AN ENVIRONMENTAL PERSPECTIVE ON DECISION-MAKING FOR THE CONTROL OF JOHNE'S DISEASE ON BEEF RANCHES

A Dissertation

by

LISA ANGELA BENJAMIN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2009

Major Subject: Biomedical Sciences

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ABSTRACT

An Environmental Perspective on Decision-making for the Control of Johne's Disease on Beef Ranches. (August 2009)

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Biosecurity practices for *Mycobacterium avium* subsp. *paratuberculosis* (Mptb), the etiologic agent for Johne's disease (JD), are predicated on the fact that fecal-oral is the major route of infection and that Mptb is present in the environment of affected farms. The objectives of these studies were to describe perceived benefits of test-negative Level 4 status in the Voluntary Bovine Johne's Disease Control Program (VBJDCP), describe producer and veterinarian attitudes towards JD relevant biosecurity practices, compare 5 JD control options using a Markov model, determine if tangential flow filtration (TFF) increases the detection sensitivity for Mptb and describe the distribution of environmental predictors for Mptb survival.

Twenty-five percent and 39% of beef producers in the VBJDCP reported that they received substantial or marginal benefits (financial and non-financial), respectively, from program participation. Producers suggested increased marketing opportunities to improve the VBJDCP.

Producers in a cross-sectional mailed survey of attitudes towards biosecurity practices were more likely than veterinarians to agree that separating JD clinical or suspects from calves or heifers; acquiring replacements or additions from JD low-risk herds, testing for JD every 10 to 14 months and test and culling clinical suspects only were useful for control of JD.

A state transition Markov model, with the environment as the source of Mptb, was used to compare 6 alternative control strategies for JD. Management and the probability of Mptb surviving 1 year in the environment were important determinants of the prevalence of subclinical JD on beef farms under the analyzed control strategies. Heterogenous distribution of environmental predictors for Mptb survival was observed in spatial risk maps.

In conclusion, although some beef producers experienced gains from participation in the VBJDCP, the perceived program benefits could be improved by increased marketing and education on the advantages of participation. Specific problem areas should be addressed. The length of time Mptb survived in the environment was an important parameter in the Markov chain model. Additionally, due to the heterogenous distribution of environmental predictors, a multiscale approach to sampling and analysis should be useful.

DEDICATION

I dedicate this dissertation to my mother, father (deceased) and grandmother who worked very hard and gave everything that they had to ensure that my siblings and I had the best future possible.

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My determination to succeed is fueled by the motivation of my committee chair, Dr Geoffrey Fosgate, and the members of my committee, Dr. Feagin, Dr. Roussel, and Dr. Ward. I thank them for their assistance throughout the course of my research. Their invaluable comments have helped me to gain a greater understanding of the principles of epidemiology and to appreciate the practical applications of these principles.

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NOMENCLATURE

AJDMAP	Australian Johne's Disease Market Assurance Program
APC	Antigen Presenting Cell
BCR	B Cell Receptors
BMP	Best Management Practices
CERS	Composite Environmental Risk Score
CF	Complement Fixation
CMI	Cell Mediated Immunity
DJC	Designated Johne's Disease Coordinator
DTH	Delayed Type Hypersensitivity
EIA	Monoclonal Antibody-based Sandwich Immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
EUV	Expected Utility Value
GIS	Geographic Information Systems
HEYM	Herrold's Egg Yolk Medium
HPC	Hexadecylpyridinium chloride
IDW	Inverse Distance Weighting
IFN-γ	Interferon Gamma
Ig	Immunoglobulin
IL	Interleukin
IS900	Insertion Sequence 900

JD	Johne's Disease
MAIS	*Mycobacterium avium, Mycobacterium intracellulare* and *Mycobacterium scrofulaceum*
MGIT	Mycobacteria Growth Indicator Tube
MHC	Major Histocompatability Complex
MN	Monitored Negative
Mptb	*Mycobacterium avium* subsp. *paratuberculosis*
NJDCP	National Johne's Disease Control Program
NJDDHP	National Johne's Disease Demonstration Herd Project
nPCR	Nested Polymerase Chain Reaction
OIE	The World Organization for Animal Health
PCHS	Premium Cattle Health Scheme
PCR	Polymerase Chain Reaction
PBMC	Peripheral Blood Mononuclear Cells
PPD	Purified Protein Derivative
QRT-PCR	Quantitative Real-Time PCR
TCR	T Cell Receptors
TFF	Tangential Flow Filtration
Th1	T Helper 1 Cells
Th2	T Helper 2 Cells
TNF	Tumor Necrosis Factor
TNF-α	Tumor Necrosis Factor α

TRA	Theory of Reasoned Action
TVJDP	Texas Voluntary Johne's Disease Program for cattle
TVMDL	Texas Veterinary Medical Diagnostic Laboratory
UV	Ultra Violet Light
VBJDCP	Voluntary Bovine Johne's Disease Control Program
VJDHSP	Voluntary Johne's Disease Herd Status Program

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1. INTRODUCTION

1.1. Johne's disease in cattle

1.1.1. *Overview*

Johne's disease (JD), a chronic gastroenteritis, occurs in domestic ruminants and wild animals including rabbits and deer (Chiodini et al., 1984; Greig et al., 1997; Nelli et al., 2008). The etiological agent for JD is *Mycobacterium avium* subsp. *paratuberculosis* (Mptb) (Twort and Ingram, 1912). The control of JD is challenging and requires a long-term commitment (Caldow and Henderson, 2000), as a result, it is important to understand the factors that influence the adoption of control programs as well as the epidemiology of the disease. Age and breed susceptibility of cattle to JD is suspected (Chandler, 1961; Rankin, 1962; Larsen et al., 1975; Elzo et al., 2006). In addition, it has been reported that in comparable environments higher doses of Mptb are required to cause infection in adult cattle than in calves (Doyle, 1953). Johne's disease is characterized by a long incubation period (Sweeney, 1996) that can vary from a few months to 14 years. Higher doses of Mptb are apparently associated with shorter incubation periods (Hagan, 1938; Rankin, 1962).

Calves are more likely than adults to develop infection and progress to clinical disease subsequent to similar exposure to Mptb (Hagan, 1938). Most clinical cases of JD are observed in cattle between the ages of 2 to 5 years (Doyle, 1956), but cattle can

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This dissertation follows the style of Preventive Veterinary Medicine.

develop clinical disease at any age depending upon the bacterial challenge (Doyle and Spears, 1951; Julian, 1975; Chiodini et al., 1984). It is possible that clinical signs of JD are not observed in some cattle infected as adults because these cattle are culled before the long incubation period is complete (Whitlock and Buergelt, 1996). Most cattle with clinical signs of disease progress to advanced disease within 3 to 4 months that includes profuse diarrhea and hypoproteinemia that can be followed by death within days (Sweeney, 1996). The appearance of clinical signs is preceded by a subclinical stage of disease. During the subclinical stage infection might not be detectable by diagnostic tests (Sweeney, 1996) and this poses a challenge for disease control because the Mptb infected animal may have been shedding the bacteria in its feces for several months at low concentrations (Smythe, 1935; Smythe, 1950; Hole, 1956; Whitlock and Buergelt, 1996). The presence of cattle with subclinical disease is problematic because most susceptible cattle acquire infections by ingesting Mptb from a contaminated environment (Sweeney, 1996).

1.1.2. *Immune responses to infection*

Acquired immunity consists of cell-mediated and humoral immune responses. Lymphocytes are involved in the acquired immune response. Lymphocytes include T cells - helper, cytotoxic and memory; B cells - memory and plasma; as well as natural killer cells. The cell-mediated immune response is mediated by T cells and B lymphocytes function in the humoral immune response. Antibodies, are soluble B receptors (BCR) shed from B lymphocytes into blood and tissue fluids.

Receptors are present on the surface of all lymphocytes. Each T cell has several copies of the same receptor on its surface, T cell receptors (TCR). These receptors are proteins that bind antigens or function in the transmission of signals to the target cell mediating an immune response against an antigen. The actions of T cells are regulated by cell to cell interactions and cytokines. Cytokines are proteins or glycoproteins that function in intercellular communication. Interleukins (IL), interferon (INF) and tumor necrosis factors (TNF) are examples of cytokines. Cytokines are typically produced in a cascade. It is possible for 1 cytokine to have a similar function as another, for example IL-12 and IL-18 both stimulate T-helper type 1 cells to produce interferon-gamma (IFN-γ). Some cytokines inhibit the action of other cytokines; for example IL-10 inhibits the action of IFN-γ. When cytokines bind to specific receptors on lymphocytes they induce or inhibit target cell division or differentiation. In addition, new receptor or protein production can be induced or inhibited subsequent to cytokine-lymphocyte binding (Coussens, 2004; Sohal et al., 2008).

In order for T cells to recognize antigens, the antigens must first be processed and presented to T cells by other cells, examples of which include macrophages and dendritic cells. CD4+ and CD8+ are immunoglobulins associated with T cell receptors. CD4+ is found on helper T cells and it binds major histocompatability complex (MHC) on antigen presenting cells, whereas CD8+ is present on cytotoxic T cells and it binds MHC class Ia molecules. One type of TCR has α and β protein chains, and the other type has γ and δ protein chains. Between 15-30% of peripheral blood lymphocytes of young ruminants are $CD4^{-}CD8^{-}$ and are mainly γδ TCR. In adult ruminants up to 60%

of T cells may have γδ TCR. Pre-activation of macrophages may occur when γδ T cells, which do not require the presence of MHC proteins to be activated, produce IFN-γ or TNF-α. T helper cells are divided into 2 major groups, helper 1 (Th1) and helper 2 (Th2) cells, depending upon the cytokines they secrete. Subsequent to stimulation by IL-12 and IL-18, Th1 cells secrete IL-2 and interferon. Th1 functions relate to delayed hypersensitivity reaction and macrophage activation. Antigen and IL-1 stimulate Th2 cells to secrete IL-4, IL-5 and IL-10. Th2 cells increase production of IgA, IgE and IgG1.

Following ingestion by cattle, Mptb attaches to the surface of M cells in Peyer's patches within the walls of the small intestine. The Mptb, transported to the base of the M cell in vesicles by a process known as transcytosis, is then expelled on the basolateral surface of the M cell. The bacteria bind to specific receptors, including complement receptor 3 or mannose receptors, on the surface of the unactivated or pre-activated macrophages and are phagocytised. Mptb may evade death in some macrophages by preventing the maturation of the phagosome into a phagolysosome. The macrophages in which Mptb cells are not killed are termed persistently infected. Pre-activated macrophages process Mptb antigens and act as antigen presenting cells (APC) by presenting antigens on MHC class II molecules to CD4+ T cells. The histocompatability molecules are glycoprotein receptors that are coded for by the MHC gene complex. There are 3 gene loci in the MHC referred to as class I, class II and class III. Class I loci code for molecules on most nucleated cells. Class II loci code for polymorphic MHCs

on the surface of APCs. T cells recognize the complex of antigen-MHC complex and are activated when the APC produces IL-1, a stimulatory cytokine.

The early cellular immune response mounted against Mptb is the chief defense against infection and persistent infection may be prevented if an adequate cell-mediated immune (CMI) response is mounted. Stressed cattle may be more susceptible to infection with Mptb because reduced functioning of γδ T cells. IFN-γ is produced by macrophages when the cytokines IL-12 and IL-18 stimulate the αβ T cell receptor of CD4+ T cells. IFN-γ and IL-2 are important in the immune defense against Mptb (Stabel, 2006). The population of cytotoxic CD8+ cells increases in response to Mptb infection. The mechanism by which these cells are recruited is not known but it is possible that MHC class I molecules present mycobacterial antigens which escape from macrophages to CD8+ T cells.

Humoral immunity is believed to have limited effectiveness for the defense against Mptb infection. Th 2 cells act to increase antibody production by B cells that reside in Peyer's patches. However, the production of B cells in early infection may also lead to stimulation of T cells and increased IFN-γ production. The detection of humoral immunity suggests bacterial proliferation and waning cell-mediated immune response (Stabel, 2000).

Fecal shedding occurs when macrophages in granulomas near the mucosal lining emigrate into the intestinal lumen. Granulomas are lesions formed in chronic inflammation which consist of fibroblasts, macrophages, loose connective tissue and blood vessels. The presence of Mptb at the site of tissue damage results in the continued

recruitment of more fibroblasts and macrophages. IL-1 secretion by macrophages regulates collagenase production by fibroblasts and leads to excessive deposition of collagen in granulomas. Granulomas form in the walls of the intestine in an attempt to contain Mptb infection. However, they are not well defined and bacteria are able to proliferate because they are shielded from the immune response in persistently infected macrophages. TNF-α produced during granuloma formation recruits new macrophages and retains the bacteria at the local site of invasion. However, local tissue injury is another consequences of high concentrations of TNF-α (Coussens, 2004).

1.1.3. *Diagnostic testing*

1.1.3.1. *Diagnostic tests in general*

Diagnostic tests are used to reduce uncertainty about the state of disease in an animal. Diagnostic tests may involve primary or secondary detection of Mptb. In primary detection, the presence of the etiologic agent is detected by procedures such as growth in culture or detection of the etiologic agent DNA using polymerase chain reaction (PCR). Secondary detection involves using tests to predict the presence or absence of the etiologic agent or of the disease based on immune responses of the host. The results of more than 1 test conducted on individuals may be interpreted in series or in parallel. In serial interpretation, the animal has to be positive on all tests to be classified as test positive. Serial interpretation increases the specificity of the combination of tests, but decreases the sensitivity. When tests are interpreted in parallel,

a test positive animal is one with positive results on at least one test. Parallel interpretation increases sensitivity, but decreases specificity.

1.1.3.2. *Fecal culture*

Bacterial culture methods directly detect viable Mptb in feces or tissue samples. Live, non-dormant Mptb must be present for growth to be observed. Culture is often considered to have 100% specificity. However, passive shedding of Mptb in feces of cattle has been reported (Sweeney et al., 1992a). One advantage of fecal culture is that it confirms that cattle are excreting viable bacteria, allowing an assessment of the current herd status.

Several strains of Mptb have been identified and are generally divided into 2 major categories, **S** or Type I (ovine) and **C** or Type II (bovine) (Bauerfeind et al., 1996). Colonies of the type I strain generally appear after 16 weeks and the faster growing type II strains may be observed in 6 to 12 weeks on Middlebrook 7H11 agar (Collins et al., 1990; Stevenson et al., 2002; Motiwala et al., 2004). As a result of the slow growth of Mptb, decontamination methods are required to eliminate microorganisms in the sample that may be present and overgrow these bacteria. Also, methods including centrifugation, sedimentation or filtration can be used to increase the analytic sensitivity of culture since there is a relatively low quantity of bacteria per unit weight of feces. However, concentration methods can also increase the concentration of contaminants (Whipple et al., 1992; Kim et al., 2005). Conventional culture methods including the Cornell method, modified Lowenstein-Jensen and Herrold's egg yolk

(HEYM) and Middlebrook 7H9, 7H10 and 7H11, use solid agar growth media. Developed to decrease losses to contamination during culture, the Cornell method, involves preincubation in a broth media containing antibiotics. Mycobactin, an iron transport carrier, is added to culture media to promote growth of Mptb. Hexadecylpyridinium chloride (HPC) is used for decontamination prior to inoculation on HEYM. Initial cultured colonies of Mptb on HEYM are small, colorless, translucent and hemispherical that become larger over time (Sockett et al., 1992a; OIE, 2004). The egg in Herrold's egg yolk medium counteracts the bactericidal activity of any remaining HPC from the decontamination procedure. On Lowenstein-Jensen medium, oxalic acid and NaOH are used for decontamination rather than HPC and this medium does not have the anti-bactericidal capability of HEYM.

The slow growth of Mptb is an obstacle to making rapid decisions on the farm. Radiometric culture has the advantage over use of solid culture media because results are available sooner (4 -7 weeks). The BACTEC 12B medium is a liquid medium containing a radioisotope-labeled nutrient source, carbon-14 labeled palmitate. The BACTEC 460 detects the carbon-14 released when palmitate is metabolized. The time to appearance of growth in radiometric culture is correlated with the inoculation dose, giving a measure of the concentration of bacteria in the sample (Lambrecht et al., 1988).

The Mycobacteria Growth Indicator Tube (MGIT) is a modified Middlebrook 7H9 broth based non-radiometric system in which fluorescence occurs when acid-fast bacteria grow and utilize the oxygen present in the culture tube. Negative sample tubes are those without fluorescence after 49 days of incubation. Positive cultures from MGIT

must be confirmed as Mptb using a PCR or mycobactin J dependency. As with BACTEC 12B, MGIT system results are more rapidly available when compared to solid culture media methods. Thomas et al. (2005) reported that the Mptb detection time from fecal and tissue samples for the MGIT system (23 days) was faster than that for BACTEC 12B (33 days). The MGIT system was also more diagnostically sensitive for detection of Mptb than was BACTEC 12B. Higher proportions of contaminated samples were reported for MGIT as compared with BACTEC culture (Cornfield et al., 1997; Whyte et al., 2000; Gumber and Whittington, 2007). Increasing the concentration of decontaminants may reduce contaminants but can also inhibit growth of Mptb.

1.1.3.3. *Polymerase chain reaction*

Polymerase chain reaction amplifies target DNA in the sample. The majority of diagnostic PCRs for Mptb target the DNA insertion sequence IS900 (Collins et al., 1989; Vary et al., 1990; Millar et al., 1996). However, IS900-like sequences have been reported in other mycobacteria including *Mycobacterium scrofulaceum* (Cousins et al., 1999). Multiplex PCR, which targets multiple DNA sequences at the same time, has been evaluated in an attempt to reduce false positives. Other DNA sequences that have been used in PCR, multiplex or otherwise, include 16S rRNA and F57 (Tasara et al., 2005; Herthnek and Bolske, 2006). Li et al. (2005) reported additional multi-copy insertion sequences, for example IS_MAP02 and IS_MAP04, that are believed to be unique to Mptb.

Nested PCR (nPCR) uses 2 different sets of primers in successive PCRs. The first set of primers in the initial reaction targets a more conserved DNA sequence. In the subsequent reaction primers designed for more specific binding sites are employed. As a result of this 2 stage process, nPCR has increased analytic sensitivity and the reported sensitivity of nPCR, targeting IS900, for detection of Mptb in bovine feces was greater than commercial PCR (Collins et al., 1993). Another advantage of the 2 stage nPCR procedure is that it reduces the quantity of potential inhibitors in the sample. However, more detailed knowledge of target sequences is needed to carry out successful nPCR when compared with other PCR assays because nPCR includes the use of a second primer which is often required to have a target sequence which is shorter sequence of that targeted by the first primer.

