstrate a consistent association between *M. paratuberculosis* test status and reproductive outcomes (culling due to fertility, calving interval and days open) for subclinical dairy cattle (Abbas et al., 1983; Buergelt and Duncan, 1978; Johnson-Ifeorlundu et al., 2000; McNab et al., 1991b; Merkal et al., 1975). This is also area of consideration for beef cow-calf producers as reproduction is pivotal to their success.

Overall, when the direct production losses (milk production, slaughter value and premature voluntary culling) are taken into consideration in a partial budget for an

average infected herd, Johne's disease costs between \$40 and \$100 USD per cow on an annual basis (Chi et al., 2002; Ott et al., 1999; Hendrick et al., 2005a). This equates to an estimated loss of \$200 to \$250 million dollars annually for the US dairy industry (Ott et al., 1999). A similar estimate is not available for the beef industry, although the production losses due to clinical or subclinical infection with *M. paratuberculosis* are no doubt significant. In reality, the direct production losses are important, but are minimal when compared to the lost access to markets created by this disease.

B. Agent

M. paratuberculosis is an obligate pathogen and has many complexities of its own. Two strains of *M. paratuberculosis* have been identified using restriction fragment length polymorphism (RFLP) (Eamens et al., 2000). However, the existence of a cattle (C) and sheep strain (S) continues to be debated (Eamens et al., 2000; Reddacliff et al., 2003; Stehman, 1996; Whipple et al., 1989). The S strain is thought to be found mainly in sheep, but may also infect goats, and less commonly cattle (Collins et al., 1993; Eamens et al., 2000; Taylor, 1953). The S strain has cultural requirements slightly different from the C strain, but little else is known about it microbiologically (Collins et al., 1990; Eppleston and Whittington, 2001; Reddacliff et al., 2003).

M. paratuberculosis is surrounded by a complex tripartite lipid-rich cell wall that enables it to persist in the environment and contributes to its resistance to low pH, high temperature and chemical agents (Manning, 2001). Environmental survivability is one important factor to the epidemiology of the disease. In terms of chemical agents, a recent study suggests that chlorination of water may not kill *M. paratuberculosis*,

especially if high quantities of the organism exist in the pretreated water (Whan et al., 2001). Similarly, *M. avium* has also been found to be more resistant to free chlorine than several other bacteria (Taylor et al., 2000).

The thermotolerance of *M. paratuberculosis* is problematic to cattle producers on several accounts. Not only does thermotolerance improve the environmental survivability of this agent, but it also increases the risk of exposure to calves and humans through pasteurized milk. In terms of pasteurization, there is still considerable debate as to the efficacy of this process (Grant et al., 2002; Stabel, 2000). Many of the differences found in the multitude of studies relate to the method of pasteurization (batch versus high temperature-short time period (HTST)), the diagnostic tests used to evaluate the samples (bacterial culture versus PCR), the samples themselves (natural versus experimental inoculation) and the presence or absence of clumped colonies (Lund et al., 2002). Once again it is beyond the scale of this review to summarize the studies surrounding this topic, however, there are several good review articles available (Boor, 2001; Lund et al., 2002; Stabel, 2000; Sung and Collins, 1998). Overall, the tolerance of this pathogen to various temperatures should not be disregarded in terms of the epidemiology of this disease.

C. Environment:

The ability of *M. paratuberculosis* to survive in the environment for an extended period of time significantly effects how Johne's disease needs to be managed. The organism has been found to survive up to 55 weeks in a dry, fully shaded environment (Whittington et al., 2004). However, moisture, application of lime, UV radiation and temperature fluctuations can all significantly impact the recovery of viable *M.*

paratuberculosis. It has been suggested that dormancy may also play a role in the survivability of this organism (Whittington et al., 2004). The ability of *M. paratuberculosis* to survive in feces (desiccated or slurry), urine, various sources of water, silage, and compost have all been investigated (Gobec et al., 2005; Jorgensen, 1977; Larsen et al., 1956; Lovell et al., 1944). The survival times reported in these studies ranged from 21 days in compost to 3 months in cattle slurry up to 19 months in tap water.

There are several environmental factors that have been associated with the survival of *M. paratuberculosis*. Soil type, aridity, and pH are just three of these associated factors. Studies completed in the United Kingdom, Netherlands, and United States (Wisconsin and Michigan), have all concluded that *M. paratuberculosis* is self-limiting in alkaline, calcareous soils (Johnson-Ifearegulu and Kaneene, 1997; Kopecky, 1977). More recently it was reported that both loamy and sandy soils are conducive to the survivability of *M. paratuberculosis* (Ward and Perez, 2004). These findings were explained by the fact that, higher organic matters in loamy soils, and lower pH in leached sandy soils, both improve the survival of this pathogen (Ward and Perez, 2004). Soil aridity and pH were identified as significant inhibitors to *M. paratuberculosis* survival outside of the host in Alberta (Scott, 2004). *M. paratuberculosis* has been cultured from water and sediment samples from rivers, lakes, and reservoirs in the United Kingdom. Positive water samples were significantly associated with recent rainfalls upstream, river height, and flow (Pickup et al., 2005).

The ability of *M. paratuberculosis* to survive in the environment has been well described but the area of the farm with the greatest risk for pathogen exposure has had

little discussion. In Minnesota dairy herds with Johne's disease, environmental samples were cultured positive in cow alley-ways (77% of herds), manure storage (68%), calving area (21%), sick cow pen (18%), water runoff (6%), and post-weaned calf areas (3%) (Raizman et al., 2004). These findings are not too surprising given that infected mature cattle shed the greatest number of organisms into the environment. Knowing the environmental distribution of *M. paratuberculosis* is useful information, especially when combined with the age-related susceptibility to Johne's disease. Further research is required focusing on the environment of beef herds.

PATHOGENESIS

The pathogenesis of Johne's disease will be discussed in terms of its relevance to the fecal shedding of *M. paratuberculosis*. However, very little research has been reported on fecal shedding, including the mechanisms for "triggering" or modulating its occurrence, the normal patterns of shedding, etc. It is commonly believed that fecal shedding is initiated through suppression or other changes of the immune system. A complete review of the immunology related to paratuberculosis infection can be found in several sources (Chiodini and Rossiter, 1996; Rideout et al., 2003).

It has been suggested that if the host is unable to contain the infection through granuloma formation, then *M. paratuberculosis* continues to proliferate and more mononuclear phagocytes are recruited from peripheral circulation (Chiodini, 1996). The granulomatous lesion will continue to expand until such a point that emigration occurs. It is thought that this migration of macrophages out of the lesion may be a result of lost or diminished macrophage inhibitory factor, or simply the effect of a space-occupying lesion. Macrophages near the epithelial lining emigrate into the intestinal lumen and are passed in the feces making the host "culture positive". The emigration of macrophages is dynamic, and varies as the lesion progresses and regresses. As each focus of infection expands, *M. paratuberculosis* is periodically shed into the feces through macrophages.