In quantitative real-time PCR (QRT-PCR), fluorescent genetic probes can be used to quantify IS900 at the termination of each amplification cycle. Fang et al. (2002) reported a relative sensitivity of 93 to 96% QRT-PCR using 2 different culture methods as the standard of comparison (Whipple et al., 1991; Whipple et al., 1992; Stabel, 1997). In addition, both QRT- PCR and nPCR when combined with a commercial extraction procedure were reported to have equivalent sensitivity to a National Veterinary Services Laboratory culture for detection of Mptb in bovine fecal samples (Fang et al., 2002; Christopher-Hennings et al., 2003). When compared with nPCR, QRT-PCR is less likely to have false positives due to cross-contamination because the reaction tubes are not opened during the procedure.

1.1.3.4. *Intradermal test*

Measures of cell-mediated immunity include the intradermal test and the interferon gamma (IFN-γ) assay. These are measures of the early cell-mediated immune response to Mptb. The intradermal test, referred to as the Johnin test, measures type IV hypersensitivity reaction to Mptb antigen injected into the skin. Johnin was originally a concentrated filtrate made from cultured Mptb. The modern Johnin test uses a purified protein derivative (PPD) of *M. avium,* or more recently Mptb. The PPD commonly used in the past was from the laboratory adapted strain of *M. avium*, strain 18 which had been misidentified as Mptb. In an attempt to use antigen more genetically related to that encountered in field conditions and to standardize the production of Johnin PPD, Steadham et al. (2002) reported the preparation of Johnin PPD from a strain of Mptb, ATCC 19698. Usually 0.1 ml PPD is inoculated intradermally, into the middle third of the neck. The skin thickness is read at time 0 and after 72 hours. An increase in skin thickness of >2mm is interpreted as a positive delayed-type hypersensitivity reaction (OIE, 2008). Delayed-type (type IV) hypersensitivity (DTH) can be detected early during infection but its utility is limited because it develops only in a proportion of subclinical cases and in almost no clinical cases (OIE, 2008). Another limitation of the intradermal test is that Johnin or *M. avium* PPD will detect DTH resulting from exposure to other mycobacterial species.

1.1.3.5.*Interferon gamma assay*

A cell-mediated immune response occurs initially with the production of IFN-γ following infection with Mptb. The IFN-γ assay measures interferon released by sensitized peripheral blood mononuclear cells (PBMC). In the assay, IFN-γ is released when bovis-PPD and avium-PPD are separately mixed with whole blood and incubated overnight. The IFN-γ released into plasma by bovis-PPD and avium-PPD challenged PBMC as well as that released by non-stimulated cells is compared. IFN-γ production is assayed using a monoclonal antibody-based sandwich immunoassay (EIA). As the disease progresses, the cell–mediated immune response wanes and the humoral response increases in intensity. Clinical signs appear in proximity with the increasing humoral response (Stabel, 2000). Kalis et al. (2003) suggested that a combination of intradermal inoculation and a IFN-γ assay be used to identify infected animals before the onset of shedding for herds involved in test and cull programs.

1.1.3.6. *Enzyme-linked immunosorbent assay*

Enzyme-linked immunosorbent assay (ELISA) and complement fixation (CF) are 2 types of serological tests that are used for JD diagnosis in cattle. ELISAs are designed to detect the level of antibody response to Mptb. The ELISA targets serum IgG1 antibodies against fractionated Mptb protoplasmic antigen (Yokomizo et al., 1983) or lipoparabinomann containing antigen (Jark et al., 1997) for greater sensitivity. Abbas et al. (1983) reported false-positive results in CF test and ELISA due to cross-reactions with antibodies against environmental mycobacteria. Pre-absorption of sera with

Mycobacterium phlei reduces false positives that may occur with ELISA because of exposure due to environmental mycobacteria (Yokomizo et al., 1983; Milner et al., 1987; Cox et al., 1991). When compared with bacterial culture, ELISAs have an advantage in control programs because of more rapid results at a lower cost. The sensitivity of serum ELISAs vary with the stage of disease and reports range from 0.50 to 0.87 for animals with clinical signs of disease and 0.24 to 0.94 for infected but subclinical cattle. Reported specificities of serum ELISAs range from 0.40 to 1.0 for infectious cattle (Nielsen and Toft, 2008). More recent serum ELISA specificities lie in the range of 0.90 to 0.99 (Sweeney et al., 1995; Jakobsen et al., 2000)

1.1.3.7. *Complement fixation assay*

Complement fixation (CF) is another serological test for JD. Antibodies against Mptb in the serum from infected animals bind to added antigen, *M. paratuberculosis* 316F or *M. avium* strain D, an aqueous bacterial extract with lipid removed. Antigen, sera, complement and sensitized erythrocytes are incubated overnight. The indicator system for fixed complement consists of erythrocytes sensitized with hemolysin. The degree of fixation is determined using visual comparison of hemolysis to a standard. Results are reported as the reciprocal of the highest dilution of serum giving 50% or more fixation. Lower sensitivity and specificity have been reported for the CF test than for absorbed ELISA. Reichel et al. (1999) reported that the sensitivity and specificity for a commercial ELISA were 31.1% and 97.9% whereas the sensitivity and specificity for the CF test were 17.0% and 97.9%. In another study of 632 dairy cattle from 9

infected herds and 196 dairy cattle in 4 herds certified free of JD based on annual whole-herd conventional fecal culture, Sockett et al. (1992b) reported a sensitivity of 38.4% and a specificity of 99.0% for the CF test. The sensitivities of the 2 ELISAs used were higher than that of the CF test, 54.6% (Allied) and 43.4% (CSL) whereas the specificities for the ELISAs were 95.4% (Allied) and 99.0% (CSL). Kalis et al., (2002) reported a specificity of 62 to 100% for the CF test relative to modified Jorgensen culture used to identify infected and non-infected cattle.

1.1.4. *Prevalence in beef cattle*

Johne's disease has been identified in many countries throughout the world, including the US, UK, Argentina, Australia and Japan. Reported seroprevalence of JD varies by region. Waldner et al. (2002) reported that beef herds in community pastures in Saskatchewan had an apparent animal-level seroprevalence of 0.8% and the herd apparent seroprevalence was 15% for a cut-off of 1 positive animal or 3.0% with a cutoff of 2 positive animals using IDEXX ELISA. Scott et al. (2007b) reported that Canadian herds in the Alberta Johne's control program had animal-level seropositivity of 1.5% and herd-level seropositivity of 29% if at least 1 positive animal was required to classify a herd as positive or 7.9% if at least 2 positive animals were required. The true animal-level seroprevalence of these beef herds in Alberta was estimated as 1.1%.

Braun et al. (1990) reported a herd-level seroprevalence of 8.6% for beef cattle in Florida. Turnquist et al. (1991) reported an animal-level seroprevalence of 4.4% and a herd-level seroprevalence of 30% for JD in beef cattle in Louisiana. Thorne and Hardin

(1997) estimated that the true prevelance of JD at the animal-level for beef cattle in the brucellosis testing program in Missouri was 8.0%, the animal-level apparent prevalence was 5.0% and the herd-level prevalence was 40% when at least 1 animal was required for the herd to be classified as positive. In 2001 it was reported that the herd-level prevalence of JD in beef cow-calf herds in the US was 7.9% and the animal-level seroprevalence was 0.4% (Dargatz et al., 2001b). Pence et al. (2003) sampled cull beef cows and bulls in sale barns in Gerogia from June 1999 to June 2000 and reported that the animal-level seroprevalence was 4.0% using a commercial ELISA. Hill et al. (2003) reported that in a subpopulation of the Alabama Brucellosis Certification beef herds the apparent animal-level seroprevalence of JD was 8.0%, the estimated true prevalence was 8.8%, and the herd-level prevalence was 54% when a herd was considered positive if at least 1 animal tested positive using a commercial ELISA. Keller et al. (2004) reported that the animal-level apparent seroprevalence of JD among 75 beef herds whose owners were considering participation in the VBJDCP in Florida was 7.4%, an estimate of the true prevalence was 11%, and the herd-level prevalence was 76% when a herd was considered to be positive if at least 1 seropositive animal was present using IDEXX ELISA. The reported animal-level seroprevalence of JD using an ELISA (Allied Monitor) in beef cattle in Texas was 25.0%, based on serum collected from cattle in the Texas brucellosis market identification program at 20 randomly selected markets (Alexander et al., 1993). Roussel et al. (2005) reported an animal-level seroprevelance of 3.0% for JD in purebred beef herds in Texas and a herd-level prevalence of 44% when a cut-off of 1 positive animal was used for a commercial ELISA.

1.2. Control programs for Johne's disease

1.2.1. *Goals of control programs*

The goals of the control programs help to determine which options are considered to be the most logical choices for adoption. Goals may be epidemiologic, economic or animal welfare related (Ge et al., 2007). Epidemiological goals include limiting the number of animals or farms that are infected. Strategies used in control programs may be classified as prevention, eradication or control. Prevention refers to measures employed in excluding Mptb from disease-free herds (interherd transmission) or in avoiding the spread of infection to susceptible cattle (intraherd transmission). Eradication seeks to eliminate the etiologic agent completely from a defined area, for example, the herd, whereas control employs measures to attain lower, biologically or economically justifiable disease frequencies (Martin et al., 1987). Eradication can be conducted locally, such as at the farm level, nationally or globally. Eradication of a disease can be achieved when the basic reproductive number, R_0 is less than 1. R_0 is defined as the number of new infectious hosts that 1 infectious host is expected to produce in an entirely susceptible population and is calculated as the product of the number of contacts per unit time, the transmission probability per contact and the duration of infectiousness (Rothman and Greenland, 1998).

Whether eradication or control is considered appropriate will depend on cost, initial disease prevalence, the impact of the disease on herd productivity and animal welfare concerns. Economic goals include facilitating compliance with international export regulations or minimizing losses due to the disease on farms. Johne's disease is

listed as a multispecies disease that is notifiable to The World Organization for Animal Health (OIE). Concerns about the ability to export may prompt individual farms or countries to adopt JD control measures.

1.2.2. *Components of Johne's disease control programs*

JD control programs typically consist of a subset of the following components: diagnostic testing to identify infected animals; culling of cattle that are likely to be infected; improving hygiene, for example, by preventing fecal contamination of feeders; and separating susceptible cattle from potentially infectious cattle. In addition, there may be regulations which restrict movement and sale of animals from infected herds. The importance of having a credible information source for program participants, both current and prospective, has been recognized and included as a major component in JD control programs. In 1997, 61% of beef cow-calf producers reported that they considered veterinarians to be a very important source of animal health information (National Animal Health Monitoring System, 1998b).

In general, the rapid identification and elimination of shedding animals as well as measures which prevent susceptible cattle from coming into contact with Mptb contaminated feces have been used in control programs (Benedictus et al., 2000). Several measures recommended for the control of JD are based on reasoning related to what is known about the epidemiology of JD rather than on scientific evidence of the effectiveness of the measure (Caldow and Henderson, 2000). JD is mainly a fecal-oral transmitted disease (Sweeney, 1996) and control recommendations aim to reduce the

exposure of susceptible animals to Mptb-contaminated feces. Johne's disease may also be transmitted in utero (Sweeney et al., 1992b; Buergelt and Williams, 2003) and some control programs also recommend culling the offspring of test-positive cows. In a recent report on beef cattle in support of this recommendation, the offspring of dams with elevated ELISA sample:positive ratios were 4.6 times more likely to be classified as "suspect" or greater than other offspring (Osterstock et al., 2008a). However, in another study it was reported that the odds ratio for the association between the ELISA result of the offspring and ELISA status of the dam was 1.35 (P=0.69), suggesting that culling based on ELISA status of the dam may not be beneficial in beef cattle (Osterstock et al., 2008b). The differences in the odds ratios for the association between ELISA results of offspring and ELISA status of the dam in the 2 latter studies may be related to herd-level risk factors.

1.2.3. *Selected control programs*

1.2.3.1. *Overall*

Control measures such as improving hygiene and test and cull are used to reduce the proportion of cattle with clinical and subclinical JD on farms (Jubb and Galvin, 2004). The only program reported to be successful in eradicating JD was a combination vaccination/culling program carried out in France in 1975 in which 140 formerly infected herds were declared free after 12 years (Hillion and Argente, 1987). Control programs also exist in other countries including the USA, Australia, Scotland, Sweden,

and Japan. Internationally, there is a dearth of reports regarding the impact of control programs on JD in beef cattle.

1.2.3.2. *The USA*

In the USA, the Voluntary Bovine Johne's Disease Control Program (VBJDCP) is the national program for the control of JD. Participating states must adopt the uniform program standards (USDA, 2002), but can also introduce more stringent standards. Veterinarians who have successfully completed a JD certification program administered for the VBJDCP may recruit and enroll producers in the state program. The VBJDCP consists of 3 components: education, management, and herd testing and classification. Initially, JD-certified veterinarians educate producers on the basic epidemiology of JD, management strategies that can be used to control and eliminate the disease, and on control and testing strategies. A management plan is then formulated based on a risk assessment conducted by the JD-certified veterinarian. The management plan targets management practices that interrupt the transmission of Mptb to susceptible members of a herd and measures that reduce the risk of introducing Mptb (such as maintaining a closed herd and introducing cattle from herds with a low probability of JD) to the farm. Diagnostic tests approved for use by the VBJDCP include serum ELISA, fecal PCR, fecal culture and tissue culture. Herds with an initial negative test are enrolled in the test-negative component of the program. Each 10 to 14 months, a herd can advance by 1 level from test-negative status Levels 1 to 4 if they have met the requirements for the

current level and the herd test result is negative. The probability of a herd being non-infected increases from test-negative Levels 1 to 4 (USDA, 2002).

The Voluntary Bovine Johne's Disease Control Program (VBJDCP) includes a National Johne's Disease Demonstration Herd Project (NJDDHP) in which data are collected investigating the impact of the control program on selected real farms. A goal of the herd demonstration project is to develop and validate model strategies for JD control (USDA, 2005b). In 2007, 22 beef herds were enrolled in the NJDDHP (Fossler, 2007). The US VBJDCP takes advantage of the role of veterinarians as providers of information since they are responsible for administering the education component of the program (USDA, 2002).

1.2.3.3. *Australia*

The National Johne's Disease Control Program (NJDCP) in Australia consists of several components which include the National Information System for Mptb, the Bovine Johne's Disease Program, and the Australian Johne's Disease Market Assurance Program (AJDMAP). The AJDMAP, initiated in 1996, is a voluntary program in which producers are required to pay for services to assess and certify herds with negative JD test status. The AJDMAP component for cattle is known as CattleMAP. CattleMAP aims to provide low risk replacements, facilitate trade between zones, provide a marketing tool and to reduce the risk of spread of Mptb infection at shows and other events. The CattleMAP module consists of 4 management and 6 livestock elements. The management elements are training, internal auditing and corrective action, records

and document control. The livestock elements include herd or property risk assessment, introduced livestock, movement of assessed animals, livestock identification, herd management plan and testing strategies. Herds may move from Monitored Negative (MN) Levels 1 to 3. Herds at MN 1 have a moderate assurance in a protected zone; MN2 herds have a high JD assurance and MN3 herds have a high assessed JD assurance that is equivalent to a herd in a JD free zone. Assurance categories are based on the analysis of the probability that the herd is Mptb infected. Herds in regions designated to be "Protected" are initially assigned to the category MN1 and these herds may advance to MN3 based on successive serological test results (Anon, 2003). Prior to 2000, no herd enrolled in the NJDCP's Test and Cull Program in Victoria had eradicated bovine JD since new reactors or cattle born before the beginning of the program were still in herds (Sykes, 2000). However, Jubb and Galvin (2004) reported a decline in clinical cases of JD among 18 infected beef herds participating in this program in Victoria between 1992 and 2002. Twenty-two clinical cases were observed mainly in the 2 years before the program was implemented. Most seropositive animals were 5 years old (range 2 to 13) and most fecal culture animals were 6 years old (range 2 to 8) (Jubb and Galvin, 2004).

Freeman and Jordan (2005) reported that over the period 1971 to 2004, 92.8% of beef herds in a sub-tropical region of New South Wales in South-Eastern Australia acquired JD by introducing infected animals. For these Australian beef herds the time period between detection and successful eradication of JD from herds was a mean of 10.4 years, where JD positive cattle were identified among cattle born on the farm, and

2.2 years, where no JD positive cattle were identified among cattle born on the farm. Western Australia was considered to be free of JD, until a case was identified in July 2006 (Martin, 2008). Previous to this outbreak, regulations for zoning and movement control of infected and suspect herds were introduced to protect the disease-free status (Kennedy and Allworth, 2000). Kennedy et al. (2005) reported that the apparent herd prevalence of Mptb infected beef cattle in Australia was 0.08% (137/176 100) . Beef cattle are considered to be at low risk for infection and an assurance category requiring biosecurity, "Beef Only," was introduced to recognize their low risk. As a result, herds in south-eastern Australia can gain access to additional markets if they are identified electronically in the National Livestock Identification Scheme and are classified as "Beef Only."

1.2.3.4. *Scotland*

The Premium Cattle Health Scheme (PCHS) for JD control is a voluntary program administered by the Veterinary Science Division of the Scottish Agricultural College (Scottish Agricultural College, 2008). Participants pay an annual membership fee and additional costs are incurred for laboratory tests, sample collection and consultancy when these services are required. Samples are collected by a veterinary surgeon or a designate of the surgeon who is not the cattle owner or an employee of the farm. The two programs under the PCHS that are relevant to beef cattle include the Status Qualifying or Control Program and the Status Accredited Monitored Free Program. The Status Qualifying or Control Program of the PCHS requires annual tests

and culling of reactors, removal of the offspring of test positive dams and vaccinations in heavily infected herds to reduce environmental contamination. If the absorbed ELISA is positive for an animal within a herd in which JD was formerly not identified, fecal culture confirmation is performed before culling. In the Status Accredited Monitored Free Program herds are certified free of JD. Two tests of all adult animals in the herd are conducted with an intervening 1-year interval. Tests are carried out on cull cows in non-herd test years. Samples are collected from cattle purchased from non-accredited herds for fecal culture and serology at the beginning of a 4-month quarantine period. The need for a quarantine period is determined by the veterinarian. The quarantine facility may be a pen or paddock which does not allow contact between the quarantined animal(s) and the rest of the herd. Animals positive by serology or fecal culture are designated as reactors and are not allowed to enter the herd (Scottish Agricultural College, 2008). According to Caldow (2005), 7 years after the initiation of the PCHS there were 608 participating herds, 130 of which had 2 or more negative herd tests.

1.2.3.5. *Sweden*

Clinical JD is a notifiable disease in Sweden and veterinarians are legally required to sample JD suspect cattle. The impetus for the introduction of a JD control program in Sweden was the discovery of an imported cow with JD in 1993. The objectives of the voluntary control program for JD initiated in Sweden in 1998 were to detect JD in pedigree beef herds and to prevent the spread of infection among cattle nationally. Eight hundred and eighteen herds were enrolled by December 2000 but this

number declined to 774 in 2001 when participating farms were required to pay administrative fees. Herds advance through 3 categories based on the number of successive negative fecal cultures. When a positive herd is discovered a stamping out policy is implemented, that is, the infected cattle are culled and infected premises are cleaned and disinfected. Farmers can only add animals from herds in a similar category or higher in the program and common grazing is allowed solely with animals from a similar category. No direct contact is allowed with non-participating animals during transport or at shows. Farmers may therefore experience difficulties with shows, transport and are limited in their choices for usage of common pastures. Only 1 infected herd was identified from the inception of the control program to 2007 (Sternberg et al., 2002; Sternberg et al., 2007).

1.2.3.6. *Japan*

Active surveillance for JD was established in Hokkaido, Japan, in 1997. Farmers were compensated at 80% of the market value for the culled cattle. All targeted herds are required to undergo testing once every 5 years. Either two ELISAs in sequence or fecal culture are used. A herd could be declared free of disease if there were 4 negative tests over a period of 3 years. Active surveillance for JD included approximately 26% of the beef breeding cattle in Japan, whereas all dairy cattle were subjected to testing at least once between 1997 and 2004 (Kobayashi et al., 2007).

1.2.4. *Factors influencing participation in control programs*

1.2.4.1. *Non-attitude related factors*

The control of JD requires a long-term commitment because of the long incubation period during which the bacteria may be shed in the feces of infectious animals (Armstrong, 1956). Rapid turnovers of veterinarians and farm managers can result in the inconsistent application of control measures and reduce the overall effectiveness of a control program. Sykes (2000) reported that one reason for failure to eradicate JD within Australian dairy herds participating in the Dairy Research and Development Corporation Project was the lack of continuity in testing and implementing management changes due to staff changes on farms during the 10-year period of the project (Sykes, 2000). This illustrates the importance of the length of time the producer plans to operate his farm in the future, since the period of time over which the JD control program is operational may not be sufficient for eradication of this chronic disease. Also, the change of management may not be under the control of the producer as marketing forces take their toll on economically unfeasible operations.

Barlow and McKenzie (2000) reported that several factors determined enrollment, non-participation and withdrawal from the voluntary Australian program to protect non-infected herds from JD. Enrollment was influenced by issues associated with breeders wanting to be assured of the quality of their stock, inter-zone movement requirements, availability of financial assistance, peer pressure to test and requirement for testing in order to gain entry to sales or shows. Factors important in non-participation were concerns about livelihood and property values if JD positive animals were identified,

absence of benefits for commercial producers, no financial support for infected herds and having to include other eligible species on the farm in equivalent JD control programs. Withdrawal concerns were related to required limitations on herd introductions and stock movement, and also test accuracy and cost (Barlow and McKenzie, 2000).

1.2.4.2. *Attitudes and control program attributes*

An attitude is a relatively long lasting organization of an individual's beliefs about an object which predisposes actions that may be in favor of or against a new idea (Rogers, 2003). The Theory of Reasoned Action (TRA) links attitudes to behavior by predicting that voluntary behavior is an outcome of the person's attitude towards the behavior and their understanding of what is considered to be the norm (Fishbein and Ajzen, 1975). Producers with positive attitudes towards a voluntary control program are more likely to enroll (Gillespie et al., 2007). Hall et al. (2003) reported that beef producers in Texas and Nebraska believed that a focus on disease prevention was their most important strategy for improving cattle health. The actual measures that the producers are willing to adopt will determine how effectively disease is prevented or controlled. According to Rogers et al.(2003), the formation of an attitude about a control program which may lead to its eventual adoption, occurs during the persuasion stage of the innovation-decision process. The formation of an attitude towards a program is influenced by the potential adopter's perception of the program's attributes. Perceived attributes of a program relevant to the formation of an attitude include the perceived

advantage to be gained by adopting the innovation, ease with which the idea is understood and implemented, and the extent to which the new idea can be observed and experimented with before full scale implementation on the farm.

1.2.4.3. *Perceived advantages of control programs*

The advantages of participating in the program relevant to attitude formation may be economic or social in nature. Gunn et al. (2008) reported that the effectiveness and economic benefits of biosecurity programs were not apparent to farmers, veterinarians and auxiliary industries in Great Britain and these served as obstacles to the adoption of these programs. Participation in JD control programs is expected to lead to increased marketing opportunities for producers since there will be low probabilities that cattle in participating herds will be infected with Mptb. In turn, these perceived benefits will act as incentives for new producers to enroll in the program. Kovich et al. (2006) reported in a survey of 21 dairy producers enrolled at Levels 3 and 4 of the Voluntary Johne's Disease Herd Status Program (VJDHSP) that 90% (19/21) of the producers perceived an economic advantage to participation and that 95% (20/21) of the producers felt that advantages outweighed the costs. Forty-three percent (9/21) of these producers responded that herd health was the major factor for participation and 29% (6/21) stated that marketability of surplus cattle was their motivation. Fifty-three percent (8/15) of the dairy cattle producers, who marketed cattle as being from a VJDSHP herd, received a VJDSHP status related premium.

In general, costs associated with JD control include direct cost of JD itself as well as the cost of participating in a control program. In 1994, the direct cost of JD to commercial beef producers in Kentucky was estimated to be $1.5 million annually (Meyer and Hall, 1994). Factors associated with costs include herd size, production level and the management of susceptible cattle. Direct losses to JD include reduced slaughter value because the return from the culled animal will be much less than its original market value. Additional losses include shorter productive life, losses due to mortality, reduced fertility, poor feed conversion and increased susceptibility to other diseases. Indirect losses include premature culling, lost opportunities for breeding, herd replacement expenses, diagnostic testing, lost genetic value (especially important for breeders) trade restrictions and decreased marketing opportunities (Hasonova and Pavlik, 2006).

The costs of participating in a JD control program include costs associated with time spent implementing the program and in sustaining required changes and costs associated with testing protocols. Testing costs include veterinary consultancy fees, costs associated with sample collection and diagnostic tests themselves. If management changes are required then these may incur costs associated with fencing and improving hygiene. Hygiene is improved by introducing measures that decrease contact between cattle and Mptb contaminated feces in the environment. Improvement of hygiene may entail costs associated with moving feeding areas off the ground and in providing access to drinking water that is less easily contaminated with feces. The areas in which the herd congregates may also be manually scraped depending on the management system.

Overall, the costs of improved hygiene include labor and materials required to make the changes that will decrease exposure to Mptb in the environment. The cost of culling animals with the inevitable loss of genetics and the need to raise or purchase replacements in a timely manner should also be considered. Actual benefits received in relation to costs could also reinforce the original decision to join the program and determine whether or not participation is continued.

Management changes made as a result of a JD control program should have the additional benefit of decreasing the incidence of other fecal-oral transmitted diseases. Also, intangible benefits, such as the pride in producing healthy cattle (Willock et al., 1999; Hood and Seedsman, 2004), are important and may be experienced.

1.2.4.4. *Ease of understanding and integration of control methods*

The disparity between the recommendations of the present program and the current practices of the farmer can help to determine the farmer's attitude towards the program. The farmer is faced with recommendations from different sources and with different intended goals. If the program recommendations conflict with present practices then the producers need to consider the feasibility of and value to be gained by introducing the new practices into the routine.

The ease with which the new idea is understood and put into practice is important. Participants in an Ovine Johne's Disease Control Program in Australia in 1999 reported that management changes required by the program proved to be challenging, unpractical or unfeasible (Hood and Seedsman, 2004). In addition,

Gillespie et al. (2007) reported that more beef producers had not adopted Best Management Practices (BMP) related to environmental quality because of unfamiliarity rather than as a result of high cost.

1.2.4.5. *Experimentation prior to implementation of control methods*

It is important for the farmer to be able to experiment with segments of the new program before full scale implementation on the farm. The VBJDCP allows the producer to remain at any stage selected among the available 3 stages. The producer is therefore able to observe the impact of the program on the herd before committing to a more stringent protocol at a higher level of the program. In addition, the National Johne's Disease Demonstration Herd Project (NJDDHP) allows for the monitoring of prevalence and incidence of JD on farms on which VBJDCP control recommendations have been implemented.

Other factors influencing attitudes include whether farmers believe that they should take financial responsibility for disease control and previous experience with control programs. In a recent survey, UK farmers reported that they should not be solely responsible for biosecurity and that the government, food retailers and auxiliary industries should share the financial burden (Gunn et al., 2008). Previous experiences with control programs may also influence the decision to participate in another control program. There may have been an erosion of trust in the body administering the program which is carried over to the current program. In a review of state Johne's disease control programs, Sykes (2000) reported that problems facing JD control in the

Netherlands included loss of credibility due to contamination of vaccines with Bovine Viral Diarrhea Virus in an infectious bovine rhinitis program, high level of contamination of fecal culture samples and detection of Mptb after 5 years of testing in herds initially believed to be free of JD.

The relationship between attitudes and behavior is complex because individuals may have positive attitudes towards ideas and yet not adopt behaviors that can be considered to be consistent with these attitudes (Rogers, 2003). Incentives offered by the entities administering the control program help to increase consistency between attitudes and behavior (Yapa and Mayfield, 1978). They act as cues to action and are useful for chronic diseases such as JD for which the incubation period lasts for months or years and can be followed by an undetectable subclinical period lasting for years. The producer may be unaware of the presence of disease in the herd because animals may be culled before they develop clinical signs. Cues to action encourage participation in a control program by signaling to the individual that there is a need to act. Due to the insidious onset of JD, incentives draw the attention of producers to the possible presence of the disease in their herds. These incentives include free or subsidized diagnostic testing and financial compensation for culled animals as well as subsidized veterinarians' fees. In 1999, producers participating in the CattleMAP program in Australia listed subsidies on tests and veterinary costs as one reason why they had decided to participate in the program. In addition one reason producers listed for non-participation was a lack of incentives for commercial herds (Barlow and McKenzie, 2000).

In order to reduce any uncertainty associated with an idea, potential adopters may look for information about it. The idea may be rejected or accepted following the information seeking stage (Rogers, 2003). In the VBJDCP, the veterinarian is the major source of information for the producer (USDA, 2002). As a result, the attitudes of the veterinarians towards biosecurity in general and the control of JD in particular are important in attracting producers to the program. In a recent survey, most veterinarians in Great Britain, reported feeling that their clients were not willing or able to invest in biosecurity (Gunn et al., 2008). Veterinarians' attitudes towards the willingness of producers to participate in control programs can determine whether or not they introduce their clients to these programs.

In epidemiology, risk is defined as the probability of an event occurring in a specified population during a certain time period. In risk analysis, risk refers to a specific form of an attitude towards a hazard. A hazard is an agent – physical, chemical or biological – with the ability to have an undesirable consequence. Different risk attitudes have been defined: risk aversion, risk neutral or risk taking. Risk aversion involves adopting actions that will avoid an undesirable consequence; risk neutral implies indifference; and risk taking accepts the undesirable consequence. The risk attitude is relevant to the selection of the optimal control option (Cher et al., 1997), since the risk attitude will determine the disease prevalence that the producer is willing to accept on his or her farm.

1.3. Decision analysis and disease control

Decision analysis involves using logic to structure a problem which can then be analyzed across alternative strategies. Economic values or preferences of stakeholders for outcomes can be included in the analysis. Decision analysis methods include decision tree analysis and Markov process analysis. In decision tree analysis the components of a decision problem are represented in a decision tree. The tree consists of decision and chance nodes which are connected by lines referred to as branches. Probabilities are assigned to each chance branch. The sum of the probabilities of the branches arising from each chance node is 1. The terminal node is the final node of each branch. A utility value or cost is assigned to each terminal node. The Expected Utility Value (EUV) for each outcome is the product of the probability and the utility of the outcome. The expected utility of the branch representing each possibility of a decision node is the sum of the expected utility of all associated outcomes.

Decision tree analysis has been used to study the epidemiology of JD and to compare the economic impact of different control measures (Collins and Morgan, 1991, 1992; Dorshorst et al., 2006). An event that occurs more than once can be represented in a recursive decision tree. However, recursive trees can soon become complex as repetitions and consequently branches of the tree increase in number. This complexity limits the practicality of using recursive trees for events over long time horizons (Sonnenberg, 1993). A Markov process model is an alternative approach to the analysis of events that happen more than once. The main components of the Markov model are transition states and transition probabilities. In a Markov model possible health states,

Markov states, of the animal are modeled. The states in a Markov model are mutually exclusive and may be recurrent, transient or absorbing. The individual has 0 probability of escaping from the absorbing state, for example, dead or culled. Transitions from one state to another represent the events of interest. In Markov process models uncertain events are modeled as transitions between specified health states rather than at chance nodes as in decision trees. The state transition in the model is determined by the transition probabilities that may be represented in a transition probability matrix. A Markov model is a unique case of a state-transition model which assumes that the probability of entering the next state, j, is dependent only on the current state, i, and not on previously occupied states. Therefore, transition probabilities are conditional probabilities, p_{ij}. The fundamental matrix solution is one basic method of evaluating a Markov process. A P-matrix is a table in which the probability of moving from stage i, row, to stage j, column, is recorded for each possible transition. The probabilities for each row of states sum to 1. Probabilities are represented in a 1-cycle transition. Only 1 event occurs during a cycle and the length of the cycle is chosen to represent a clinically relevant time period. Each state in the Markov model can also be assigned different utility values (Hunink, 2001). The Markov chain model has the disadvantages of assuming that probabilities remain constant from one cycle to the next. In addition, a single transition probability is used to represent all possible routes of transition from one state to another (Sonnenberg, 1993).

Deterministic and stochastic Markov models of JD transmission in herds can be created. Probability distributions are used for each parameter in stochastic models as

opposed to single parameter probability estimates in deterministic models. As a result, the output in deterministic models is the most likely outcome, whereas, the iterative methods used in stochastic models compute the outcome as a range of possible values, reflecting the uncertainty in the data being analyzed. In the deterministic model, the new animals infected in each state is the product of the transition probability and the number of animals in that state at the end of the immediately preceding time period (Taylor, 2003).

The majority of state transition models for JD described in the literature are for dairy cattle. Collins and Morgan (1991, 1992) described an epidemiological model of JD in dairy cattle based on a modified Reed-Frost model, with no immune state, to determine the probability of being infected. Juste and Casal (1993) developed a Markov chain model to compare JD control strategies in sheep. Pouillot et al. (2004) developed a model for the intra-herd dynamics of JD in French beef and dairy herds. Humphry et al. { , 2006 #5}described a state transition model in which the environment of a beef suckler farm rather than direct contact between animals was the major route of infection with Mptb. Groenendaal et al. (2002; 2003; 2003) presented a stochastic simulation model of JD in dairy and beef cattle in The Netherlands and of dairy cattle in Pennsylvania.

Models of disease are useful when the disease is chronic because different scenarios can be rapidly investigated, within minutes, as opposed to the several years that may be required to observe the disease in a longitudinal study. However, models have several limitations that should be kept in mind when interpreting results. A model

is only a reflection of the system and it has to be meticulously examined for areas in which it differs from the system it is constructed to represent (Taylor, 2003). Another concern is that producers, veterinarians, control program administrators, local subsidiary agencies and global agencies all have different perspectives on the control program and these differences may lead to conflicts since the option considered to be optimal may vary by perspective. For example, the eradication of JD may be a national goal in order to secure international markets, but for the individual commercial producer, this goal may not be cost-effective.

1.4. Environmental predictors of Johne's disease

1.4.1. *General*

There is experimental evidence, mainly laboratory, that environmental factors can increase survival of Mptb (Lovell et al., 1944; Larsen et al., 1956). In addition, Whittington et al. (2001) conducted field experiments to measure the association between Mptb survival and environmental factors in Australia. Ward and Perez (2004) identified a spatial cluster of herds with greater than median herd seroprevalence of JD associated with soils with low silt content (OR = 0.72) in northeast Indiana. According to Roussel et al. (2005), location was associated with JD seroprevalence in Texas purebred beef cattle herds. Environmental factors suspected of influencing survival of Mptb include soil type, soil pH, soil calcium, soil iron, and soil moisture (Johnson-Ifearulundu and Kaneene, 1999; Schroen et al., 2000). Other possibilities include,

surface water, shade, and temperature (Whittington, 2001; Katayama et al., 2004; Whittington et al., 2005).

1.4.2. *Soil type and moisture*

Regional and national studies have identified associations between the occurrence of clinical cases of JD or JD seropositivity and environmental factors, such as soil type. Kopecky (1977) concluded that JD in infected dairy herds in Wisconsin is associated with acidic and not calcareous soils. Calcareous soils are alkaline and contain calcium or magnesium carbonate. Scott et al. (2007b) reported that cattle and herds in areas with high soil pH (>7.0), southern latitudes, and arid climates had a moderately reduced risk of Mptb infection (Odds Ratio = 0.40) in Alberta, Canada. Other support for an association between low pH and JD was found in a cross-sectional questionnaire study conducted in Avila (central Spain) with the objective of evaluating the relationship between soil type and herd-level ovine and caprine JD seropositivity (Reviriego et al., 2000). The estimated odds ratios were 25.9 on entisol soils (low pH) and 3.5 on inceptisol soils when compared with the referent category of alfisol soils (high pH). Johnson-Ifearulundu and Kaneene (1999) reported an association between acidic soil, increase in iron content and JD seropositivity in dairy cattle of Michigan using a case-control study. In a study conducted in New South Wales (Australia), researchers reported an association between the age at which sheep died due to clinical JD and soil type (Lugton, 2004).