Several longitudinal studies have investigated the temporal patterns of diagnostic results from cattle and other species infected with *M. paratuberculosis* (Barrington et al., 2003; de Lisle et al., 1980; Kurade et al., 2004; Manning et al., 2003; van Schaik et al., 2003a). These studies have all reported considerable variation in the responses of

animals to *M. paratuberculosis* for the various tests. This suggests that there are many herd and cow factors that govern these different response patterns. One problem with many of these studies is the small numbers of animals investigated. However, such studies do provide some inferences as to what may be happening both immunologically and in regards to fecal shedding of *M. paratuberculosis*.

For a cow, the periparturient period is commonly regarded as the time of most immune suppression. However, studies have failed to consistently show an increase in fecal shedding during periods of stress. One such study demonstrated that force-feeding infected cattle during the peripartum period resulted in improved immunological status, but no difference in fecal shedding during this time (Stabel et al., 2003). This study only had 6 cows in each treatment group with variable stages of infection and it is also possible that the follow-up of these cows was not long enough. Perhaps “stressful” periods provide a “trigger” for shedding, but only when the intestinal lesions are sufficiently advanced.

Corticosteroids have also been used to try and stimulate fecal shedding of cattle. Dexamethasone (0.1 mg/kg IM) and prednisolone (0.3 mg/kg IM) were given once daily for 6 days to 10 female cattle (Wentink et al., 1988). These cattle ranged in age from 1 to 3 years and were purchased from 3 infected herds. No clinical signs were present in any of these animals prior to treatment. In two of the cattle, *M. paratuberculosis* was cultured from the ileum and mesenteric lymph nodes, but only one of the two shed *M. paratuberculosis* into its feces after treatment. Overall, corticosteroids were found to alter the immunological reactivity of these cattle, but not to such an extent that clinical disease was developed.

The ability of animals to clear infection has been documented experimentally (Chiodini et al., 1984). Our current understanding of how such phenomena may occur is limited by our knowledge of the immune response to *M. paratuberculosis*.

Unfortunately many of the immunological studies completed to date have been focused around diagnosis rather than host-agent interactions (Chiodini, 1996).

DIAGNOSIS

In the discussion of diagnostic tests, it is paramount that the definition of sensitivity and specificity are made extremely clear from the onset. Sensitivity and specificity are two measures that are used to determine the accuracy of a diagnostic test. Sensitivity is the proportion or percentage of truly diseased animals that test positive on the test being evaluated (Greiner and Gardner, 2000b). The specificity is the proportion of non-diseased animals that test negative on the test being evaluated (Greiner and Gardner, 2000b). These definitions should not be confused with those used in the laboratory, where sensitivity refers to the smallest amount of a substance or organism that is detectable and specificity is the ability to identify only the desired chemical or agent. From the epidemiologic definitions given above, it becomes clear that in order to calculate sensitivity and specificity, two populations of animals must be present, one with the disease of interest and one without. The test used to determine the true infection status is commonly referred to as the “gold standard” (Greiner and Gardner, 2000b).

Unfortunately for several diseases like paratuberculosis, no “gold standard” exists (Enoe et al., 2000). More recently, it has become in vogue to calculate the sensitivity and specificity of diagnostic tests without a reference test. Maximum likelihood estimation and Bayesian methodologies have been applied to generate such estimates (Branscum et al., 2004; Enoe et al., 2000). It is beyond the scope of this review to cover the details of these techniques. However, some precaution should be taken as the estimates calculated by each of these methods are based on several assumptions (Enoe et al., 2000). First, the two tests are assumed to be conditionally independent, meaning that

the classification errors in the reference test and test in question are independent and conditional on the true disease state. A second assumption is that the accuracy of both tests remains constant over different populations. When maximum likelihood estimation is used, the populations being compared are also assumed to have a different prevalence. Similarly, the Bayesian approach is influenced by the prior sensitivity and specificity estimates chosen and the distributions that are assumed for each. Overall, these techniques are another tool that can be utilized in the validation of diagnostic assays.

The validation and application of veterinary diagnostic tests in epidemiologic terms have already been succinctly described (Christensen and Gardner, 2000; Greiner and Gardner, 2000a; Greiner and Gardner, 2000b; Greiner et al., 2000). The aim of this review will be to discuss the literature surrounding the performance and interpretation of the commercial antemortem diagnostic tests for paratuberculosis that are commonly used in North America. However, a brief overview of the methodology of each test will also be given to provide an appreciation as to how it may influence the performance of the test. To simplify this discussion, the diagnostic tests have been broken down into two broad categories: agent detection tests and indirect diagnostic assays.

A. Agent Detection Tests

The first diagnostic tests to be discussed are the agent detection tests. These include bacterial culture (both traditional and broth media) and polymerase chain reaction (PCR) probes. As the name of this category of tests implies, these tests identify *M. paratuberculosis* directly or a genetic sequence from it.

In a commercial diagnostic laboratory setting, the most common sample for bacteriologic isolation or PCR is fecal samples. Although cultivation of tissue samples is done, these samples are usually often taken from post mortem specimens. The difficulty associated with fecal samples is the massively large number of organisms present in the sample. In all bacteriologic culture methodologies, a series of decontamination and concentration steps are used to selectively enhance the growth of *M. paratuberculosis* in the sample. The double-incubation or Cornell method of decontamination incubates the samples in hexadecylpyridinium chloride (HPC) solution and brain-heart infusion broth to initiate germination of bacterial and fungal spores (1989; Whitlock and Rosenberger, 1990). A second incubation of the sample in a powerful antibiotic cocktail is then used to kill the germinated bacteria and fungi, but may also decrease the number of viable *M. paratuberculosis*. The same is true for filtration of samples. Sedimentation and centrifugation techniques have also been described to increase the yield of *M. paratuberculosis*, but these may also concentrate contaminating microbes that are present in the sample (Stabel, 1997). Once samples are decontaminated, they are ready for culture.

In North American diagnostic laboratories, traditional culture of *M. paratuberculosis* typically utilizes Herrold's egg yolk medium. The fastidious nature of the organism results in a standard incubation period of 12 to 16 weeks at 37°C. Such a long duration of incubation increases the risk of overgrowth by contaminating *Bacillus* and mold species and thus the importance of proper sample preparation. One of the unique characteristics of *M. paratuberculosis* is its dependence on mycobactin J for in vitro growth. Mycobactin J is essential as the organism has no ability to acquire and

chelate iron. One of the original methods for confirming the presence of *M. paratuberculosis* was to inoculate several tubes containing mycobactin J and one tube without. If colonies characteristic of *M. paratuberculosis* were present in the tubes with mycobactin J and not in the tube without, a diagnosis of paratuberculosis was made. A second attribute of *M. paratuberculosis* is its positive reaction to Ziehl-Neelsen or acid-fast staining.