The presence of soil moisture can increase survival of Mptb. In a series of experiments conducted in Australia, Schroen et al. (2000) reported that the absence of moisture significantly reduced the survival of Mptb in soil. In earlier experiments with Mptb, Lovell et al. (1944) simulated the effect of different environmental conditions on Mptb and reported that Mptb survival was longer in a sample kept moist, 208 days, than in a duplicate sample kept dry, 152 days. Soil moisture helps to prevent microbial desiccation.

1.4.3. *pH and iron*

In general, the pH of soil depends on the parent material from which the soil is formed, for example, granite rocks tend to form acid soils and limestones form alkaline soils. However, other factors also affect soil pH including the extent of mineral weathering subsequent to soil formation and biological processes. For example, microbial respiration will lower the pH, whereas denitrification and the action of urease on urea will lead to higher pH (Elsas et al., 2007). When tiled-oligonuleotide DNA microarrays were used to examine the transcriptional profile of Mptb cultures following exposure to an acidic environment for 3 hours, it was reported that 402 genes were down-regulated and 195 were up-regulated. The large number of genes regulated and the inclusion of general stress-response genes among the activated genes suggest that pH is important to Mptb and the existence of mechanisms to cope with the pH challenge (Wu et al., 2007). Associations between *Mycobacteria* species other than Mptb and pH have also been described in the literature. Brooks et al. (1984) reported a high

correlation between the complex *Mycobacterium avium, M. intracellulare* and *M. scrofulaceum* (MAIS) and soils with high acidity and organic matter. A positive correlation between elevated numbers of MAIS and high zinc and fulvic acid of water samples was reported in addition to strong correlations between elevated MAIS numbers and humic and fulvic acid (Kirschner et al., 1992).

The hypothesized association between calcium in lime and JD may be better attributed to the correlation of Ca with CO_3 and hence more alkaline soils. In an early observation, Smythe (1935) noted that along the north coast of Cornland, England the prevalence of clinical JD was low in districts with up to 25% calcium carbonate but that cows developed clinical disease within weeks of being sold to farms in an adjacent area with acid soil lacking lime. As a result of this Cornish anecdote and other evidence, it has been hypothesized that Mptb infected cattle on alkaline soils were less likely to progress to the clinical stage of JD than were similar cattle on more acid soils (Kopecky, 1977). In addition, Jansen et al. (1948) reported that the number of clinical JD cases in Friesland province in the Netherlands increased as the calcium percentage and pH of the soil decreased. Later, based on international observations of JD cases in areas with and without limestone or when lime was added to soil, Richards (1989) proposed that low pH enhanced and high pH was self-limiting with respect to JD. In Michigan, the literature was reviewed for evidence of an association between pH, iron and JD and a 3-year longitudinal study was subsequently conducted to investigate these associations. It was concluded that every increase in soil pH of 0.1 was associated with a 5% decrease in the number of seropositive cattle (Johnson-Ifearulundu and Kaneene, 1999). In one

experiment, a reduction in seropositivity of cattle was observed when calcium in the form of lime was added to the soil of farms on which there were herds with positive ELISA test results (Johnson-Ifearulundu and Kaneene, 1999). In experiments conducted in Australia, however, a significant association between soil pH and Mptb survival was not observed (Schroen et al., 2000).

According to Johnson-Ifearulundu and Kaneene (1999), a 10 ppm increase in soil iron content was associated with a 5% decrease in the number of test-positive cattle, thus supporting the hypothesis that iron plays a role in the epidemiology of JD. This suggests that soil iron availability might prolong the survival of Mptb in the environment. Iron is mainly in the practically insoluble form $Fe(OH)_3$ and at pH 7 has a solubility of about 1.4×10^{-9} M. The solubility of Fe^{3+} increases by 10^3 for each 1 unit drop in pH so that at pH 5 the solubility is 10^{-3} and some bacteria that can grow at this pH do not need a carrier molecule for the uptake of iron (Ratledge, 2004). Siderophores are compounds produced by bacteria that allow them to uptake insoluble iron. Mptb does not normally replicate in the environment because, unlike other mycobacteria, it is unable to produce the intracellular siderophore known as mycobactin or the extracellular carboxymycobactin that is produced by other pathogenic mycobacteria (Francis et al., 1953; Snow, 1965, 1970). However, Mptb can replicate in amoeba in the laboratory (Whan et al., 2005; Mura et al., 2006) and there is speculation that Mptb can replicate within amoeba present in water.

1.4.4. *Surface water*

Water sources were associated with JD seropositivity in beef herds in Texas (Roussel et al., 2005). In addition, Whittington et al. (2003) reported that there was faster growth of Mptb in environmental samples from low lying areas of sampled sheep farms that drained into dams and water courses. Since radiometric (BACTEC) culture was used, faster growth suggested a higher initial concentration of bacteria in samples from these areas. Bacteria, likely accumulated as a result of landscape features, and contaminated water sources associated with low lying areas. Mptb has been isolated from rivers and catchment in South Wales and Northern Ireland (Abbey, 1952; Pickup et al., 2005; Whan et al., 2005; Pickup et al., 2006). It has been reported that a bovine strain of Mptb survived for more than 2.5 years in fresh pond water under experimental settings (Pickup et al., 2005).

1.4.5. *Other predictors*

According to Larsen et al. (1956) shade improves Mptb survival. In addition, it was reported that Mptb survived up to 55 weeks in fully shaded dry environments but only up to 9 weeks on grass in 70% shade (Whittington et al., 2004). The ultraviolet (UV) spectrum can be divided into long wave UVA (400 – 320 nm), medium wave UVB (320 – 280 nm) and short wave, or germicidal, UVC (280 – 100 nm) bands. Ninety-eight percent of the ultraviolet radiation at the earth's surface is UVA. Ultraviolet light did not affect the survival of Mptb in experiments conducted in Australia in which replicates received UVA light at 250 milli-Watts per cm^2 and controls received 10

mW/cm^2 (Schroen et al., 2000). However, Katayama et al. (2004) reported that the quantity of UVB needed to eliminate Mptb was equivalent to the amount that summer pastures will be exposed to over a period of 2 hours and that UVB light was unable to penetrate grass leaves at the measured intensities (2 - 1085 kJ/m^2).

The impact of solar energy on soil temperature is partially determined by soil moisture, soil type and depth and the vegetation present (Elsas et al., 2007). According to Shroen et al. (2000) dry soil and high soil temperature (30°C) were associated with decreased survival of Mptb. Temperatures tend to be lower in shaded areas and observed associations of increased survival of Mptb with shade may be due to lower temperatures (Whittington et al., 2004). Soil moisture is a major factor determining the extent to which a unit of net solar radiation increases the temperature of a unit of soil (Elsas et al., 2007).

1.4.6. *Identification of* Mycobacterium avium *subsp.* paratuberculosis *in the environment*

Mptb has been isolated from the environment of farms with domestic ruminants (Raizman et al., 2004; Berghaus et al., 2006). Whitlock et al. (1992) isolated Mptb from 5/11 dairy farms using samples collected from pastures and exercise lots. Mptb has also been recovered from a range of topographic sites on infected sheep farms (Whittington et al., 2003). Campbell et al. (2007) targeted areas from the environment of beef farms that were most likely to be contaminated and isolated Mptb from within chutes (4/26), the ground surrounding cow feeders (2/21), inside cow feeders (2/13), close-up pens

(1/7), bullpens (1/10), turnout pens (1/18), calf shelters (1/18) and calving pens (1/26). Raizman et al. (2004) recommended targeted sampling of cow alleys and manure storage areas as a screening strategy for JD in dairy cattle.

MGIT culture and PCR can be used to detect Mptb in environmental samples. Decontamination procedures are usually required to remove faster growing bacteria that may overgrow Mptb. PCR assays may experience inhibition from other substances present in the sample. The reported detection limit of radiometric culture was 3 Mptb per gram of feces (Lambrecht et al., 1988). Clumping is characteristic of mycobacteria and this poses an obstacle to the quantitative estimation of Mptb in the environment. Also, dormancy of Mptb may occur when environmental conditions are unfavorable leading to limited success of radiometric culture. Inhibition of reaction components may also lead to false-negative PCR results (Stinear et al., 2004a). PCR inhibitors may act by directly decreasing polymerase activity or limiting polymerase availability by competing with the target template. According to Tsai and Olson (1992) humic acids and iron can decrease the analytic sensitivity of PCR. Analytic sensitivity refers to the lowest concentration of the target substance that can be detected by the test. On the other hand, epidemiologic sensitivity can be defined as the proportion of test positive among the truly positive samples.

High concentrations of magnesium and potassium ions can also cause the amplification of nontarget DNA (McPherson and Moller, 2006). Real-time PCR has been reported to detect as few as 10^2 Mptb cells in 20 ml of experimentally spiked water (Rodriguez-Lazaro et al., 2005).

1.4.7. *Spatial interpolation*

Spatial interpolation is used to predict values at unobserved locations based on measured values. Interpolation methods may be deterministic or geostatistical (stochastic). In deterministic interpolation, surfaces are created from measured points based on the extent of similarity between points or the amount of smoothing. The statistical properties of measured points are used in geostatistical interpolation. Deterministic methods may either be local or global. Global methods use all available data to develop predictions for the entire area of interest and local methods provide predictions only in a small zone around observed locations.

Inverse distance weighting (IDW) is a local deterministic method since it involves applying a mathematical function repeatedly to small subsets of the total set of observed data points and then linking these surfaces to form a composite encompassing the entire study area. IDW assumes that the value of an attribute at some unobserved location is a distance-weighted average of data points within a neighborhood or window surrounding the point. Weights are calculated by moving average methods. Each observed point has a local influence that diminishes with distance. A disadvantage of IDW is that altering the weights of neighboring points changes the output surface. Deterministic methods such as IDW are limited since they neither provide estimates of variance for the value at unsampled locations nor do they provide information on the

number of points required to calculate the local average, the size orientation and shape of the neighborhood sampled and whether or not the interpolation of weights as a simple function of distance is appropriate.

Kriging produces prediction and error surfaces using the spatial correlation between the distance and direction of sample points to determine the variation in the surface. Weights are used in kriging to predict values of the variable at unmeasured locations. Kriging weights are selected to give the best linear unbiased estimate of the value of the variable at a particular point. The variable of interest is known as a regionalized variable and its behavior is governed by regionalized variable theory. According to the regionalized variable theory each spatial variable has 3 major components: (1) A structural, deterministic, component with a constant mean or trend, $m(\mathbf{x})$, (2) A random, stochastic, spatially correlated component , $\varepsilon'(\mathbf{x})$, and (3) a residual error term representing spatially uncorrelated random noise, $\varepsilon''(\mathbf{x})$. The value of the random function Z at position **x** is represented by the expression, $Z(\mathbf{x}) = m(\mathbf{x}) + \varepsilon'(\mathbf{x}) + \varepsilon''(\mathbf{x})$. The intrinsic hypothesis of the regionalized variable theory is defined by 2 assumptions about the autocorrelated errors: (1) stationarity of difference and, (2) variance of differences. According to the stationarity of difference, the mean is constant across the region if no trend or drift is present. Also, it is assumed that the variance of differences depends only on the distance and angular directions between sites. Therefore

the variance of differences is the same for any two points with the same distance apart and direction regardless of which two points are selected, that is, $E[[Z(\mathbf{x}) - Z(\mathbf{x} + \mathbf{h})]^2] = E[[\varepsilon'(\mathbf{x}) - \varepsilon(\mathbf{x} + \mathbf{h})]^2] = 2\gamma(\mathbf{h})$. As a result, $\varepsilon'(\mathbf{x})$ in the preceding equation can be replaced by $\gamma(\mathbf{h})$, the semivariogram function, $Z(\mathbf{x}) = m(\mathbf{x}) + \gamma(\mathbf{h}) + \varepsilon$". The semivariogram function can be estimated from sample data if the conditions of the intrinsic hypothesis hold $\hat{y}(\mathbf{h}) = 1/2n\Sigma_{i=1}^{n}[z(x_i) - z(x_i + h)]^2$ where, n is the number of pairs of observed values of attribute z separated by distance **h.** The experimental semivariogram is a plot of semivariance, $\hat{y}(\mathbf{h})$, against the distance between locations, **h**, a separation vector in space with associated direction and distance. The semivariogram provides the weightings of neighboring measurements used in ordinary kriging. The range is the lag distance at which the variogram levels off. Within a range, closer points are assumed more similar than more distant points (Burrough and McDonnell, 1998). Samples within the range are spatially correlated and a data point separated from an unobserved point by a distance greater than the range will not contribute to the interpolation. Semivariance is low when there are local spatial effects and highest when spatial dependence is absent (Durr and Gatrell, 2004), that is, as distance increases the squared difference of paired locations, or semivariance, increases and higher values on the y-axis of the semivariogram are observed.

The empirical semivariogram only contains information about the spatial autocorrelation of points at measured locations. In order to predict points at unmeasured locations a model, for example spherical or exponential, is fit to the empirical semivariogram. The model is a continuous function which ensures that predictions have

positive variances. The value of at the unmeasured location is calculated as a linear combination of neighboring values. Each measured value is weighted. Kriging weights are determined by solving a system of linear equations. The "Best" estimator is selected so that the variance of the errors is as small as possible, though not necessarily 0. The estimator is also required to be "Unbiased" with a mean of errors equal to 0. In effect, the Best Linear Unbiased Estimator is selected for ordinary kriging.

Interpolation has traditionally been used in geology and mining to predict the distribution of soil variables such as pH (Goovaerts, 1998). The probability of contamination substances important for environmental monitoring, such as lead and nickel can be determined using interpolation (Goovaerts, 1999; Becker et al., 2006). In addition, applications for interpolation have been found in ecology where it is used to predict the distribution of plants, bacteria and soil dwelling insects (Rossi et al., 1992; Goovaerts, 1998; Ritz et al., 2004)

2. BENEFITS OF OBTAINING TEST-NEGATIVE LEVEL 4 CLASSIFICATION FOR BEEF PRODUCERS IN THE VOLUNTARY BOVINE JOHNE'S DISEASE CONTROL PROGRAM*

2.1.Introduction

Disease control measures, including improved hygiene, testing, and culling, are employed for the management of Johne's disease (JD) because the therapeutic treatment of this chronic enteritis of domestic ruminants and wild animals is not practical (Emery and Whittington, 2004). JD is a fecal-oral transmitted disease and factors that bring susceptible cattle into contact with feces contaminated with *Mycobacterium avium* subsp *paratuberculosis* (Mptb), the etiologic agent for JD (Manning and Collins, 2001), are believed to increase the risk of introduction and transmission on farms.

The perception of benefits can influence the outcome of an individual's decision about adopting a disease control program. This is because the prospective program participant takes into consideration the perceived benefits and costs associated with program adoption when forming an attitude towards the program (Wraight et al., 2000; Rogers, 2003). An individual with a favorable attitude towards a program may behave in a manner that is logically consistent with his attitude by adopting the program(Fishbein and Ajzen, 1975). Perceived benefits were important to 29% (6/21) of dairy producers at the highest levels of the JD control program in Minnesota who reported that their major reason for enrolling was to increase the marketability of surplus

*Part of the data reported in this section is reprinted with permission from "Benefits of obtaining test-negative Level 4 classification for beef producers in the Voluntary Bovine Johne's disease control Program by Benjamin, L., Fosgate, G.T., Ward, M.P., Roussel, A.J. Feagin, R.A. and Schwartz, A.L., 2009. *Prev. Vet. Med.,* doi:10.1016/j.prevetmed.2009.06.007, Copyright [2009] by ELSEVIER.

cattle (Kovich et al., 2006). The increased marketability was due to the added value of cattle from a herd with low probability of being infected with Mptb.

The costs of implementing JD control measures are also important because the producer will not likely participate in the program if the costs exceed the benefits. An imbalance between perceived costs and benefits was reported by producers exiting the CattleMAP, a JD control program in Australia (Kennedy and Allworth, 2000). Apart from economic considerations, intangible gains and losses are associated with control programs and should be considered to accurately describe the impact of the program. For example, producers in a JD control program in Victoria, Australia, reported that they experienced decreased self-esteem when their herds were identified as JD affected (Hood and Seedsman, 2004). Conversely, the resulting pride associated with producing healthier animals is a possible benefit of a JD control program.

The receipt of perceived benefits from a control program helps to maintain participation by reinforcing the individual's original decision to adopt the program (Rogers, 2003). Although the actual benefits received by beef producers with test-negative Level 4 status in the Voluntary Bovine Johne's Disease Control Program (VBJDCP), the level with the highest likelihood of being a non-infected herd are unknown, it is expected that benefits of participation are greater than the costs. The objective of this study was to describe the beef producers' perceived benefits of attaining test-negative Level 4 status in the VBJDCP.

2.2.Materials and methods

All protocols were reviewed and approved by the Institutional Review Board at Texas A&M University (Protocol 2004-0423).

2.2.1. *Description of program*

The VBJDCP is the national program for the control of JD in the US. Coordinators of the VBJDCP are appointed in each state that adopts the program. Uniform program standards have been developed and participating states must implement these, but can also introduce more stringent standards. Producers are recruited in the state program by veterinarians who have successfully completed the JD certification program administered for the VBJDCP.

The VBJDCP consists of 3 components: education, reduction of management risk factors based on a risk assessment, and an annual test and cull of cattle 2 or more years of age. Management practices that interrupt the transmission of Mptb to susceptible members of a herd are recommended and include providing clean calving areas, reducing cow/calf pair density, decreasing the contamination of food and water with manure and separating younger from older cattle. In addition, measures that reduce the risk of introduction of Mptb (such as maintaining a closed herd and introducing cattle from herds with a low probability of JD) may also be recommended. Available tests for JD diagnosis include serum ELISA, fecal PCR, fecal culture and tissue culture.

The first step for herds that wish to participate in the VBJDCP is to develop a risk assessment based management plan performed by a JD-certified veterinarian. Herds

with a negative initial test are enrolled in the test-negative component of the program. Each 10 to 14 months, a herd can advance by 1 level from test-negative status Levels 1 to 4. Herds at test-negative Levels 1, 2, 3 and 4 have increasing probability of being non-infected (USDA, 2002).