Other systems of culturing *M. paratuberculosis* that have become more common are the use of liquid or broth media. The techniques employed were extrapolated from previous work done on the isolation of *Mycobacterium tuberculosis* (Cummings et al., 1975; Kirihaara et al., 1985). This method of culture involves inoculating a sealed vial with liquid medium, commonly Middlebrook 12B medium in the BACTEC system, and then measuring the growth of bacteria. At the current time, there are three different systems for measuring the growth of bacteria. The first system to be utilized was the BACTEC radiometric culture, in which a radioisotope (^{14}C) is incorporated into palmitic acid in the medium (Damato and Collins, 1990). As bacterial growth occurs, $^{14}\text{CO}_2$ is released into the headspace of the vial. Each week, these vials are tested using the BACTEC 460TB analyzer to determine the amount of radioactivity present in the headspace gases. These measures are indexed and once the growth index is greater than a predetermined value, the sample is considered positive and in need of confirmation. The necessary incubation period of samples is eight weeks, or half of that for traditional culture (Collins et al., 1990). However, many positive samples reach the growth index by 3 to five weeks of incubation (Whittington et al., 1998). Another system, ESP Culture System II (TREK Diagnostics, Sun Prairie, WI), measures bacterial growth by

measuring pressure changes within specially designed vials (Kim et al., 2002). This system is considered safer than the BACTEC system as there is no radioisotope involved (Grant et al., 2003). The third system, MGIT (Mycobacterium Growth Indicator Tube) Culture System (Becton Dickinson, USA), relies on fluorometric technology to determine the growth of organisms (Grant et al., 2003). When positive growth MGIT tubes are placed on an UV transilluminator (365 nm wavelength) a vivid orange fluorescent glow is emitted at the base of the tube and at the meniscus (Grant et al., 2003). As respiring bacteria consume the dissolved oxygen in the MGIT broth medium, more fluorescence exists (Grant et al., 2003). The important realization with all culturing systems is that none of them definitively grow *M. paratuberculosis*, so another test must be utilized to confirm its presence. The most commonly used test is a PCR probe.

PCR probes for *M. paratuberculosis* have been described in the literature for well over a decade and a half (McFadden et al., 1987a). After similar decontamination steps to those described above, the bacteria present in the sample must be lysed to extract their DNA. A particular sequence of DNA is then amplified through the PCR process. All of the PCR probes used commercially today are based upon identifying insertion sequence 900 (IS900) within the *M. paratuberculosis* genome. With over 99% homology between the genome of *M. paratuberculosis* and *M. avium*, the availability of other sequences is quite restricted (McFadden et al., 1987b). Other sequences like IS1311 have been identified, however, IS900 is repeated approximately 8 to 20 times in the *M. paratuberculosis* genome, making it a good target for amplification (Olsen et al., 2002). It is often suggested that the difference between various PCR assays are the primers. These primers are oligonucleotide sequences that are used to direct and initiate the

polymerase enzyme in its replication of the desired DNA fragments. Once the DNA is amplified, it must then be detected. PCR assays commonly use electrophoresis to separate the DNA fragments according to their electrical density. Another approach also used today is dot-blot hybridization, which in simplified terms is the use of specific oligonucleotide sequences to hybridize with part of the amplified DNA fragments. Enzymes are bound to the nucleotide sequences and produce a colour change when the appropriate substrate is provided. Overall, the techniques associated with such probes are very technically sophisticated and time consuming. Although automation of these procedures is making these tests simpler to perform, they are still relatively expensive. The greatest advantage of PCR probes over the culturing methods is that results can be received from a laboratory within 3 days as compared to several weeks or months.

The performances of each of these tests have been evaluated independently in several reports. There is only one report that compares conventional (traditional) fecal culture, radiometric fecal culture (BACTEC system) and a commercial PCR probe (IDEXX HerdChek PCR Probe, IDEXX Laboratories, Portland, Maine) (Sockett et al., 1992a). The objective of this paper was to compare these three diagnostic tests in subclinically infected cattle. Two populations of dairy cattle were utilized, one with Johne's disease and another without. The specificity of the two culturing systems was assumed to be 100%. Using 3 herds containing 214 cows that were certified free of Johne's disease after 3 years of negative annual herd fecal cultures, the PCR probe was estimated to have a specificity of 100%. Nine herds (641 cows) with a prevalence ranging from 7% to 61% were used to determine the sensitivities of the three diagnostic tests. The test sensitivities of conventional fecal culture, radiometric fecal culture and

DNA probe were $45.1\% \pm 7.2\%$, $54.5\% \pm 7.3\%$ and $33.5\% \pm 6.9\%$, respectively.

McNemar's chi-square test indicated that there were significant differences in sensitivity among all three tests. Although the design of this study is good, some concern still exists in regards to the gold standard test used in the sensitivity analysis. All 641 cattle had fecal and blood samples taken at the time of sample collection. Cows that were positive on any of the three fecal tests were considered infected. Any cattle that were negative on fecal culture, but positive on a serum ELISA, had biopsies of ileum and regional lymph node collected surgically or at slaughter. These tissue samples were evaluated through histopathology and mycobacterial culture. The sensitivity of these two tests was not reported. A second area of concern relates to the number of ELISA and fecal culture negative cattle that were truly infected. One recent study reported that of conventional fecal culture negative cattle, 26% were positive on tissue culture, and conversely, of fecal culture positive cows, only 66% were positive on culture of the ileum and adjacent lymph node (Pavlik et al., 2000). Unfortunately, ileum and adjacent lymph node biopsies were not collected from the ELISA and fecal culture negative cattle in the Sockett study. Of the 641 cows in the study, 559 were negative on a single traditional fecal culture. If we assume that 26% of the conventional fecal culture negative cattle would have been tissue culture positive, then an additional 43 positive cows may have been present. The re-estimated sensitivity values for conventional fecal culture, radiometric fecal culture and DNA probe would be 36%, 44% and 27%, respectively. Therefore, the reported sensitivity values are higher than they truly are for subclinically infected cattle (stage 2).

It should be acknowledged that when these tests are used in an infected population, the cattle would be at various stages of the disease. The sensitivity of these

tests in clinical cattle (stages 3 and 4) has not been determined, however it has been estimated to be >90% (National Johne's Working Group, US Animal Health Association). Whitlock et al. (2000) followed a cohort of newborn calves, yearlings and mature cows from 10 paratuberculosis infected herds over a period of 4 years. All 954 animals were initially tested with fecal culture and culture negative animals that were not culled were re-tested every 6 months for 3 ½ years. Over the four years, 210 animals were identified as fecal culture positive and 79 of these animals were detected on the first fecal culture, giving a sensitivity of 38%. Unfortunately, culled cattle were not followed to slaughter in this study. It was estimated, however, that an additional 111 positive cows were culled, giving a single fecal culture an overall sensitivity of 25% when all stages of disease are included. Fecal culture is generally only used to test mature cattle within a herd. When the culture results from only mature cattle were evaluated, the overall sensitivity of a single conventional fecal culture was estimated to be 33% for subclinically infected cattle (Whitlock et al., 2000). This sensitivity value is quite similar to the 36% estimate calculated from a study discussed previously (Sockett et al., 1992b).

One of the issues with culture-based tests is that decontamination methodologies vary between laboratories and ultimately influence the sensitivity (Collins et al., 1990; Eamens et al., 2000; Kalis et al., 1999; Stabel, 1997). Eamens et al. (2000) concluded that radiometric methods are more sensitive than conventional culture when the same decontamination process is used. These results agree with the differences in sensitivity between the culturing methods estimated in the Sockett study (1994). Alterations to the

media commonly used for both radiometric and conventional culture can also influence the growth of *M. paratuberculosis* (Damato and Collins, 1990; Jorgensen, 1982).

The use of the IDEXX PCR probe directly on feces is less sensitive than culturing methods, however, both conventional and radiometric culturing systems are commonly confirmed with PCR (Lein et al., 1990; McFadden et al., 1987a; Sockett et al., 1992a). This is essentially using two tests in parallel to increase the overall sensitivity. In fact, culture is a great amplification process for increasing the amount of *M. paratuberculosis* in a sample and thus its DNA. Coupling agar enrichment with PCR has demonstrated an improvement in the quantity of organisms that can be detected (Secott et al., 1999). Immunomagnetic separation (IMS) techniques to aid in the extraction of DNA prior to PCR are also being developed (Chui et al., 2004; Grant, 1998; Grant et al., 2000). The use of IMS and PCR on spiked milk and fecal samples appears promising with the research completed to date (Chui et al., 2004; Grant et al., 2000). One concern with using direct fecal PCR and PCR for confirmation of culture results, is the potential risk that other Mycobacteria might acquire or already contain IS900 or IS900-like genetic elements (Englund et al., 2002; Kim et al., 2002). This would then question the specificity of the test. Further research is being directed at identifying other novel DNA sequences for the testing of *M. paratuberculosis* (Bannantine et al., 2002).

The bacteriologic culture of pooled fecal samples has also been evaluated for the detection of *M. paratuberculosis* in dairy herds. It has been reported in two separate studies that 63% to 81% of age-clustered pools with one or more infected cows, will test positive on bacterial culture and that the herd sensitivity of age-clustered pooled fecal samples ranges from 73% to 94% (Kalis et al., 2000; Wells et al., 2003). Additionally,

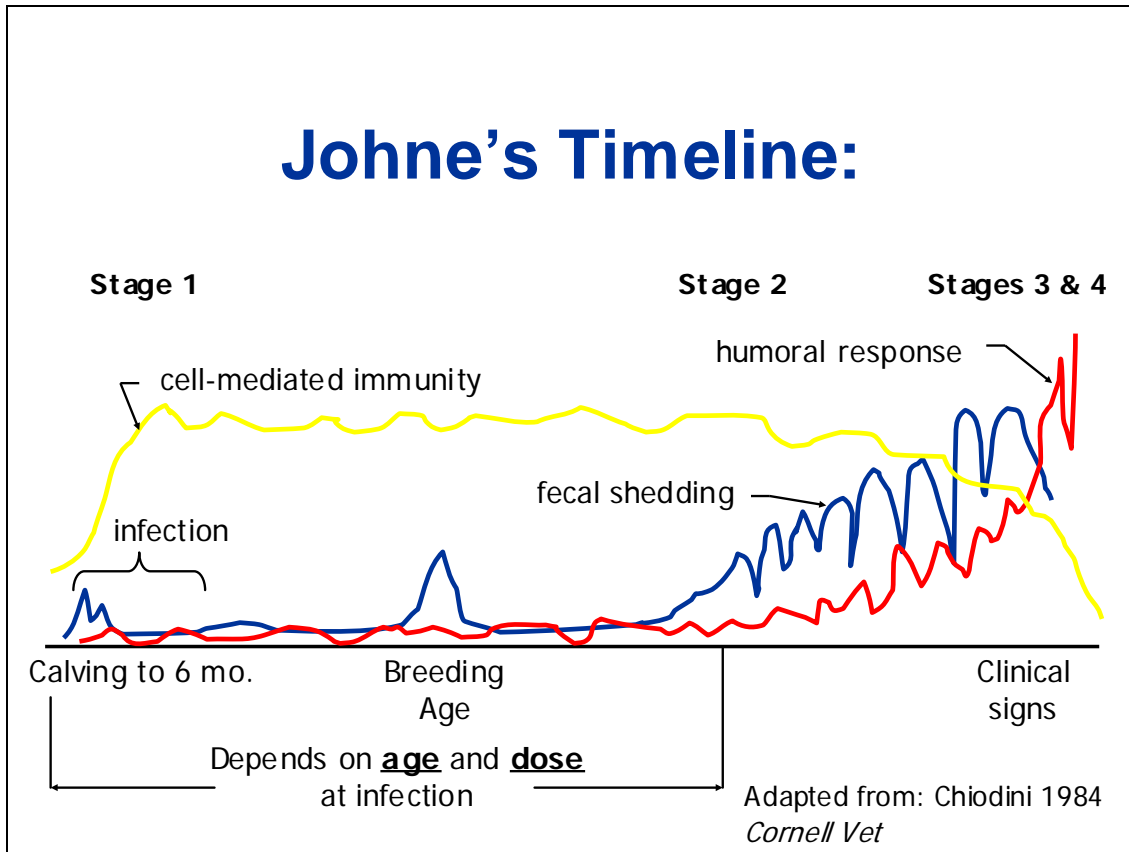
4% to 7% of fecal pools cultured positive, despite being comprised of negative individual samples. Not surprisingly, the higher the mean number of colony forming units in a fecal pool the greater the likelihood that the sample will culture positive (Wells et al., 2003). Pooling fecal samples from 5 cows will detect more infected cows than pools of 10 cows, however, herd prevalence and size are two other important considerations if pooled sampling is going to be valid and economical (McKenna et al., 2005a; van Schaik et al., 2003b; Wells et al., 2002). More specifically, a stochastic model predicts that if pooled fecal samples are going to be more cost effective than individual cultures when used to determine a herd's infection status, then the optimal pool size is directly proportional to prevalence and herd size (van Schaik et al., 2003b). For example, the pooling of fecal samples was not cost efficient in a 100-cow herd with a low prevalence (<5%) because herd sensitivity was only 53%.