2.2.2. *Development and administration of questionnaire*

The administered questionnaire was a mixture of 26 closed and open-ended questions related to herd demographics, reasons for attempting to achieve test-negative Level 4 status, tangible and intangible benefits of Level 4 status, difficulties experienced in attempting to maintain Level 4 status and suggestions for improvement of the VBJDCP. It was estimated that there were 58 beef producers with test-negative Level 4 status based on initial contact with JD coordinators. Packages, consisting of an introductory letter and a stamped self-addressed card for producer contact information, were sent to state JD coordinators, who then forwarded the packages to the test negative Level 4 producers in their state. The participating states were Kentucky, Michigan, Minnesota, Missouri, New Jersey, North Dakota and Ohio. Each producer that returned contact information was subsequently mailed a cover letter and questionnaire booklet. The questionnaires contained no information that could be used to identify respondents. Responses to open-ended questions were summarized, categorized, and recorded in a spreadsheet.

The 5-category outcome variable "Received monetary and non-monetary benefits because of participation in the VBJDCP" was dichotomized by collapsing the 2

categories, “Significant Benefits” and “Marginal Benefits” into the single category, “Benefits” and by collapsing the 2 categories “Significant Losses” and “Marginal Losses” into the single category “Losses.” The exposure variables analyzed using Fisher’s exact test included the dichotomous variables (Yes/No), herd is registered; cattle operation is a major source of income, and marketing was the major reason for enrollment. The 3-category variable “Provides other producers with information about the VBJDCP” was dichotomized by collapsing the categories “Often” and “Sometimes” into a single category. The 3-continuous variables were dichotomized based on their median values. Descriptive analysis of closed and open-ended questions and Fisher’s exact test were conducted using commercial software (SPSS Version 12.0.1 for Windows, SPSS Inc., Chicago, IL). Fishers’ exact test or Pearson’s chi-square was interpreted at the 5% level of significance.

2.3. Results

Forty of the estimated 58 producers (69%) from Ohio (20), Minnesota (11), Missouri (4), Kentucky (4) and North Dakota (1) returned cards with their contact information. Thirty-nine out of hese 40 producers (98%) returned completed questionnaires. The median herd size was 35 (range 5−250) head of cattle. The median number of hectares used for cattle operations was 66 (range 4.5−486). The median cows per hectare was 0.7 (range 0.2−3.5). Angus was the most frequently (55%; 21/38) reported breed. Sixty-six percent (25/38) of operations were registered (purebred cattle operations that sold cattle as replacements or for commercial production).

Twenty-nine percent (11/38) of producers reported that the beef herd surveyed was their major source of income. The median (range) length of time enrolled in the VBJDCP program was 6 years (3−11). A majority (71%; 27/38) of producers reported that they were sometimes approached by other producers for information about their experience with the VBJDCP. Seventy-six percent (29/38) of producers responded that veterinarians were a source of information about JD.

Forty-three percent of producers (16/37) reported that herd health/disease monitoring were major reasons for enrollment. Twenty-seven percent (10/37) of producers reported that increased marketing opportunities were a major reason for enrollment, and 5% (2/37) reported enrolling in the VBJDCP because of JD suspect/positive cows.

Classification at test-negative status Level 4 in the VBJDCP led to increased marketing opportunities for more than one-third (13/35) of producers. In Table 2.1 are the results of the cross-tabulation of the numbers of producers reporting the extent of benefits that they received in the VBJDCP as a result of their test-negative Level 4 status. Overall, 25% (9/36) of the producers reported that they had received significant and 39% (14/36) marginal benefits (financial and non-financial) as a result of participation in the VBJDCP. All evaluated associations between the outcome (received benefits) and exposure variables were non-significant. The median (range) added value that producers expected to receive for bulls was $100 (0−$500) and that actually received was $18 (0−$200). In addition, for heifers, the median (range) expected added value was $100 ($2−$1000), but only $10 ($2−$200) was received. The median (range)

reported annual benefit was $0 (0−$10000) and the median (range) annual cost of implementing and sustaining the VBJDCP on ranches was $200 (0−$5000). Thirteen percent (4/32) of producers reported that they observed a decrease in disease on their operations that they attributed to changes made as a result of the VBJDCP.

The most common problems reported by producers (27%; 10/37) in complying with VBJDCP requirements were associated with tests and testing. Other problems included lack of buyer education concerning JD, insufficient public relations for the program, and project administration. The majority of producers (48%; 16/33) spent an additional 0 to 5 hours per year working on their operations as a result of the VBJDCP. The management factor change most often reported (35%; 8/23) by producers was restricting purchases/closing the herd.

The measures most frequently suggested by producers for improvement of the VBJDCP were increased cost sharing (32%; 11/34), and improved public relations (32%; 11/34), with the aim of increasing marketing opportunities.

2.4. Discussion

The majority of producers reported that they received either significant or marginal benefits (monetary and non-monetary), 64%, as a result of their participation in the VBJDCP. However, only one-half of those producers who received benefits reported that they had also experienced increased marketing opportunities. The mean added value of cattle sold and the annual benefit received were less than what producers expected.

Table 2.1: Numbers of producers (%) and perceived benefits of test-negative status Level 4 in the Voluntary Bovine Johne's Disease Control Program (VBJDCP) stratified by herd-level factors.

			Benefits of being involved in the program (monetary and non-monetary)						
Variable	Level	Total	Significant benefits	Marginal benefits	No difference	Marginal losses	Significant losses	P value*	Total missing responses
Number of test eligible cattle in herd	<50	23	4 (11)	7 (19)	7 (19)	5 (14)	0	0.163	3
	50 to 99	8	3 (8.3)	4 (11)	1 (2.8)	0	0		
	≥100	5	2 (5.6)	3 (8.3)	0	0	0		
Number of hectares used for cattle	<10	6	2 (5.6)	1 (2.8)	3 (8.3)	0	0	0.671	3
	10 to 99	14	1 (2.8)	6 (17)	4 (11)	3 (8.3)	0		
	≥100	16	6 (17)	7 (19)	1 (2.8)	2 (5.6)	0		
Registered herd	Yes	28	9 (26)	11 (31)	4 (11)	4 (11)	0	0.568	4
	No	7	0	3 (8.6)	3 (8.6)	1 (2.9)	0		
Cattle operation is major source of income	Yes	9	3 (8.6)	2 (5.7)	2 (5.7)	2 (5.7)	0	0.367	14
	No	16	6 (17)	2 (34)	5 (14)	3 (8.6)	0		
Length of time involved in the program (years)	3 to 5	14	3 (8.6)	9 (26)	1 (2.9)	1 (2.9)	0	0.629	4
	6 to 8	14	3 (8.6)	2 (5.7)	6 (17)	3 (8.6)	0		
	9 to 11	7	2 (5.7)	3 (8.6)	1 (2.9)	1 (2.9)	0		
Provides other producers with information concerning VBJDCP	Often	2	1 (2.9)	1 (2.9)	0	0	0	0.221	4
	Sometimes	15	6 (17)	1 (31)	5 (14)	3 (8.6)	0		
	Never	8	1 (2.9)	2 (5.7)	3 (8.6)	2 (5.7)	0		
Major reason for enrollment was increased marketing	Yes	7	2 (15.4)	1 (0.04)	1 (0.04)	3 (11.5)	0	0.062	5
	No	27	5 (19.2)	13 (50.0)	7 (26.9)	2 (0.08)	0		

*Based on Fisher's exact test or Pearson's chi-square

There was a higher percentage of herds in the 50 head and above group in this survey than reported nationally in 1997 (National Animal Health Monitoring System, 1998a). Research suggests that larger farms are more likely to adopt biosecurity practices, such as those recommended by the VBJDCP, than are smaller farms (Dutil et al., 1999). The percentage of registered-only farms in the present survey was 10 times that of the national average reported for beef producers in 1997 (National Animal Health Monitoring System, 1998a). It is possible that those producers who operate registered farms are convinced of a responsibility to maintain their reputations as breeders of disease-free animals. Twice as many producers in the present study reported that their beef herd was the major source of income compared to what has been reported nationally (National Animal Health Monitoring System, 1997a). Producers whose beef herd is their major source of income and owners of registered farms may be more risk-averse and more likely to protect their investments. In addition, these producers may have been attracted to the possibility of increased marketing opportunities associated with participation in the VBJDCP.

The added value per head of cattle received for bulls and heifers reported in this survey was less than reported expectations of the producers. This finding is important because the perception of benefits to be gained by prospective participants in the program can lead to less favorable attitudes towards the program. In addition, the lower added value of cattle will not re-enforce the original decision for those who enrolled in the program because of perceived economic benefits.

The percentage of producers who considered other producers as sources of information about JD was about 1/3 of the reported percentage of producers who considered other producers as sources of information about animal health in the 1992−1993 Beef Cow/calf Health & Productivity Audit for animal health (National Animal Health Monitoring System, 1998b). This finding is important since prospective participants can acquire practical information about the possible impact of a new program from those producers who have already introduced it in their herds. The transfer of practical information about the program could convince prospective participants about the program feasibility (Rogers, 2003). The National Johne's Disease Demonstration Herd Project (NJDDHP) systematically gathers this feasibility information about control suggestions (Hartman and Wells, 2004).

Although the experience of disease in their herds may act as a motivating factor for producers to adopt preventive measures (Wraight et al., 2000; Rogers, 2003), the prospect of benefits can also be useful in attracting participants to disease prevention programs. Ninety-five percent of dairy cattle producers enrolled at the highest levels of the JD control program in Minnesota reported that they received benefits in the form of improved animal health and premiums from sales of surplus replacement cattle (Kovich et al., 2006). In comparison, only 37% of producers in the present survey were successful in marketing their cattle based on enrollment in the JD control program. The difference in success of the dairy and beef producers may, in part, be due to the lower prevalence of JD in beef than in dairy cattle (Thoen, 1988) and consequently a greater awareness of the disease among dairy producers. In 1997, 70% of beef operations

reported that they had not heard of JD prior to the survey (National Animal Health Monitoring System, 1998a), whereas only 10% of dairy operations reported the same in the previous year (USDA, 2005a). In addition, only 12% of beef operations felt that JD was an important problem for the industry (National Animal Health Monitoring System, 1994). As a result, buyers of beef cattle may be less likely to pay a premium for cattle that are free of a disease that they are largely unaware of and which they otherwise do not believe to be a problem. A possible solution to this problem is to increase the focus on the transfer of information, the initial stage of the decision process, by providing targeted groups of producers with user-friendly information about the impact of JD on their herds and how to get involved in a control program. This increased awareness could help shape producers' attitudes towards the control of JD since they will be more informed when assessing the benefits of participation.

The annual average cost of bovine tuberculosis (TB) per infected cow (1200 lbs) was $1838.22 (2003 US dollars) estimated as the sum of the cull cow slaughter price, replacement cow price, calf price and retained carcass (Gilsdorf et al., 2006). Costs of the bovine TB eradication program to the producer, such as labor to round-up the animals, were assumed to be negligible and not included in the analysis by Gilsdorf et al. (2006). In the VBJDCP, the costs associated with replacing cattle apply to herds with test-negative Level 4 status only if they test positive because test-positive herds are deprived of their status and must begin again to advance through the successive classification levels from Level 1 once they have eliminated the test-positive animals from the herd. Any benefit derived from marketing cattle with a low probability of

having JD will, of course, not be available to herds during the period in which they attempt to regain test-negative status.

Test-negative status Level 4 producers did not spend much additional time on activities related to VBJDCP participation. This minimal time requirement for VBJDCP activities may be because some of these producers had previously adopted the required management practices. The testing requirement could have been marketed, by veterinarians, to those producers who already were testing their animals for other reasons as an additional benefit for little further commitment. Another marketing tool could be the decreased prevalence of diseases other than JD that was reported by producers.

Questionnaire studies such as the present study are limited because they are an imprecise method for gathering data. Also, since the questionnaire was self-administered the possibility exists for the misinterpretation of questions. Future studies are needed to determine which differences exist between enrolled producers and the average beef producer in the US with respect to their perceptions about the VBJDCP, and the implications of these differences for the success of the program. In addition, on-farm studies are necessary to more accurately measure relevant costs and benefits. This was a cross-sectional study and exposures and outcomes were measured at the same time. Thus, the reported costs and benefits did not necessarily occur in the same year. Since it is generally assumed that the present value of the dollar is greater than its future value, future costs are typically discounted in order to compare them with benefits received at some later point in time (Petitti, 2000).

It should be kept in mind that the US state with the highest proportion of beef herds, Texas, was not represented in this study. The resources dedicated to the VBJDCP are expected to vary by US state and this might impact the producers' perception of the program. There was a high proportion of registered herds in this study and, as a result, the results of this study might be more applicable to registered herds. Registered cattle might be more highly priced than non-registered cattle leading their owners to perceive greater benefits from the adoption of a preventive program such as the VBJDCP.

2.5. Conclusions

Overall, 25% of producers reported that they had received benefits (financial and non-financial) as a result of their participation in the VBJDCP. Increased publicity about the program is suggested as a method of improving the success of the VBJDCP in the beef cattle industry, by leading to increased marketing opportunities.

3. ATTITUDES TO BIOSECURITY PRACTICES ON BEEF FARMS WITH REFERENCE TO JOHNE'S DISEASE CONTROL

3.1. Introduction

Johne's disease (JD) or paratuberculosis is a chronic enteric disease of domestic ruminants, and has also been reported in white-tailed deer, rabbits and other wild animals (Daniels et al., 2001; Daniels et al., 2002; Judge et al., 2005; Raizman et al., 2005). The infective agent is *Mycobacterium avium* subsp. *paratuberculosis* (Mptb). There is a long period between infection and the development of clinical signs; for example, a calf infected in the first 3 months of life may not show signs of disease until it is 2 years of age or older.

Several countries have designed and implemented control programs for JD. To increase the chances of success of these programs it is necessary to investigate the factors which determine whether or not prospective participants adopt the program (Kennedy and Benedictus, 2001; USDA, 2002; National Research Council Committee on Diagnosis and Control of Johne's Disease, 2003). One such factor is the formation of an attitude. An attitude, a relatively long-lasting organization of an individual belief about an object, predisposes actions that can be either in favor of or against a new idea (Rogers, 2003). Information about the attitudes of current and potential participants in prevention programs can be used to design or modify programs to increase the chances

of success by increasing program adoption. An example of a relationship between behavior and attitudes is documented by cattle producers in Texas and Nebraska who reported that their major health management practice to reduce risk was an emphasis on disease prevention (Hall et al., 2003). Risk, in this instance, can be defined as the specific form of the producers' attitudes towards the potential disease hazard (Frewer et al., 2004) and, the disease prevention emphasis represented their behavior.

The relationship between attitudes and behavior is complex. Different behaviors may be linked to attitudes of different intensities (Frewer et al., 2004). The belief that JD is a problem in livestock production has not translated into higher enrollment in JD control programs. Even in dairy cattle production, in which the prevalence of JD is relatively high, enrollment is typically poor. For example, despite the fact that 93% of surveyed Wisconsin dairy producers believed that JD was important, only 9% of these producers reported enrollment in the state JD control program (Hoe and Ruegg, 2006). Also, 78% of Scottish cattle farmers in a JD "hot-spot," identified in Veterinary Intelligence Surveillance Reports from the Veterinary Laboratories Agency in Great Britain, reported that they believed that JD was a problem but only 38% of these farmers reported implementing any control measures against this disease (Daniels et al., 2002). This disparity between attitude and behavior may, in part, be related to the absence of "cues-to-action." The implementation of preventive programs for chronic diseases is challenging because vivid images (cues-to-action) that are usually associated with acute

diseases are not present to convince potential program participants of the need to act. These cues-to-action, such as discussing the program with a peer who has had a positive experience, are important in increasing motivation to adopt the preventive program (Rogers, 2003).

Available methods of JD control include test and cull, elimination or minimization of management risk factors and vaccination. Tests used for the control of JD include fecal and tissue culture (sensitivity 30 to 50%, specificity 99%), serum enzyme-linked immunosorbent assays (ELISA; sensitivity 25% for nonclinicals and 85% for clinically affected cattle, specificity 98 to 99%) and fecal PCR (sensitivity 40%, specificity 99%) (Sockett et al., 1992a; Sweeney et al., 1995; Stabel, 1997; Whitlock et al., 2000). The results of PCR can be available within 3 days, whilst culture results require a minimum of 8 weeks. ELISAs are the least expensive tests to perform and the results can be available within the shortest period of time. Vaccination is a potential control option, but there are concerns about the efficacy of available vaccines and it may be difficult to interpret results of serological testing in the post vaccination period (Harris and Barletta, 2001). In addition, many states, Texas included, restrict the use of the JD vaccine due to interference with tuberculosis (TB) skin testing. This policy is not likely to change since 6 states have reported TB infected cattle herds within the past year. Various factors are believed to increase the risk of introduction of Mptb onto farms and the transmission of this infective agent within herds. Since the infective agent

for JD is shed in the feces of clinically affected animals and some Mptb infected animals at the subclinical stage, the basic management practices targeted for JD control in beef herds include sanitizing calving areas, reducing the density of cow-calf pairs, minimizing manure contamination of food and water, and separating younger calves from older cattle (TJWG, 2003).

The level of compliance with the requirements of preventive programs influences their success (Volmink and Garner, 2007). The ability and willingness of producers to alter management and environmental practices are affected by several factors, such as the perceived risk posed by the disease, cost of the program, farm size, herd density, as well as the feasibility and the compatibility of program requirements with other farm practices. The aims of this study were to describe attitudes towards JD control among beef cattle producers and veterinarians, and to compare producer attitudes towards recommended Texas Voluntary Johne's Disease Program (TVJDP) control measures with veterinarians' beliefs about the willingness of producers to comply with the same.

3.2. Materials and methods

All protocols were reviewed and approved by the Institutional Review Board at Texas A&M University (Protocol 2004-0423).