Preliminary results from a single beef herd study, suggest that pooling 4 to 5 age-clustered samples is more effective than random pooling of samples (Jensen et al., 2005). Research in Alberta has shown that when compared to individual fecal culture, the cow level sensitivity of fecal pooling of 5 and 10 cattle in test positive herds was 73% and 77%, respectively in beef herds and 74% and 63% respectively in dairy herds (Scott, 2004). Herd level sensitivity of fecal pools of 5 and 10 cattle was 92% and 83%, respectively in beef herds and 78% and 78%, respectively in dairy herds. Dairy cattle fecal samples were also twice as likely to develop fungal overgrowth as compared to beef cattle. It has been suggested that this may be due to the common practice of feeding silage to dairy cattle.

B. Indirect or Immunological Tests

There are several indirect or immunological tests to detect *M. paratuberculosis* infection in cattle. In fact there are two broad categories that are based on the predominant immune reaction that is being evaluated; these include the cell-mediated and humeral assays. A cell-mediated immune (CMI) response is thought to be the primary and protective reaction to *M. paratuberculosis* infection (Collins, 1996). The nature of this immune response is more generalized and as a result many of the diagnostic tests evaluating CMI reactions are lacking specificity. Many antigens can stimulate IFN- γ production, or alter body temperature, and measuring the size of skin reactions is often subjective, thereby producing equivocal results (Collins, 1996; Kalis et al., 2003). It is for these reasons that the interferon-gamma (IFN- γ), intravenous Johnin and intradermal testing, respectively, are not commonly used today. Despite current limitations, more research is being done to try and improve the functioning of IFN- γ assays (Huda et al., 2004; Jungersen et al., 2002; Kalis et al., 2003; McDonald et al., 1999; Stabel and Whitlock, 2001). As suggested above, the real advantage of these assays is that they have the ability to detect younger animals than humeral or agent detection tests. One further logistical challenge for the IFN- γ test is that it requires live lymphocytes, from the white buffy coat of separated whole blood. To acquire such cells, heparinized blood samples must be submitted to the laboratory and analyzed within 12 to 16 hours for the results to be meaningful (Collins, 1996).

Figure 2: A schematic to illustrate the presumed changes in fecal shedding and immune response over time in an infected bovine.



The second immunological response to be discussed is the humeral or antibody reaction to *M. paratuberculosis*. This immune response is thought to occur later in the disease process and as a result the diagnostic tests manufactured to measure antibodies have a lower sensitivity when compared to other diagnostic tests. Once again, several diagnostic assays have been developed to detect antibodies in either serum or milk. The most commonly used serological test today is the enzyme-linked immunosorbant assay (ELISA). Some laboratories still offer agar gel immunodiffusion (AGID) or complement fixation tests (CFT), however, these are no longer commonly used and limited discussion will be directed around them.

An ELISA for paratuberculosis was first described in the literature in 1978 (Jorgensen and Jensen, 1978). However, it wasn't until 1985 that researchers found that pre-absorbing sera with *M. phlei* cells removed cross-reacting antibodies and this considerably improved the specificity of the assay (Yokomizo et al., 1983; Yokomizo et al., 1985). A commercial absorbed ELISA testing service was first offered in North America in 1988 for the detection of antibodies to *M. paratuberculosis* (Allied Monitor, Fayette, MO) (Collins and Sockett, 1993). In 1992, the first USDA license was granted for an Australian developed diagnostic test kit that would be marketed in North America by IDEXX Laboratories Inc. (Westbrook, ME) (Collins and Sockett, 1993). Today there are several absorbed serum ELISA test kits available from manufacturers globally. The sensitivity and specificity of commercial absorbed ELISAs commonly used in North America have been reported to range from 43% to 65% and 98.9% to 99.8%, respectively (Collins et al., 1991; Cox et al., 1991; Milner et al., 1990; Reichel et al., 1999; Ridge et al., 1991; Sockett et al., 1992b). The stage of disease was acknowledged in all of these studies as influencing the accuracy of the ELISA, but was not investigated thoroughly. However, two studies reported to date, have evaluated commercial ELISAs in multiple groups of infected cattle at various stage of disease (Dargatz et al., 2001a; Sweeney et al., 1995). The results were remarkably similar with the sensitivity varying from 15% in light-shedding, subclinical cattle to as high as 88% in clinical cases of paratuberculosis. The overall sensitivity and specificity of the ELISA using the manufacturers recommended cutoff was $45\% \pm 4.8\%$ and $99\% \pm 0.9\%$ (Sweeney et al., 1995). Whitlock et al. (2000) estimated that in a typical infected population of cattle, 95% of the detectable individuals will be subclinical (stage 2) and 5% will be clinical cases (stage 3).

The percentage of low, medium and high subclinical fecal shedders is 70%, 5% and 20% of the identified infected animals, respectively. If the sensitivity values estimated in the Sweeney study were applied to such a population, the estimated sensitivity of the serum ELISA would be 25% (Whitlock et al., 2000). An important consideration is that this estimate excluded cattle that were in stage 1 of the disease. The sensitivity of two commercial absorbed serum ELISAs were recently determined to be 8.8% and 6.9% relative to tissue culture in a slaughterhouse study of culled dairy cattle (McKenna et al., 2005b). These estimates may in fact be realistic measures of the serum ELISA sensitivity when cattle in all stages of disease are considered. The lack of sensitivity and agreement between these are a significant concern.

The varying estimates of serum ELISA sensitivities can be overwhelming and confusing to a veterinarian and producer. As indicated above, consideration must be given to the stages of disease present in the study population. The reference or gold standard test should also be in question. In several of the studies published in the early 1990's (Milner et al., 1990; Ridge et al., 1991; Sockett et al., 1992b), the fecal culture techniques used were not as sensitive as those utilized today (Whitlock et al., 2000). Similarly, fecal culture is not as sensitive as tissue culture. As a result, the sensitivity values reported are slightly inflated (McKenna et al., 2005b; Whitlock et al., 2000). Another concern is that of agreement between the different ELISAs. When the agreement between ELISAs were examined, it was found that these tests were in agreement 18% to 85% more often than what would be expected due to chance alone depending on which tests were being compared (Collins et al., 2005; McKenna et al., 2005b). These issues need to be considered when attempting to interpret the results of

one specific test. Identification of cattle in the first stage of paratuberculosis should remain a priority for future research, but perhaps the ELISA sensitivities that are reported to practitioners should only include animals in stages 2 and 3 of the disease as these are the animals believed to be of highest risk to fellow herd mates and are the animals that current tests can actually identify.

The recommended use for all serum ELISAs has been the identification of infected herds. Although many producers and veterinarians interpret the individual ELISA results for each cow. Two methods that have been discussed to improve the accuracy of decisions made for an individual cow include serial testing and multiple cutoffs (Collins, 2002; Hirst et al., 2002). Strategic sampling of cows over time to monitor their serologic status may aid in management decisions (Hirst et al., 2002). Serial testing can be even more powerful if multiple cut points are used to increase the specificity of the test. It has been reported that as the ELISA optical density or sample to positive control (S/P) ratio increases, the likelihood that a cow is infected with *M. paratuberculosis* increases exponentially (Collins, 2002; Collins and Sockett, 1993). The probability that a cow is infected (post-test odds) can be calculated at various cut-points, however, the prevalence of Johne's disease in herd (pre-test odds) must also be taken into consideration. Overall, caution must be exercised when ELISAs are interpreted at the individual cow-level.