3.2.1. *Study population*

The 3 major types of beef operations in the US are cow-calf, stocker and feedlots. A cow-calf operation may be either seedstock or commercial. Purebred cattle in either seedstock or commercial operations can be registered, or are eligible for registration. In the US during 1997, 6% of all operations bred registered cattle only, whilst 73% bred commercial cattle and 21% bred a mixture of both registered and commercial cattle (National Animal Health Monitoring System, 1998a). In cow-calf operations, cattle are typically raised on pasture. Calves or yearlings, about 250 kgs, may be sold to stocker operations where they are raised until they are approximately 364 kgs. Spring calves may be sold directly from cow-calf operations in the fall as feeder calves when they are 7 to 8 months old. These cattle are then marketed from the feedlots as finished cattle at 12 to 16 months. In 1999 there were 142 feedlots in Texas with a capacity of 1000 or more animals (USDA, 2000). Texas had the largest proportion (15%) of beef cow operations in 1996 (National Animal Health Monitoring System, 1998b). The target populations for this study were beef producers (all types) with farms larger than 50 head of cattle and veterinarians who had worked with these producers.

3.2.2. *Program description*

The TVJDP for cattle is based on the US Voluntary Bovine Johne's Disease Control Program (VBJDCP) of 2002. The VBJDCP recommends specific biosecurity

measures to control JD in cattle. Veterinarians are certified for the TVJDP by participation in a training course, which is available from the Texas Animal Health Commission. Certified veterinarians are monitored by a designated Johne's disease coordinator (DJC). Veterinarians recruit producers for voluntary participation and play critical roles in all components of the TVJDP. There are 3 basic components of the TVJDP: education, management and herd testing and classification (TJWG, 2003).

In Texas, producers participate either in the management phase or the herd classification phase of the TVJDP. The first step in either phase of the program involves a risk assessment and herd plan completed by a Johne's disease certified veterinarian. Testing is an optional part of the management phase that is mandatory to enter the herd classification phase. Fecal and tissue culture, fecal PCR and ELISA can be used in the TVJDP. Herds entering the classification phase are classified as test positive or negative based on the results of the specified JD tests and those in the test negative program may advance from Levels 1 to 4 depending on the results of successive annual tests. The increasing levels signify an increasing probability of the herd being free of JD. In Texas, risk assessments and herd plans are submitted to the DJC for every participating herd, but test results are submitted only when the owner wishes to enter the herd classification component of the program. In states where JD is reportable the state veterinary officer receives all test results.

3.2.3. *Sample selection*

The sample size calculation was performed to compare attitudes with a desired power of 80%, an alpha of 5% and a hypothesized outcome proportion, with respect to agreement with the attitude question, of 50% in the veterinarians' group and 60% in the producers' group (EpiInfo 3.4.1 Centers for Disease Control and Prevention, Atlanta, GA, 2007). The required sample size was 408 per group. For the survey of producers, a membership roster from a producer organization in Texas was used to randomly select members that reported owning ≥50 cattle. A response proportion of 40% was assumed and the total number of producers on the list frame, 4887, was systematically sampled at an interval of 4 up to a total of 1100. All 840 veterinarians with membership in a state professional organization and reported working with cattle in August of 2006 were selected for participation.

3.2.4. *Questionnaire development and administration*

Two questionnaires were developed to measure the attitudes of producers and veterinarians towards the TVJDP. The questionnaire sections were based on problem areas identified in a survey of test-negative Level 4 producers in the VBJDCP (Benjamin et al., 2009b). The questionnaires included open-ended and closed ordered general questions about herd demographics and more specific questions about the VBJDCP, including expected and perceived benefits of enrollment, reasons for enrollment,

problems experienced with the program and suggestions for improvement. The questionnaires were pre-tested by 2 veterinarians familiar with beef cattle production. The questionnaires were printed in booklet form and the first page was a signed introductory letter that described the purpose of the study.

The producers' questionnaire was 10 pages and consisted of 53 questions that covered the following areas: 1) Herd demographics and producer goals, 2) Information sources for JD and cattle diseases, 3) Education requirements for herd health, 4) General preventive practices, 5) Herd movement/contacts, 6) Basic knowledge about JD, 7) Herd handling practices, and 8) Attitudes towards JD and the TVJDP.

The veterinarians' questionnaire was 10 pages and consisted of 46 questions that addressed the following areas: 1) Demographics of beef clients, 2) Attitudes towards JD and the TVJDP, 3) Participation in the TVJDP, 4) Perceived client attitudes towards JD and the TVJDP, 5) Information sources for JD, 6) Whether there is a need for promotion of the TVJDP and 7) Adequacy of compensation paid to veterinarians for risk assessments and testing related to the TVJDP.

The questions in both surveys were a mixture of 5 category Likert-scale, dichotomous (yes/no) and numerical free-response. The Likert-scale consisted of the following categories: strongly agree, agree, undecided, disagree and strongly disagree. In addition, 6 questions on general preventive practices with categories often, sometimes or never were included in the producers' survey and 1 question with the same categories

was included in the veterinarians' survey.

Included in each survey packet was a first class stamped return envelope. Questionnaires were mailed to producers in August 2006 and veterinarians in November 2006 using first class mail. No reminders were sent, nor were other attempts made to contact subjects subsequent to the initial mailing of questionnaires.

3.2.5. *Analysis of data*

Responses were recorded in a database and analyzed using commercially available software (SPSS Version 12.0.1 for Windows, SPSS Inc., Chicago, IL). Statistical tests performed were interpreted at the 5% level of significance. Analyses included descriptive statistics, chi-square analysis of associations between exposures and outcomes, and chi-square analysis of the association between age and herd size. Frequencies were computed for familiarity with JD and actual practices or practices willing to adopt. Mean, median, range and frequencies for responses to questions on 5-point Likert scales, yes/no and, often/never/sometimes categories were computed for variables. The primary outcome for the survey of producers was: "I have no JD problem on my ranch," and for the veterinarian survey was: "JD ranches."

Variables used to compare the attitudes of producers and veterinarians to control measures for JD originally recorded on a 5-point Likert scale were dichotomized for analysis. Comparison variables were as follows: separate JD clinical cows from calves

and heifers, separate weaned heifers and bull calves from mature cows, separate bred heifers and yearling bulls from mature cows, replacements or additions from JD low-risk herds, test for JD every 10 to 14 months, test purchased cattle for JD, quarantine new cattle and test and cull clinical suspects only. Pearson's chi-square or Fisher's exact test P values and prevalence ratios were computed for all comparisons.

Confounding was defined as a change of 15% in the effect measure and was determined using multivariable logistic regression. Univariate analysis was carried out using cross tabulations and 2 x 2 tables. Variables significant at $P<0.2$ were included in multivariable logistic regression analysis. The outcome for the survey of producers, "I have no JD problem on my ranch," was dichotomized from the original 5-point Likert scale and the potential confounding factors were herd size and age of producer. The exposures were: separate JD clinical cattle from calves or heifers; separate weaned heifers and bull calves from mature cows; separate bred heifers and yearling bulls from mature cows; get replacements or additions from JD low-risk herds; test for JD every 10 to 14 months; test purchased cattle for JD; quarantine new cattle; test and cull clinical suspects only; and JD is responsible for significant losses in beef cattle production in Texas. All variables originally measured on the 5-point Likert scale were dichotomized by collapsing the strongly agree and agree categories into a single category, and the strongly disagree and disagree categories into a second category. Undecided responses were considered missing values and were excluded from analysis. Herd size was

categorized as small:≤150, large:≥151 head of cattle, and age of producer as younger: <44, older:≥45 years.

The outcome for the veterinarians' survey was the calculated variable "JD ranches", whether or not the veterinarian had diagnosed cases of JD. For "JD ranches" the category "no" represented the number of veterinarians who reported having no clients with JD in their herds during the past 3 years and "yes" represented veterinarians who had clients with JD in their herds during the past 3 years. The potential confounding factor was the possession of JD certification. The exposures assessed were the same as the producer questionnaire.

3.3. Results

3.3.1. *Descriptive analysis*

Two hundred and eighty-seven producers (26%) returned completed and partially completed surveys. Two producers returned completely blank surveys. One hundred and eighty-three veterinarians (22%) returned completed and partially completed surveys. Three surveys did not reach veterinarians due to incomplete addresses and 27 blank/incomplete surveys were returned.

The median age of the major decision–maker responding to the producer survey was 61 years (range 31 - 91). Fifty-six percent (149/264) also had employment independent of their ranches. The majority of producers or their families, 67%

(180/267), planned to raise cattle indefinitely whilst, 2.0% (5/267) planned to raise cattle for the next 1 to 5 years only. The most frequent highest educational level of producers was a bachelor's degree (37%) and the least common was less than a high school diploma (2.0%).

The median herd size of producers responding to the survey was 150 head of cattle (range 10 - 4000). A significant association was not detected between herd size and age of producer (P=0.23). The median hectares used for these cattle was 809 (range 12 - 4047). The median head of cattle per hectare was 0.22 (range 0.01 - 7.57). The median length of the calving season was 4 months (range 2 - 12). The most frequent type of herd was unregistered commercial cow-calf (80%; 229/285). In addition, the majority of herds, 71% (189/265), used some form of rotational grazing either exclusively or in combination with other methods of grazing, such as management-intensive or continuous (single pasture).

Many veterinarian respondents worked with large animal mixed species, 48% (74/155). The second most frequent practice type, 44% (68/155), included veterinarians who worked with some percentage of small animals in addition to beef cattle. Seven veterinarians worked with 1000 or more clients, but the majority of veterinarians (132/139) worked with 500 or fewer clients. The median percent of clients who raise registered cattle was 10% (range 0 - 100). The majority (76%) of client operations were cow-calf commercial. One quarter of veterinarians (39/154) reported never developing

herd health programs for clients, whilst the majority of veterinarians, 59% (91/154), sometimes developed such programs.

3.3.2. *General beliefs about disease control*

Over the 5 years preceding the survey, the majority of herds, 73% (183/253) reported adding between 1 and 49 animals on average per year. Although 90% (188/209) of producers believed that there is a financial benefit in operating a closed herd and 95% (239/251) of producers believed that a closed herd benefits disease control, only 9% (8/253) reported having added no new animals during the preceding 5 years. Twenty-three percent (60/259) of producers reported that they use or are willing to use artificial insemination.

The majority of operations, 68% (182/266), purchased cattle from private sales and 38% (110/265) of operations purchased cattle from public auction markets. Seventeen percent (45/272) of producers either often or sometimes spread cattle manure on their pastures. Fifty-seven percent (143/251) of producers reported that they test purchased cattle for disease (unspecified) before adding the cattle to the herd. Seventy-three percent (201/277) of producers reported that cattle from neighboring ranches often or sometimes gain access to their premises. Nineteen percent (49/258) of producers reported that their ranch was enrolled in at least 1 of the brucellosis and tuberculosis certification programs for cattle. Most producers were interested in receiving additional

herd health education or information from trade magazines, 71% (185/262) or seminars, 83% (219/265).

3.3.3. *Familiarity and experience with Johne's disease*

The majority of responding producers, 73% (204/281), had heard of JD. Veterinarians reported that the median proportion of client-owned ranches with clinically diagnosed JD was 4% (range 0 - 80). Fifty-three percent, (66/124), of veterinarians had 1 or more client-owned ranches with confirmed JD. In addition, 9% (13/98) of veterinarians reported that JD had been eliminated from at least 1 of their clients' herds after implementation of a JD control program.

3.3.4. *Attitudes of producers towards Johne's disease control*

Fifty-nine percent (49/83) of the producers agreed or strongly agreed that JD was responsible for substantial losses in beef cattle productivity. Nineteen-percent (37/193) of producers agreed/strongly agreed that they knew how to control and prevent JD from becoming a problem on their ranch. Fifty-two percent (15/29) of producers agreed/strongly agreed that serum ELISA was good for confirming JD clinical suspects and 80% (41/51) of producers agreed/strongly agreed that fecal culture was good for confirming clinical suspects. Table 3.1 is a crosstabulation of the responses of producers to the question "I have no JD problem on my ranch" with herd size, herd type, whether

other selected speices are present on the farn and the age of the producer. Separate JD clinical suspects from calves and heifers was the only variable significantly associated with the outcome "I have no JD problem on my ranch" in the univariate analysis and was entered into the multivariable logistic regression along with age of the producer and herd size. Age of the producer and herd size were not significant predictors and meaningful confounding was not identified.

3.3.5. *Attitudes of veterinarians towards Johne's disease control*

Table 3.2 summarizes the responses of veterinarians to questions relevant to the control of JD. Fifty percent (48/91) of veterinarians agreed/strongly agreed that JD is responsible for substantial losses to the local beef cattle industry. Fifty-one percent (73/144)**,** 34% (47/138) and 58% (83/144) of veterinarians had submitted samples for fecal culture, PCR and serum ELISA, respectively, to test JD suspect cattle during the previous 3 years. The majority of veterinarians agreed/strongly agreed that the rapidity of fecal PCR, 94% (84/89), and serum ELISA, 95% (87/91), were useful for decision-making whereas, less than half of the veterinarians, 41% (42/102), agreed/strongly agreed that the rapidity of fecal culture was useful for decision-making. The variables separate JD clinical suspects from calves and heifers, separate weaned heifers and bull calves from mature cows, separate bull calves from mature cows, and separate bred heifers and yearling bulls from mature cows were significant in univariate analysis and

Table .3.1: Number (%) of responses of 285 producers to the question "I have no Johne's disease problem on my ranch" in a survey of beef producers' attitudes towards the Texas Voluntary Johne's Disease Program in cattle, 2006.

Variable	Level	"I have no Johne's disease problem on my ranch."				
		Strongly Agree	Agree	Undecided	Disagree	Strongly Disagree
Herd size	< 50	2 (15.4)	4 (30.8)	6 (46.2)	0 (0.0)	1 (7.7)
	50 to 99	11 (18.0)	27 (44.3)	17 (27.9)	1 (1.6)	5 (8.2)
	100 to 299	19 (18.0)	46 (52.6)	38 (35.2)	2 (1.9)	3 (2.8)
	300 or more	14 (19.4)	34 (47.2)	17 (23.6)	4 (5.6)	3 (4.2)
Herd type	Commercial cow-calf (registered)	7 (17.5)	19 (47.5)	11 (27.5)	2 (5.0)	1 (2.5)
	Commercial cow-calf (not-registered)	39 (17.8)	97 (43.7)	72 (32.4)	6 (2.7)	8 (3.6)
	Seed stock herd (registered)	10 (23.8)	22 (52.4)	6 (14.3)	1 (2.4)	3 (7.1)
	Seed stock herd (not-registered)	7 (30.4)	8 (34.8)	8 (34.8)	0 (0.0)	0 (0.0)
	Feedlot	4 (16.7)	9 (37.5)	8 (33.3)	0 (0.0)	3 (12.5)
	Mixed	3 (15.0)	11 (55.0)	6 (30.0)	0 (0.0)	0 (0.0)
	Other	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Other species present	Goats	7 (14.0)	25 (50.0)	17 (34.0)	1 (2.0)	0 (0.0)
	White-tailed deer	8 (17.8)	21 (46.7)	12 (26.7)	2 (4.4)	2 (4.4)
	Sheep	6 (24.0)	10 (40.0)	9 (36.0)	0 (0.0)	0 (0.0)
	Dairy cattle	1 (50.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)
Producer age	25 to 44	1 (0.4)	7 (2.8)	11 (4.4)	0 (0.0)	3 (1.2)
	45 to 64	22 (8.7)	63 (25.0)	40 (15.9)	3 (1.2)	3 (1.2)
	65 or more	23 (9.1)	42 (16.7)	25 (9.9)	3 (1.2)	6 (2.4)

Table 3.2: Responses (%) of 153 veterinarians to questions relevant to the control of Johne's disease in a survey of the attitudes of veterinarians towards the Texas Voluntary Johne's Disease Program in cattle, 2006.

Variable	Level	Strongly Agree	Agree	Undecided	Disagree	Strongly Disagree
Fecal culture	Useful JD information	7 (4.8)	74 (51.0)	44 (30.3)	15 (10.3)	5 (3.5)
	Cost-effective	3 (2.1)	34 (23.5)	67 (46.2)	38 (26.2)	3 (2.1)
	Rapidity useful for decision-making	5 (3.5)	37 (25.5)	43 (29.7)	38 (26.2)	22 (15.2)
Serum ELISA	Useful JD information	9 (6.4)	76 (53.9)	47 (33.3)	8 (5.7)	1 (0.7)
	Cost-effective	5 (3.6)	63 (44.7)	62 (44.0)	11 (7.8)	0 (0.0)
	Rapidity useful for decision-making	10 (7.2)	77 (55.4)	48 (34.5)	4 (2.9)	0 (0.0)
PCR	Useful JD information	9 (6.5)	83 (60.1)	43 (31.1)	3 (2.2)	0 (0.0)
	Cost-effective	2 (1.5)	33 (24.1)	75 (54.7)	26 (19.0)	1 (0.7)
	Rapidity useful for decision-making	7 (5.1)	77 (56.2)	48 (35.0)	5 (3.7)	0 (0.0)
Producers should eliminate JD in commercial cow-calf herd		4 (2.8)	31 (21.8)	42 (29.6)	60 (42.3)	5 (3.5)
Producers should eliminate JD in registered cow-calf herd		55 (38.5)	76 (53.2)	9 (6.3)	3 (2.1)	0 (0.0)

JD = Johne's disease

were entered into the multivariable logistic regression model along with JD certified. The only variable significant ($P<0.05$) in the model was JD certified. Meaningful confounding between exposure variables and the outcome JD ranches was not identified.

3.3.6. *Familiarity and experience with Texas Voluntary Johne's Disease Program*

Results related to veterinarian familiarity and experience with the TVJDP are summarized in Table 3.3. The proportions of veterinarians who had educated producers on the cause, stages and transmission of JD were 78% (116/149), 62% (91/147) and 68% (100/147), respectively. Sixty-four percent (94/148) had discussed management strategies to control or eliminate JD with producers. Sixty-seven percent (97/145), 53% (76/144) and 67% (96/144) had discussed Johne's fecal culture, fecal PCR and ELISA respectively. Sixty-seven percent (99/147) had discussed the interpretation of tests for JD with one or more clients. In addition, 83% (124/149) of veterinarians reported that they had heard of the TVJDP. However, only 39% (58/149) of veterinarians had recommended the TVJDP to producers during the previous 3 years. Twenty-nine percent (53/183) of responding veterinarians were certified to participate in the TVJDP for cattle. Of the veterinarians who were not certified, 40% (40/99) had considered participation in the TVJDP's JD certification program. More JD-certified veterinarians, 85% (44/52), often or sometimes developed herd health programs for clients than did non- certified veterinarians, 69% (70/101; $P = 0.018$). Certified veterinarians had

enrolled a median of 0 (range 0 - 25) herds. Fifty-five percent (47/86) of veterinarians did not feel that the compensation from the TVJDP for risk assessments and sample collection was adequate. Twenty percent of producers (42/212) reported that they were familiar with the TVJDP and 16% (33/204) reported that they had considered participation in the TVJDP.

3.3.7. *Comparison of veterinarians' opinions and producers' beliefs*

Detailed in Table 3.4 are comparisons of veterinarians' opinions about producers' willingness to adopt specific practices and the responses of producers related to the same practices. Ninety-eight percent (129/132) of veterinarians believed that higher cow/calf density increases the risk of transmission of Mptb and 83% (66/80) of producers agreed that decreasing cow/calf pair density is useful for control. Producers (68/75) were 0.93 (95% CI= 0.86-1.00) times as likely as veterinarians (129/132) to agree that drinking feces-contaminated water increases transmission of Mptb.

Table 3.3. Familiarity and experience of 153 veterinarians with the Texas Voluntary Johne's Disease Program in cattle, 2006.

Variable	Level	Yes	Total	Percent
Educated producers about the following (previous 3 years)	The basic cause of JD	116	149	77.9
	The stages of disease	91	147	61.9
	Transmission of etiologic agent	100	147	68.0
Discussed management strategies to control or eliminate JD (previous 3 years)		94	148	63.5
Discussed available tests with clients (previous 3 years)	Fecal culture	97	145	66.9
	Fecal PCR	76	144	52.8
	ELISA	96	144	66.7
Discussed interpretation of tests for JD (previous 3 years)		99	147	67.3
Recommended TVJDP to beef clients (previous 3 years)		58	149	38.9
Heard of TVJDP		124	149	83.2
Participated in the TVJDP training program		55	152	36.2
Read the document which describes the TVJDP		71	150	47.3
Performed a risk assessment for the TVJDP for 1 or more clients		25	147	17.0
Discussed classification levels for herds in the TVJDP in the previous 3 years		36	146	24.7
Feel that the veterinarian's compensation for the TVJDP is adequate		39	86	45.3

JD = Johne's disease
TVJDP = Texas Vlountary Johne's Disease Program in Cattle

Sixty-three percent (163/257) of producers reported that they were willing to provide separate feeding and drinking areas for cows and weaned calves. As far as practices relevant to quarantine and isolation were concerned, 79% (200/252) of producers either had or were willing to provide a separate pasture/pen for newly purchased cattle whilst 90% (236/262) of producers either had or were willing to provide a separate pasture/pen for sick animals.

3.4. Discussion

The median age of producers who responded to this questionnaire (61 years) was higher than the average age of U.S. operators (57 years) in the beef ranching and farming sector as previously reported (Allen and Harris, 2005). This is important since Hall et al. (2003) reported that producers in Texas and Nebraska who were 41 years of age or younger had greater participation in herd health management training programs and spent more hours participating in these programs than other age groups. Although it was reported that cow-calf producers with large herds in Quebec were younger (44 ± 11 years) than producers with small herds (50 ± 13 years) (Dutil et al., 1999), a significant association was not detected between herd size and age in the present study.

Table 3.4: Comparison of veterinarians' opinions about producers' willingness to adopt practices relevant to Texas Voluntary Johne's Disease Program and agreement of producers with the same practices, 2006.

Variables		Strongly Agree or Agree	Total	Percent	PR	95% CI	P value*
Separating JD clinical from calves or heifers	Producer	117	122	95.9			
	Veterinarian	88	121	72.7	1.33	1.18-1.49	<0.001
Separate weaned heifers and bull calves from mature cows	Producer	69	83	83.1			
	Veterinarian	96	117	82.1	1.01	0.89-1.15	0.421
Separate bred heifers and yearling bulls from mature cows	Producer	60	76	78.9			
	Veterinarian	78	108	72.2	1.09	0.93-1.29	0.194
Repalcements or additions from JD low risk herds	Producer	162	166	97.6			
	Veterinarian	90	105	85.7	1.14	1.05-1.24	<0.001
Test for JD every 10 to 14 months	Producer	54	71	76.1			
	Veterinarian	11	104	10.6	7.19	4.05-12.76	<0.001
Test purchased cattle for JD	Producer	119	142	83.8			
	Veterinarian	84	107	78.5	1.07	0.94-1.21	0.015
Quarantine new cattle	Producer	89	129	69.0			
	Veterinarian	83	108	76.9	0.90	0.77-1.05	0.229
Test and cull clinical suspects only	Producer	47	78	60.3			
	Veterinarian	35	100	35.0	1.72	1.23-2.38	0.001

PR = prevalence ratio, JD = Johne's disease

* Based on uncorrected Pearson chi-square test

Farm biosecurity refers to measures taken to prevent the introduction and spread of diseases among livestock. Producer attitudes toward control programs designed to strengthen farm biosecurity can determine whether or not the program in question is adopted and the extent to which measures are instituted by enrolled producers. The introduction of an infectious agent may be prevented by biosecurity measures such as testing and quarantine of new herd additions. Overall, only 0.2% of US beef cattle operations required new cattle additions to be tested for JD in the 3 years preceding a national survey (National Animal Health Monitoring System, 1998a). In the present study, a majority of beef producers, 63%, reported that they either have or are willing to have a separate pen or pasture as a part of a JD control program. Nationally, of the 0.6% of cow-calf operations which introduced dairy heifers and cows onto their operations 57% did not quarantine or separate new cattle despite the fact that JD is more prevalent in dairy cattle (National Animal Health Monitoring System, 1997a). In addition, of the 14% of cow-calf operations which introduced beef cows that had been bred at least once, 66% of these operations did not separate or quarantine new animals from the rest of the

herd (National Animal Health Monitoring System, 1997a). In contrast, in the present study, a large percentage of producers stated that they either had or were willing to have a separate pasture or pen for newly purchased cattle. In addition, 69% of producers in this study agreed/strongly agreed that quarantine of cattle was important for control of JD and the majority of veterinarians, 80%, agreed/strongly agreed that producers would be willing to make this change. The difference observed between the responses of producers to the National Animal Health Monitoring System and to the present surveys may be due to the often observed disparity between positive attitudes towards a practice and actual adoption of the practice, since the national survey asked about practices and, the related question in the present survey addressed both willingness to adopt the practice and current practice.

The potential exists for Mptb to be introduced from adjacent ranches if cattle that are shedding Mptb can gain access to the premises. A majority of producers in this study reported that cattle from neighboring herds had entered their premises. Contact with neighboring herds poses a challenge for biosecurity because Mptb infected cattle may introduce or re-introduce JD to farms with JD negative status. In the US, 6% of operations had cattle that had been off the premises for fairs or shows in 1996 (National Animal Health Monitoring System, 1997a).

The producer's perception of the hazard posed by JD is likely to influence the decision to participate in a control/preventive program for this disease. Sixty percent of

producers agreed/strongly agreed that JD was responsible for significant losses to the beef industry, but only 50% of veterinarians shared a similar opinion. The majority of producers, 90%, however, agreed/strongly agreed that they had no JD problem on their ranches at the time of the survey. The discordance between producer perception of the importance of the disease and their assessment of their own herd may be influenced by their unwillingness to admit, even on an anonymous questionnaire, that they have a problem with JD.

Producer knowledge about JD control should be increased, since 42% of producers who were familiar with JD disagreed/strongly disagreed that they knew how to control and prevent the disease. In addition, the majority of veterinarians agreed that the TVJDP should be more widely promoted among producers and veterinarians. The producers also need to be better informed about the interpretation of test results. Serum ELISA is an attractive option because of relatively low cost and rapidity of results. The utility of a rapid test is important in decision-making on the farm because pasture space available for quarantine or separation of cattle may be limited, or a breeding opportunity may be lost if a bull is not replaced in a timely manner.

Veterinarians were less likely than producers to agree that producers would adopt the following practices: separate JD clinical cows from calves and heifers; source additions or replacements from JD low-risk herds and test their herds every 10-14 months. Veterinarians were also less likely to agree that JD should be controlled by

testing and culling clinical suspects only. One reason for these differences in attitudes between producers and veterinarians may be that the responses of the veterinarians are based on their experiences with a wider range of beef production types than are the producers. Veterinarians may be more willing to believe that producers would adopt the VBJDCP if veterinarians had exposure to farms on which the program has been successfully implemented. It is possible that producers with available land to isolate clinically diagnosed JD cows from calves will have a more favorable attitude towards the introduction of this practice. The factors associated with sourcing low-risk replacements may be further examined using "willingness to pay" techniques in order to determine the value of this practice to producers. It is expected that attitudes of producers towards annual testing will be influenced by factors including herd size, cost and ease of gathering cattle for testing. Testing and culling clinical suspects only allows the producer to avoid the costs associated with implementing management changes and annual testing. Test and cull will not prevent the introduction of disease to the herd and does not take into consideration that subclinical cases are not easily detected diagnostically despite that fact that they are capable of shedding Mptb.

Valid study results are those that are obtained from a study without bias. The major categories of bias are information, selection and confounding. Non-response bias, a type of selection bias, is of concern when questionnaire surveys are conducted. Non-response bias is observed when the population value for a measured association differs

between those who did respond and those who did not respond. It is possible that producers who did not respond to the survey had not encountered JD and found the specific questions about control challenging to answer. One producer refused to be interviewed by returning a survey with a note stating that she did not participate in surveys. The attitudes of veterinarians who frequently worked with large farms and those who worked with few, small farms might have been different. Also, although researchers were unaware of the identity of respondents, it is conceivable that some producers with JD-affected herds may not have responded because they wished to avoid being associated with the negative connotations of the disease. Veterinarians who no longer treated cattle also returned incomplete questionnaires and this is another probable reason for a proportion of the unreturned questionnaires. The power of the study is expected to be lower than that assumed in the sample size calculation because the actual sample sizes achieved were lower than required.

The list frame from which herds were selected for participation in this study consisted of herds that were previously reported to have 50 or more head of cattle. Consequently, there was a larger proportion of the herds in this study (95%) with >50 head of cattle compared to beef cattle herds in the US (23%) (USDA, 2007). In addition, the median size of respondent farms, 809 hectares, was greater than that of the average farm size for Texas, 228 hectares (USDA, 2007). As a result, the conclusions of this study may be less applicable to smaller herds. The disease control issues facing smaller

herds and the limitations to adopting control programs may be different from those facing larger herds. The participation of larger herds in this survey is important because Dutil et al. (1999) reported that cow-calf producers with larger herds (≥ 40 cows and or heifers) in Quebec adopted preventive measures more frequently than did producers with smaller herds.

Other limitations to findings include the cross-sectional design of this study and the potential for confounding of the measure of association. This study of producer and veterinarian attitudes is cross-sectional and may not measure attitudes or practices at the time that the JD problem developed because attitudes and practices can change over time. Confounding is another concern although it was not identified in the multivariable logistic regression analysis conducted for variables in the producer and veterinarian studies. Producers participating in this study were more likely to be commercial cow-calf and have larger herds. The responses of Texas producers and veterinarians in the current survey may not be applicable in localities in which there are more pronounced changes in management with seasons, for example, herds kept indoor during the winter.

3.5. Conclusions

More producers should be informed about the existence of JD control programs and of the appropriate methods for control of this disease. In addition, there should be increased communication between veterinarians and producers concerning the ability of producers to implement farm biosecurity strategies.

4. MARKOV CHAIN ANALYSIS OF JOHNE'S DISEASE CONTROL STRATEGIES IN BEEF CATTLE

4.1. Introduction

Veterinarians can use decision analysis techniques that incorporate producer benefits to determine which of several alternative disease control strategies is best for a herd. Markov chain (state transition) modeling, a decision analytic technique, has been used to compare control strategies for Johne's disease (JD) in cattle (Collins and Morgan, 1991; Humphry et al., 2006), and in sheep(Juste and Casal, 1993).

Johne's disease is a chronic enteritis of ruminants and wildlife caused by infection with the bacterium *Mycobacterium avium* subsp. *paratuberculosis* (Mptb) (Twort and Ingram, 1912). Herd-level seroprevalence of JD in beef cattle range from 8.6% (Braun et al., 1990) to 75.5% (Keller et al., 2004), and within-herd seroprevalence ranges from 0.4% (Dargatz et al., 2001b) to 25.2% (Alexander et al., 1993). Within-herd seroprevalence estimates for Texas Longhorns ranged from 0-12.9% (Osterstock et al., 2008a).

Fecal-oral is considered to be the most important route of transmission for JD (Sweeney, 1996). Mptb is shed in the feces of infected animals and can survive in the environment for prolonged periods (Lovell et al., 1944; Larsen et al., 1956; Whittington et al., 2004). The environmental load and survival of Mptb have been incorporated into deterministic and stochastic models of JD transmission (AusVet Animal Health Services, 2002; Humphry et al., 2006).

Most calves are infected within the first 6 months following birth (Larsen et al., 1975) and the incubation period for JD is highly variable, ranging from months to 2 or more years (Hagan, 1938). Most clinical cases are observed in animals 2 to 5 years old (Doyle, 1953; Chiodini et al., 1984) . In general, adult cattle can be infected by Mptb (Doyle, 1953) but are less likely to develop clinical disease than are cattle infected as calves (Hagan, 1938; Rankin, 1962; Larsen et al., 1975).

Control methods are recommended for JD because it is more practical to prevent infection than to treat this disease in domestic ruminants (Thoen, 1988). Indirect methods for testing, such as serum enzyme-linked immunosorbent assays (ELISA), detect evidence of an antibody response to Mptb exposure and can be used in a control program to identify Mptb infected herds. The sensitivity of serum ELISA is low in subclinical infection but higher in animals with clinical signs of disease (Dargatz et al., 2001a).

The objective of this study was to develop a deterministic, dynamic Markov chain (state transition) model to compare 6 control strategies for JD in beef cattle.

4.2. Methods

4.2.1. *General description of the beef herd*

The modelled beef herd was a US seedstock cow-calf operation with 150 head of adult cows. It was assumed that the herd size was constant and the rate of replacement for reasons other than JD was related to age (Greer, 1980). Except for the initial introduction of a single purchased infected animal, cows culled for reasons other than

paratuberculosis were replaced by calves grown on the farm but cows culled as a result of control programs were replaced by purchased heifers.

Only one control strategy is used for an entire timeline. Contact between dams and calves and among calves is assumed to be homogenous. The maximum age of a cow in the breeding herd was assumed to be 10 years. A zero culling rate was assumed for calves and heifers. Animals culled as a result of any control strategy were replaced with purchased heifers which entered the herd at first calving at the age of 2 years (Pouillot, 2004).

4.2.2. *Control strategies*

The 6 control strategies compared were Do nothing, Cull clinical suspects only, Test and cull every year, Test and cull every 2 years, Management only and Test with management. Johne's disease is assumed to enter the herd when an animal with subclinical infection is purchased. A probability is associated with introducing infected cattle purchased to replace those culled as a result of a control program. The Test and cull, Management only as well as the Test and cull with management strategies models were initiated the year following the appearance of the first clinical case in the Do nothing strategy.

No measures were employed to control the spread of JD in the Do nothing strategy. In the Cull clinical suspects only strategy, serum ELISA assays were conducted on clinical suspects and test-positive animals were culled. Seropositive cattle were culled after annual herd ELISA testing was conducted in one Test and cull strategy.

In the other Test and cull strategy, serum ELISA herd tests and subsequent culling of seropositive cattle were carried out once every two years. In the Management only strategy environmental contamination was decreased by 50% in the first year that the control program was initiated. Initial management changes were combined with herd ELISA assays and culling of seropositive animals for the test and cull with management strategy. Test positive animals were culled at the end of the year in which they were discovered for all strategies involving testing and culling. The serum ELISA was assumed to have a sensitivity of 0.290 (Collins et al., 2005) for cattle in the subclinical stage of JD and a specificity of 0.985 (Pitt et al., 2002).

Table 4.1 details model parameter values. Factors important for the transmission of Mptb via the environmental route include excretion rate in subclinical and clinical cattle, probability of Mptb surviving 1 year, dose of bacteria required to cause infection and the infectious area that susceptible cattle are exposed.

4.2.3. *Markov chain model*

Five states representing the major stages of JD were defined for the Markov chain model: disease free (susceptible as calves, not susceptible from the age of 2 years onwards), subclinical (infectious), clinical (infectious) and culled/dead. The disease-free (susceptible calves), disease-free (not susceptible adults), subclinical and clinical stages were recurrent states. Cattle in recurrent states could stay in the same state or move to another state with each ensuing cycle based on transition probabilities. The culled/dead state was an absorbing state from which the animal was not able to exit. The length of the Markov chain cycle was 12 months because test and cull in the current national

program in the US is carried out on an annual basis. The analysis was conducted for a 22-year period.

Figure 4.1 is a flow diagram of the assumed intra herd spread of JD. The Reed-Frost equation (Abbey, 1952) was used to calculate the number of cases (Collins and Morgan, 1992). The environment was assumed to be the major source of infection. The probability of adequate contact was defined as the likelihood during a time period that a susceptible animal will have sufficient contact with Mptb in the environment for disease transmission to occur (Martin et al., 1987). The Reed-Frost equation can be represented as follows:

$$C_{t+1} = SUSCEP_t(1-Q^{C_t}) \qquad (4.1)$$

SUSCEP is the number of disease-free susceptible cattle, less than 1 year old, and Q is the probability of not making adequate contact with Mptb in the environment during the time step. Control strategies were compared over a range of Q. The exponent of Q in the Reed-Frost equation, C_t, usually defined as the number of subclinical and clinical cases, was replaced with units of Mptb in the the environment(Humphry et al., 2006).