One other concern with the ELISA and all other diagnostic tests are the consistency and reproducibility of results both within and between laboratories. Some variation in results is expected, however, proper monitoring of the coefficient of variation (CV) within the results of negative control samples is essential (Collins et al., 1993).

PREVENTION AND CONTROL

A clear understanding of the epidemiology of Johne's disease is pivotal in the creation of prevention and control strategies. The 1997 NAHMS Beef Survey found that 92.2% of beef producers were either unaware of Johne's disease or only recognized the disease by name (USDA, 1999). Any successful management of this disease will require a significant focus on producer education. Many of the management recommendations that are currently in place around the globe are simply based on the principle of decreasing the exposure of susceptible young stock to infective feces, milk and colostrum. Although these recommendations are logical, many have not yet been substantiated. Further more, the best management practices described for beef herds have primarily been derived from dairy cattle research. Given the differences in management between the beef and dairy industries in North America, the need for beef specific research is critical.

Milk, colostrum and feces are all means of transmission for *M. paratuberculosis*. However, feces contain the largest quantity of bacteria and provide the greatest risk to susceptible young stock. The largest source of infective feces is from subclinical and clinical shedding cattle and in particular the super-shedding cattle. Unfortunately, many of the subclinically infected cattle remain undetectable.

Several control points or risk factors have been found to reduce the risk of Johne's disease in dairy herds. Calving management is critical to any control strategy. More specifically, the cleanliness of the calving pen, number of cows present, length of time before a calf is removed, and the washing of udders prior to calving or at the time of

colostrum collection, have all been associated with paratuberculosis herd status (Goodger et al., 1996; Johnson-Ifeorlundu and Kaneene, 1998; Wells and Wagner, 2000). The housing and feeding of pre-weaned calves is also important (Collins et al., 1994; Goodger et al., 1996; McNab et al., 1992; Obasanjo et al., 1997). The inability of beef producers to remove calves from cows after calving introduces additional challenges when trying to manage this disease.

The application of lime or manure onto pasture, use of exercise lots for cows, contact with other cattle, cow nutrition and water sources have been associated with Johne's disease herd status for dairies (Daniels et al., 2002; Goodger et al., 1996; Johnson-Ifeorlundu and Kaneene, 1998; McNab et al., 1992). Dairy herds with a previous diagnosis of paratuberculosis, a large herd size (>300-600 cows), purchased replacement animals, or simply poor general management, are also at increased risk of Johne's disease (Daniels et al., 2002; Hirst et al., 2004; Johnson-Ifeorlundu and Kaneene, 1998; McNab et al., 1992; Obasanjo et al., 1997; Wells and Wagner, 2000). The access of wildlife, especially rabbits, to pasture or other feedstuffs, has also been identified as a significant risk factor on dairy farms in the United Kingdom (Daniels et al., 2002).

Cow-calf producers commonly engage in management practices that increase the risk of disease introduction to their cattle such as importing cattle, inconsistently testing for various diseases in imported animals, failing to use quarantine procedures, and the use of communal grazing (Sanderson et al., 2000). Management practices that have been found associated with the *M. paratuberculosis* seropositivity of beef herds include: the history of having a dairy-type nurse cow on farm (Odds Ratio=2.1), use of seasonal

calving, i.e. spring calving versus any other time of year (OR= 2.2), the use of running streams as a water source (OR=2.2), previous clinical signs of Johne's on farm (OR=2.8), and having *Bos indicus* rather than *Bos taurus* cattle on farm (OR=17.4). When controlling for the effects of other risk factors, the use of a dairy-type nurse cow and seasonal calving became non significant but cattle species and water source still remained significant risk factors (Roussel et al., 2005). Clearly, more research needs to be done in order to fully understand all of the potential factors important in management of *M. paratuberculosis* in beef herds in Western Canada.

The role of vaccination in the control of paratuberculosis is uncertain at this time as the results of many vaccine trials are varied. Vaccination does appear to reduce the incidence of clinical disease and fecal shedding, and may be economical for herds with high culling rates due to clinical disease (Kalis et al., 2001; Kormendy, 1994; Larsen et al., 1978; Uzonna et al., 2003; van Schaik et al., 1996), however, several of the reported studies were not conducted using commercial vaccines or natural exposure to the pathogen (Chiodini et al., 1984; Kalis et al., 2001; Kormendy, 1994; Larsen et al., 1978). Further complications associated with vaccination include granuloma formation at the site of inoculation, interference to serological diagnosis, and the risk of accidental self-inoculation by veterinarians (Chiodini et al., 1984; Patterson et al., 1988; Spangler et al., 1991). If vaccination is going to be used it is recommended that it be given within the first 30 days of life (Thoen and Haagsma, 1996).

The use of monensin as a feed additive may help to reduce the burden of *M. paratuberculosis* on positive farms (Hendrick et al., 2005b; Whitlock et al., 2005b). Monensin sodium belongs to the class of antimicrobials called ionophores and its

spectrum of activity includes several Gram-positive bacteria, some *Campylobacter* spp., *Serpulina* spp., *Mycobacterium* spp. as well as coccidia and toxoplasma (Ipharraguerre and Clark, 2003; Prescott et al., 2000; Liu, 1982). Its minimum inhibitory concentration (MIC) for *M. paratuberculosis* recently reported to be 0.3 micrograms per ml (Brumbaugh et al., 2004). Recent research found that monensin decreased passive fecal shedding by 55% and tissue infection by 63% in an experimental calf infection model (Whitlock et al., 2005b). Similarly, the quantity of *M. paratuberculosis* shed in the feces of infected cows was marginally reduced when monensin was fed (Hendrick et al., 2005b). Further research into the potential use of monensin for Johne's disease control is required.

Test and slaughter programs have been suggested as a primary means to control Johne's disease. However, there are several factors affecting the profitability of such programs. These include the prevalence of infection, test accuracy (sensitivity and specificity), and estimated production loss (Collins and Morgan, 1991). A stochastic dynamic computer simulation model suggested that test and cull strategies or vaccination do not reduce the prevalence of Johne's disease in dairy herds (Groenendaal and Galligan, 2003). Calf hygiene strategies were the most economical part of a paratuberculosis control program for midsize US dairy farms in this model. These results are similar to those reported in another study where the use of both calf management and test-and-cull methods provided the quickest means of controlling paratuberculosis in a simulation model (Collins and Morgan, 1992).

The USDA principles of Johne's disease control for beef producers include: reducing exposure and infection of replacement cattle on farm, identifying and removing

the most highly infected cattle, and preventing introduction of infection by screening sources of off-farm replacements (USDA, 1999). The following recommendations, based on first principles of disease control, have been made with the focus on the beef industry (Hansen and Rossiter, 2000; Rideout et al, 2003):

1. Reducing manure build-up of pens and pastures where late-gestation cattle are kept.
2. Keep the calving area clean at all times and maintain a low cow density in these areas.
3. As soon as bonding has occurred, move cow-calf pairs to a clean pasture.
4. Avoid exposing calves to manure build-up by frequently moving location of feedbunks, waterers, and creep-feeders.
5. Once calves are weaned, do not put them on pastures used by cows.
6. Annually test the entire herd and avoid calving-out or raising offspring from any test-positive cattle.
7. Calve first-calf heifers in a separate location from mature cows.
8. Use separate equipment for handling manure and feed.
9. Do not spread manure on land used for grazing, esp. for young stock.
10. Purchase replacement animals only from test negative herds and when this is not possible assess herd status through owner and veterinarian statements.

Devising control strategies is important, but little is known on how well they are perceived and utilized by producers. In Australia, it was found that 48% of dairy farmers adopted none of the long-recommended control measures even though they ranked

Johne's disease as their number two calf-hood infection concern (Wraight et al., 2000). One study has found that regardless of herd infection status, producers who had tested their herd for paratuberculosis were more likely to be using management strategies, as compared to producers who were not testing (Naugle et al., 2004). The motivation of these producers to sell breeding stock may have influenced these results, that is, producers with a negative herd status are only likely to test and use specific management strategies if it guarantees that their animals can be sold for a premium. Overall, the value and need for further research and Johne's disease management education programs for producers and veterinarians is clearly indicated.

NATIONAL JOHNE'S DISEASE CONTROL PROGRAMS

National government-funded animal disease control programs are typically focussed on specific diseases that are widely recognized for their economic or public health importance. Governments have begun to develop control programs for Johne's disease due to increasing evidence of significant production losses, possible restrictions to international trade, and its zoonotic potential. It has been suggested that because DNA from *M. paratuberculosis* has been found in 69% of patients with Crohn's disease that *M. paratuberculosis* may be a factor in the causation of the disease (Sechi et al., 2004). There are few national control programs across the world at this time and none specifically for the beef industry at this time. The United States, Australia, and the Netherlands have all attempted to control Johne's disease in their cattle industry primarily by focusing on their dairy industries. All of these control programs are a combined effort with government and industry providing funding and logistical support. A proposal for a Canadian National Voluntary Johne's Disease Prevention and Control Program is currently being developed with the support of the Canadian Animal Health Consultative Committee, Dairy Farmers of Canada and the Canadian Cattlemen's Association (CAHCCAR, 2002). This program is being developed based on the experience of national control programs that have been in place in other countries such as Australia, the Netherlands, and the United States. There is currently a voluntary Johne's disease control program in place in the province of Alberta which should be easily adapted to the proposed national program. The national Johne's disease control programs for Australia, the Netherlands, and the United States, as well as the provincial control program for

Alberta will be briefly described followed by a summary of the Canadian National Voluntary Johne's Disease Prevention and Control Program that is currently being developed.

A. Australian National Voluntary Johne's Disease Control Program

Australia started the National Johne's Disease Market Assurance Program for Cattle in 1996 (NJDP, 2003; AAHA, 2003a). There has been a steady increase in herd participation in this program from approximately 180 in 1996 to 1623 herds 2003 (AAHA, 2003b). They have developed a marketing system that involves assurance scores being assigned to farms based on multiple factors including farm location, past history of Johne's disease on farm, testing history, animal movement history, calf rearing practices, and other management factors. Herds can then progress through levels of assurance based on annual negative herd tests from MN1 (Monitored Negative 1) to the highest level of MN3 (Monitored Negative 3). The required method of testing is not specified but must be approved by the Chief Veterinary Officer (CVO). The entire herd, up to a maximum of 100 cattle, is required to be retested every two years to maintain their level. Herds are also required to have an accredited veterinarian monitor their management practises annually. Herds can also be classified as non-assessed which means there is no history of Johne's disease in the herd or suspect for multiple reasons such as failing the management inspection of an accredited veterinarian but this classification still requires that no cattle have tested positive in the herd. Infected herds have a test positive animal and restricted herds are herds that were infected but are taking part in an approved test and control program and have had at least one negative herd test

at least one year after the last known test positive animal was culled (AAHA, 2003a). Animals coming from herds of a higher assurance level should demand a higher price pressuring the industry to move towards further adopting Johne's control practices. Australia has also broken the country into regions to allow for more control of animal movement in and out of certain regions. Australia has found that the beef industry has a very minimal Johne's problem and has elected to use the Beef Only program which allows beef producers to advertise that their herds have had no potential contact with dairy cattle. Herds must prove that there had been no dairy cattle in direct contact with any of their herd for 5 years and no indirect contact within the last 12 months with the exception of dairy cattle certified as Johne's free through the government program (Kennedy, 2005).

B. The Dutch National Voluntary Johne's Disease Control Program

In 1991, The Netherlands began a Johne's disease control program. Their original program consisted of herd fecal cultures every six months and after five rounds of testing only 46% of the participating herds remained Johne's negative in spite of having no clinical disease (Kalis et al., 1999). This led to producers feeling penalized for participating in the program. They have adjusted their program to focus on the reduction of Johne's disease in the national dairy herd while being primarily concerned with reducing the level of *M. paratuberculosis* in the milk supply. The current program includes a management assessment focussed on reducing the spread of infection as well as an intensive program of herd status classification. Herds can be classified as free of disease (level 10), unsuspected (level 6-9), or infected (level 1-5). The program requires

annual testing of adult cattle. In order to improve a herd's classification level, pooled fecal samples (5 cows/pool) are required and the ELISA is acceptable for maintaining a herd's present status. Positive ELISA results confirmed with fecal culture and a positive culture result leads to a classification level reduction for the herd. There is logistical and funding support to encourage entrance and participation in the national control program (Kalis, 1999).

C. United States National Voluntary Johne's Disease Control Program

The American program allows for some flexibility on some details such as the inclusion of on farm risk assessments, herd management plans, and market based incentives/disincentives (Rideout et al, 2003), in order to encourage individual states to participate. This program consists of 3 parts including 1) education of producers, 2) on-farm risk assessments and herd management plans, and 3) herd level classification based on herd testing. The initial testing (test used determined by state officials) is done on 30 randomly selected cattle over three years of age. Environmental testing has been accepted as an acceptable herd test however if a herd is found positive based on this test, individual tests must be completed on the entire herd. Animals positive on a screening test can be appealed, but must be tested with an official Johne's test such as fecal culture or PCR. If this test is negative the herd can retain its previous classification level but is required to be retested during the next round of herd testing. If this test is positive the owner can make another appeal requiring either a necropsy or surgical biopsy of the lymph nodes and ileum. If the animal is still declared positive the herd is declared positive. In order for a herd to improve a classification level at least ten months must

pass without a positive case. If a herd is not tested for 14 months, it is declared a maximum risk herd or herd of unknown status. It is possible for herds to fast track to level four within two years involving three tests. Level one is passed with a signed declaration that the herd has had no seen or diagnosed cases of Johne's disease within the previous five years (USDA, 2005)

D. Alberta Johne's Disease Control Program and Canadian National Voluntary Johne's Disease Control Program

Alberta Agriculture Food and Rural Development implemented a Voluntary Johne's Disease Herd Status Program in September 2001. The proposed Canadian National Voluntary Johne's Disease Prevention and Control Program has intentionally been developed very similarly to the Alberta program. Some changes were made based on recent research such as the exclusion of the use of ELISA testing beyond level 1 due to evidence of ELISA inaccuracy. The proposed Canadian National Voluntary Johne's Disease Prevention and Control Program is comprised of two major components (CAHC, 2006). The first component consists of a Best Management Practices (BMP) Assessment based upon a herd risk analysis of critical control points in the avoidance of transmission of *M. paratuberculosis*. This assessment by trained veterinary personnel would lead to tangible recommendations based upon the assessment. The second component of the program would involve a herd testing protocol specific to the desires of the herd and their prevalence. It is proposed to have two separate pathways for farms to follow. Herds that have a low prevalence and want to certify that they are indeed a low prevalence herd will enter the Johne's Disease Status Pathway (JD-SP). This pathway allows herds to

demonstrate their low herd prevalence through annually repeated testing procedures in association with BMP assessments. Known infected herds, or herds that simply are not interested in vigorous testing could follow the Johne's Disease Prevention Pathway (JD-PP). These herds participate through annual BMP assessments, including a stringent follow-up assessment to determine if they have made any advances on management changes recommended from their last assessment. The BMP assessment is an evaluation of a herd's level of management with respect to the recommendations for decreasing the risk of new *M. paratuberculosis* infections. A BMP Assessment will be developed that is specific to cow-calf operations. Herds may participate in this aspect of the program without taking part in the second component, the Voluntary Johne's Disease Herd Status Program.

The proposed Herd Status Program starts at Stage level 0 (infected) and progresses to the highest level of 4. All herds start at stage 0 and to advance to stage 1, the herd must complete a BMP assessment and have a negative herd test consisting of 30 cattle in their second or greater lactation. If an animal is ELISA positive, the producer can run fecal cultures on the ELISA positive animals. If these are negative the herd proceeds to stage 1, however, any positive culture or no further testing maintains the herd at stage 0. A herd must wait for at least 10 months prior to re-applying for stage advancement.

To proceed from Stage 1 to Stage 2, a herd must undergo another BMP assessment and pooled fecal cultures (10 cattle/pool) must be performed on all cattle in their second or greater lactation. If all pools are negative, the herd would advance to the next level. If at least one pool from the herd is positive, the producer can: 1) culture the individual cows in the positive pool to identify which cows are positive. The producer

can then elect to cull the infected cows and remain at Stage 2, or 2) allow the cow(s) from the positive pool to remain in the herd and the herd would fall back to Stage 0. A herd that is at Stage 2 may choose to no longer remain in the Advancement strategy and opt to follow the Maintenance strategy instead. In order to maintain their status, a herd must have a BMP assessment at least every two years and complete environmental sampling. If all environment samples are negative, they maintain their status. If any samples are positive, they can use pooled culture followed by individual culture to identify which cow is positive. If a positive cow is found it must be culled in order to maintain status, otherwise the herd moves back to Stage 0. In order to maintain status, testing must be performed at least once during a maximum of a two-year period. To advance from Status 2 to 3, and 3 to 4, the same testing and control program would be required as for advancement from Stage 1 to 2. For Maintenance at Stage 3 or 4, a recent BMP assessment (at least once every 2 years) must be performed along with environmental sampling. If all samples are negative, they maintain status. If the samples are positive, they must attempt to identify the positive cows with pooled fecal cultures of ten cows per pool and then individual culture. If a positive cow is identified, the herd can elect to cull the infected cow and drop one Stage; otherwise they drop to Stage 1. Again, in order to use the Maintenance program, testing must be done at least once during a two-year period

This program, once operating, should have multiple benefits including the reduction of Johne's disease in the national herd, promoting future research on Johne's disease, monitoring the prevalence of Johne's in the enrolled herds across the country as well as monitoring the impact of management. The lack of a funding component may reduce the level of participation by producers due to the substantial cost of a test and cull

strategy. Increased involvement of the producers and their governing organizations is going to be critical to any control program success. In order for this proposed program to be effective it will require strong support from industry and government.

FUTURE RESEARCH CONSIDERATIONS

Significant gaps remain in our knowledge of the effects *M. paratuberculosis* has on beef cattle and the beef industry as a whole. Conducting further research specific to the beef industry will better enable producers to develop effective and efficient control programs and reduce any negative impacts *M. paratuberculosis* has on affected farms and on the entire industry. Some suggested areas of future research that should be considered include:

1. Further monitoring of the prevalence of *M. paratuberculosis* at the individual and herd level across Canada.
2. How *M. paratuberculosis* is transmitted within and between herds in the beef industry.
3. The level of environmental contamination that occurs on beef farms and how this changes over time.
4. Management practices used in the beef industry and their effect on *M. paratuberculosis* infection need to be understood so that appropriate recommendations can be made.
5. Existing diagnostic testing protocols need to be better understood to economically and efficiently diagnose individuals and herds as infected or non infected. The development of new testing methods and strategies need to be considered.

6. The epidemiology of *M. paratuberculosis* on beef farms needs to be more fully understood. The effect of wildlife species is one area that should be considered as beef cattle have frequent exposure to a wide variety of species.
7. The economic impact of Johne's disease on the beef industry needs to be determined to evaluate its relative significance.
8. Research is needed to better position the beef industry in regards to the potential zoonotic link to Crohn's disease.

CONCLUSIONS

The prevalence of *M. paratuberculosis* in Canadian beef herds is considered very low. There is little doubt that herds infected with Johne's disease may suffer severely. However, the economic loss to the beef industry as a whole is of questionable significance at this time. What has made Johne's disease an issue is the potential zoonotic threat that it presents. The beef industry is in a unique situation given its low prevalence of Johne's disease to put into motion a strategy to limit further spread of the disease. The control of Johne's disease nationally will be an immense undertaking due to the insidious nature of this disease and the relatively poor performance of tests that are currently available. There is a need to develop best management practices specific to the beef industry with consideration given to the biology and ecology of the disease. Implementing Johne's disease control programs is an important proactive step forward. However, *M. paratuberculosis* has plagued the cattle industry for many years and will likely continue for the foreseeable future.

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