Table 4.1: Parameter values for Markov chain model of intra-herd spread of Johne's disease in beef cattle

	Parameters	Values	References
Subclinical to clinical (over 1 year)	Calf	0	
	Heifers	0	
	Cow	0.15-0.20	Whitlock and Buergelt (1996)
Cull/death (over 1 year)	Voluntary culling of heifer	0	
	Voluntary culling of cow	Appendix	Greer et al. (1980)
Environment	Loading from cow with clinical JD*	5×10^{12}	Chiodini et al. (1984) and Whittington et al. (2000)
	Loading from cow with subclinical JD*	2.5×10^{12}	assumed
	Probability of Mptb surviving for 1 year in the environment	1×10^{-5}	Schroen et al. (2000) and Whittington et al. (2004, 2005)
	Infectious dose	2×10^{9}	Hines II et al. (2007)

* Johne's disease

The total environmental contamination with Mptb from subclinical and clinical cases which survived the time period was used in the calculation of units of Mptb capable of causing infection. One unit of C was 2×10^9 colony forming units of Mptb, the quantity recommended by the Johne's Disease Integrated Project Animal Model Standardization Committee as sufficient to cause infection in susceptible calves experimental exposed via the oral route (Hines et al., 2007). The probability of adequate contact with Mptb in the environment over a specified time period, one year in this model, is the only factor driving transmission through successive time periods and its relationship to Q is represented in equation 4.2:

$$P_a = 1 - Q^{C_t} \tag{4.2}$$

Equations for the state transitions for calves (homegrown replacement calves from beginning of year 0 to end of year 1) are as follows:

$$SUSCEP_{t+1} = N - SUBCLIN_t \tag{4.3}$$

$$SUBCLIN_{t+1} = P_a * SUSCEP_t \tag{4.4}$$

SUSCEP refers to susceptible cattle, that is, those less than 1 year old. N is the number of calves retained to replace routine yearly culls. SUBCLIN refers to subclinical infectious cattle which were infected as calves.

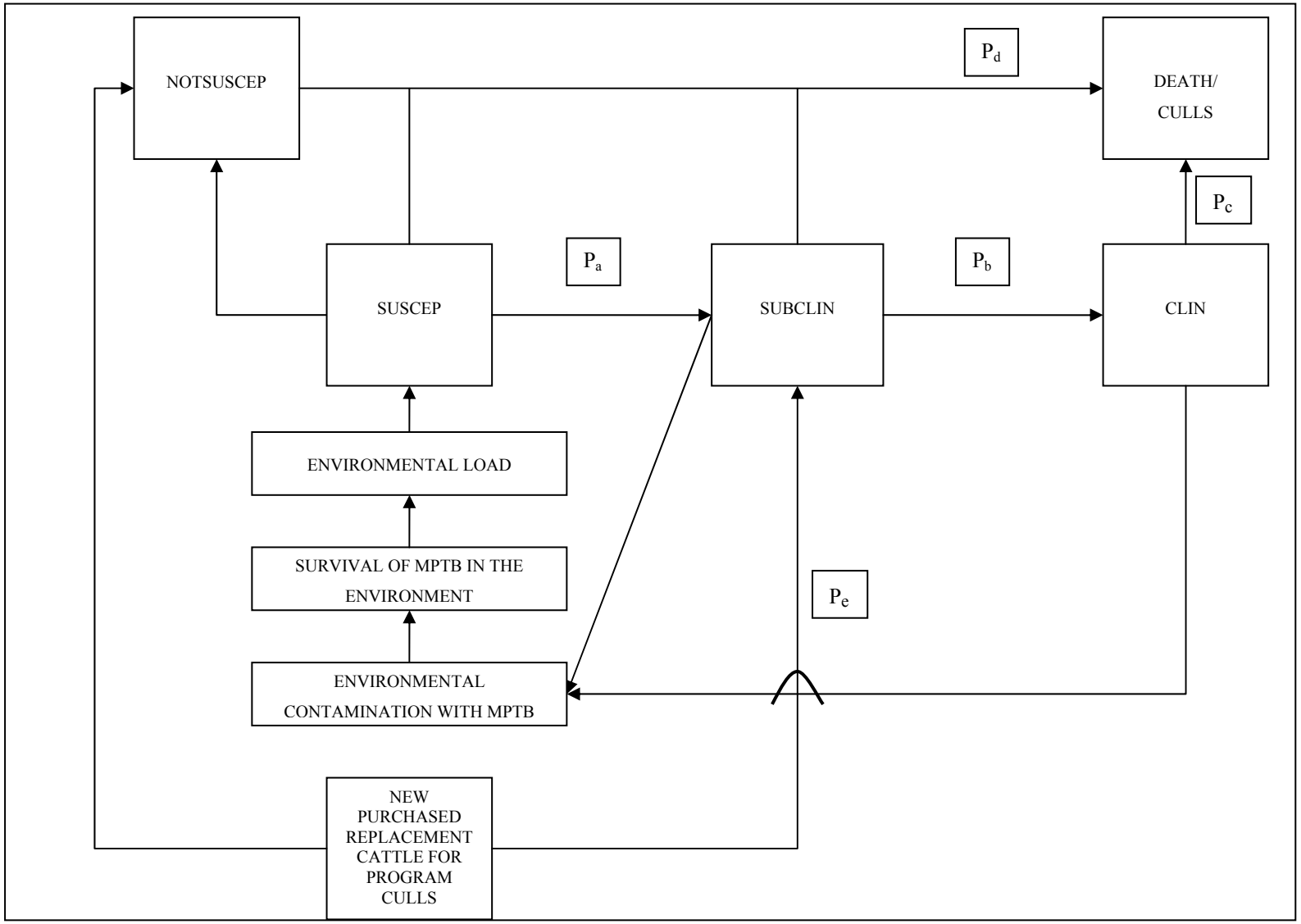

Figure 4.1: Flow diagram for Markov chain model of intra-herd spread of Johne's disease in beef cattle

JD dynamics for adult cattle from 2 to 10 years of age is as follows:

$$NOTSUSCEP_{t+1} = (1 - \text{Binomial}(n, p)) * NEW_{NOTSUSCEP,\,t} - P_d * NOTSUSCEP_t + NOTSUSCEP_t \quad (4.5)$$

$$SUBCLIN_{t+1} = (1 - \text{Binomial}(n, p)) * NEW_{SUBCLIN,\,t} - P_b * SUBCLIN_t - P_d * SUBCLIN_t + SUBCLIN_t \quad (4.6)$$

$$CLIN_{t+1} = P_b * SUBCLIN_t \quad (4.7)$$

where NOTSUSCEP is the state for disease-free cattle that are not susceptible to infection and are those previously SUSCEP cattle which become NOTSUSCEP when they reach the age of 2 years. New additions (heifers), $NEW_{NOTSUSCEP}$ and $NEW_{SUBCLIN}$, were purchased to compensate for reductions in the herd size from control program culling. The probability of introducing infected replacement cattle, $P_e = \text{Binomial}(n, p)$, is based on reported prevalence levels of JD in the beef cattle population from which the replacements originated and is assumed to be 0 in initial simulations of disease transmission in the hypothesized herd when no control measures have been introduced. CLIN refers to cattle with clinical disease. Clinically infected animals were assumed to be culled within the 1-year time step. The probability of cattle progressing from subclinical to clinical disease was P_b, the probability of cull/death for cattle with clinical

disease, P_c, was assumed to be 1 in this model and P_d was the probability of normal culls and deaths on the farm.

The number of homegrown replacement cattle is equivalent to the number of cattle normally culled yearly and is calculated as follows:

$$N_{t+1} = CULLED_{t+1} = P_d * (NOTSUSCEP_t + SUBCLIN_t + NEW_{NOTSUSCEP,\,t} + NEW_{SUBCLIN,\,t}) + P_c * (CLIN_t) \quad (4.8)$$

Transition probabilities in Tables 4.1 and Appendix D-1 represent the probabilities of moving from one state to another during the Markov cycle. Transition probabilities from the literature, where available, were used to develop a matrix of transition probabilities for each control strategy. Also the probability of transmission during contact, P_a, in the Reed-Frost equation was iteratively adjusted to generate prevalence levels of JD reported in the literature.

4.2.4. *Model implementation*

Heifers are assumed to calve at 2 years of age and each cow gives birth to one calf annually. The first calf is weaned when the first calf heifer is 31 – 34 months (Taylor and Field, 1999) so calves are assumed to be separated from their dams within the 1-year time step in which they are born. Replacement heifers, whether raised on the farm or purchased, enter the adult herd at the age of 24 months. Replacement cattle grown on the farm are added to maintain the herd at a constant size when cattle are lost

to death and routine culls. Home-grown replacement cattle may be in the SUSCEP or SUBCLIN states depending on disease transmission on the farm. Cattle with clinical JD were assumed to be culled and replaced within the one-year time step. Cattle culled based on modelled control programs were assumed to be replaced by purchased cattle.

The model was coded in an EXCEL spreadsheet as a variation of the modified Markov model described by Carpenter (1988).

4.2.5. *Sensitivity analysis*

Uncertainties in the epidemiology of Mptb include the incubation period of disease, the point at which infectiousness begins and the dose required to cause disease in cattle of different ages and breeds. A sensitivity analysis was conducted to investigate the effect of altering parameter values on simulated JD prevalence. Parameter values subjected to sensitivity analysis were diagnostic sensitivity and specificity of ELISA, the probability of introducing an infected calf to replace culled cattle, probability of Mptb surviving over the 1-year cycle and shedding level of Mptb.

ELISA sensitivity was assumed to range from 0.29 to 0.60 for cattle with subclinical infection (Ridge et al., 1991; Collins et al., 2005). ELISA specificity was assumed to range from 0.90 to 0.99 (Sweeney et al., 1995; Jakobsen et al., 2000). Within-herd prevalence was assumed to be 0.09%.

Herd prevalence was assumed to be 50% for purchased cattle. The purchase of replacement cattle with subclinical JD will reintroduce JD to the herd. The probability of introducing an infected calf to replace cattle culled in a control program was based on

the herd prevalence of JD beef herds in the population from which the replacement originated. Sampling was based on a binomial distribution, (1, p), where p is the herd prevalence of JD. All cattle to replace those culled as a result of a control program in any year were purchased from the same infected or uninfected herd. The number of infected replacement animals is determined by the within-herd prevalence of JD for infected herds.

The level of Mptb shed in feces was assumed to range from 2×10^7 to 5×10^{12} (Chiodini et al., 1984; Nelli et al., 2008) with a calculated median of 2.5×10^{12}. In the Management only as well as in the Test and cull with management strategy, the level of environmental contamination was reduced by 10, 25, 50, 75 and 90% in order to simulate the effect of the Management strategy.

The effect of control strategies on prevalence at two different values for the probability of Mptb surviving over the 1-year cycle, above and below the assumed value, were compared.

4.3.Results

Figure 4.2 summarizes the prevalence of subclinical JD for the simulations of 6 control strategies, Do nothing, Cull clinical cases only, Test and cull yearly, Test and cull once every two years, Management only and Test and cull with management over

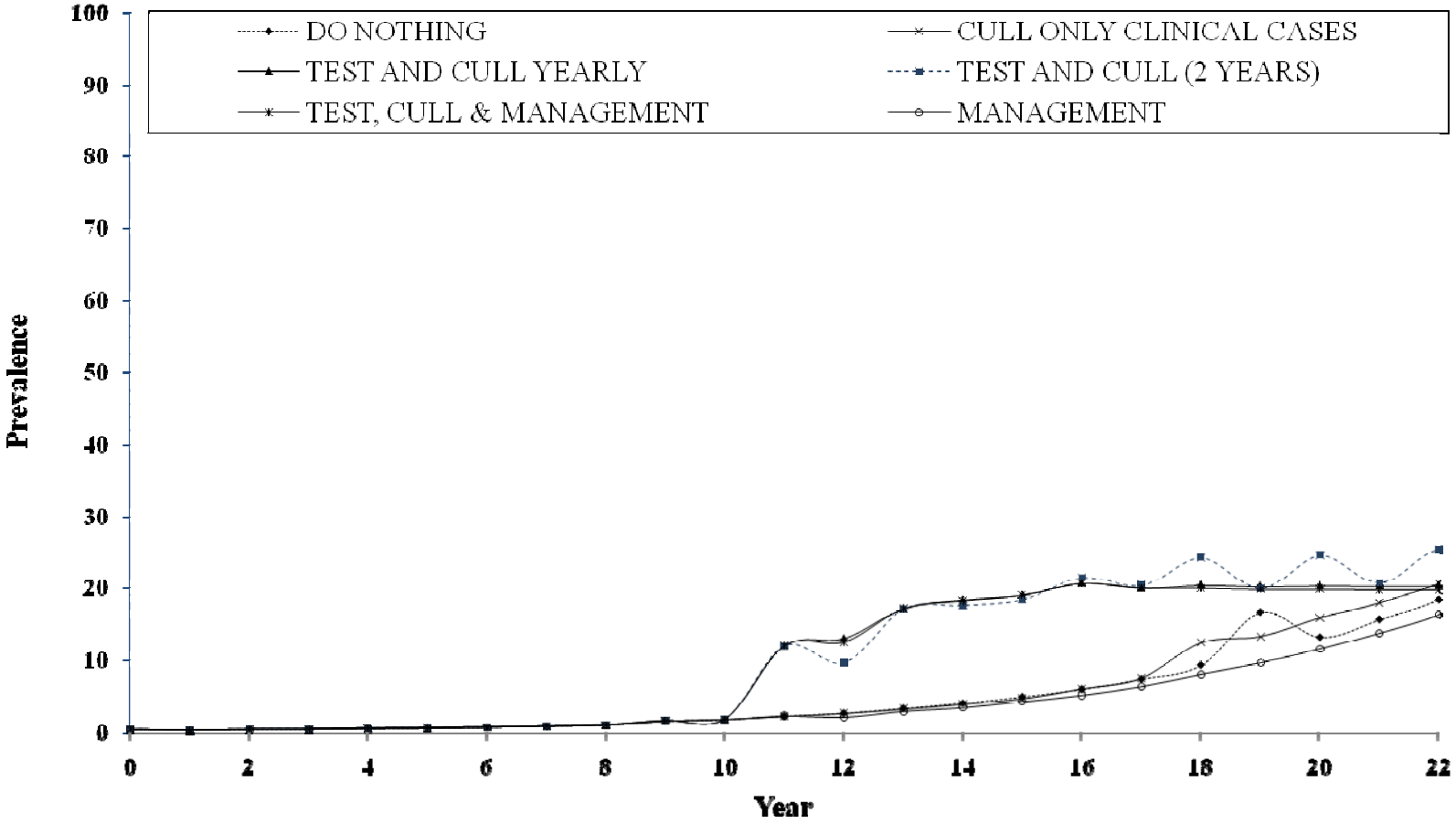

Figure 4.2: Prevalence of subclinical Johne's disease for 6 control strategies: Do nothing, Cull clinical cases only, Test and cull yearly, Test and cull once every two years, Management only and Test and cull with management over 15 years following the introduction of intervention strategies in year 7.

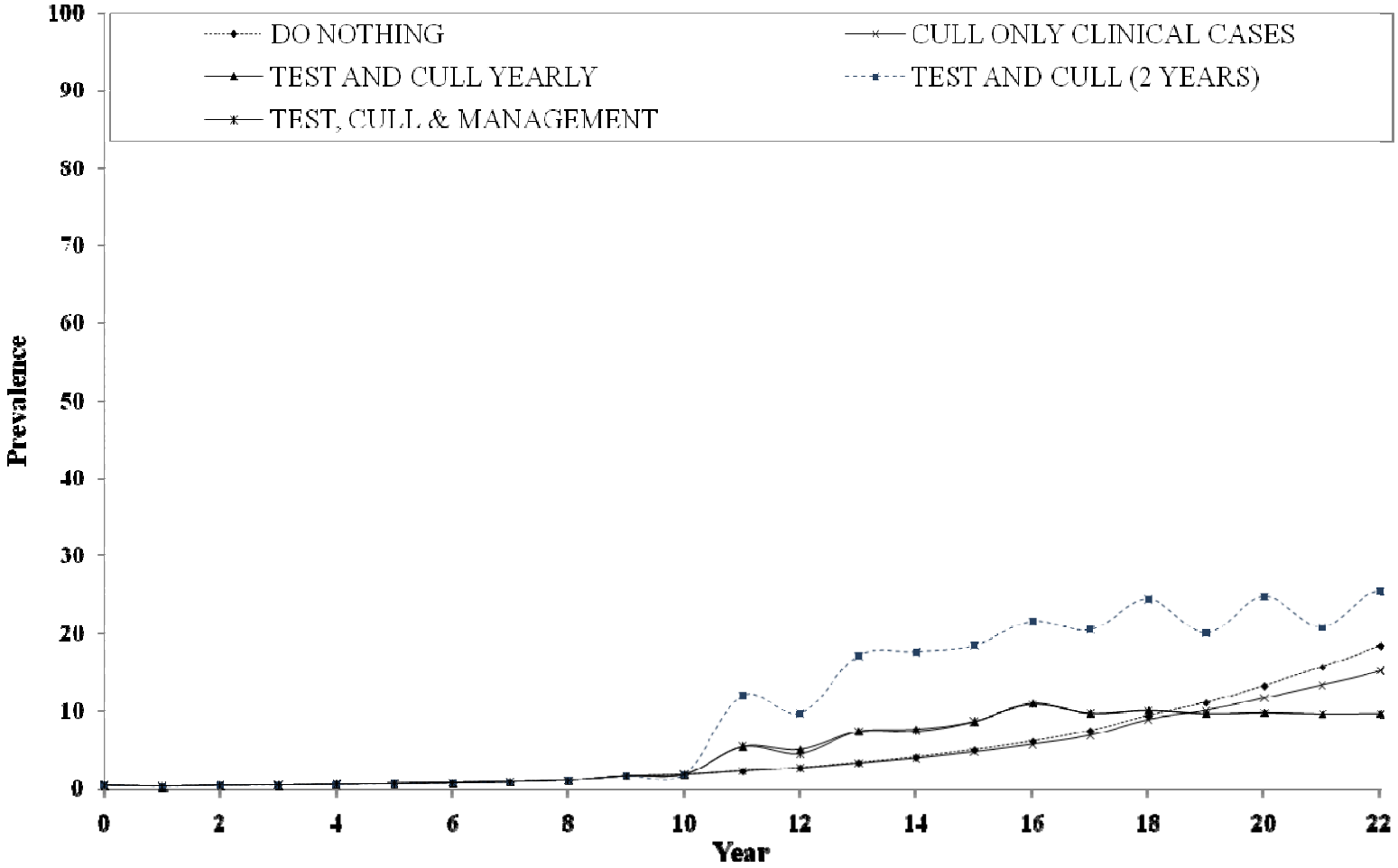

Figure 4.3. Prevalence of subclinical Johne's disease simulated by a deterministic, dynamic Markov chain model of a beef cattle herd with 150 head of cattle at for replacement cattle acquired from herds with Johne's disease herd prevalence of 12%.

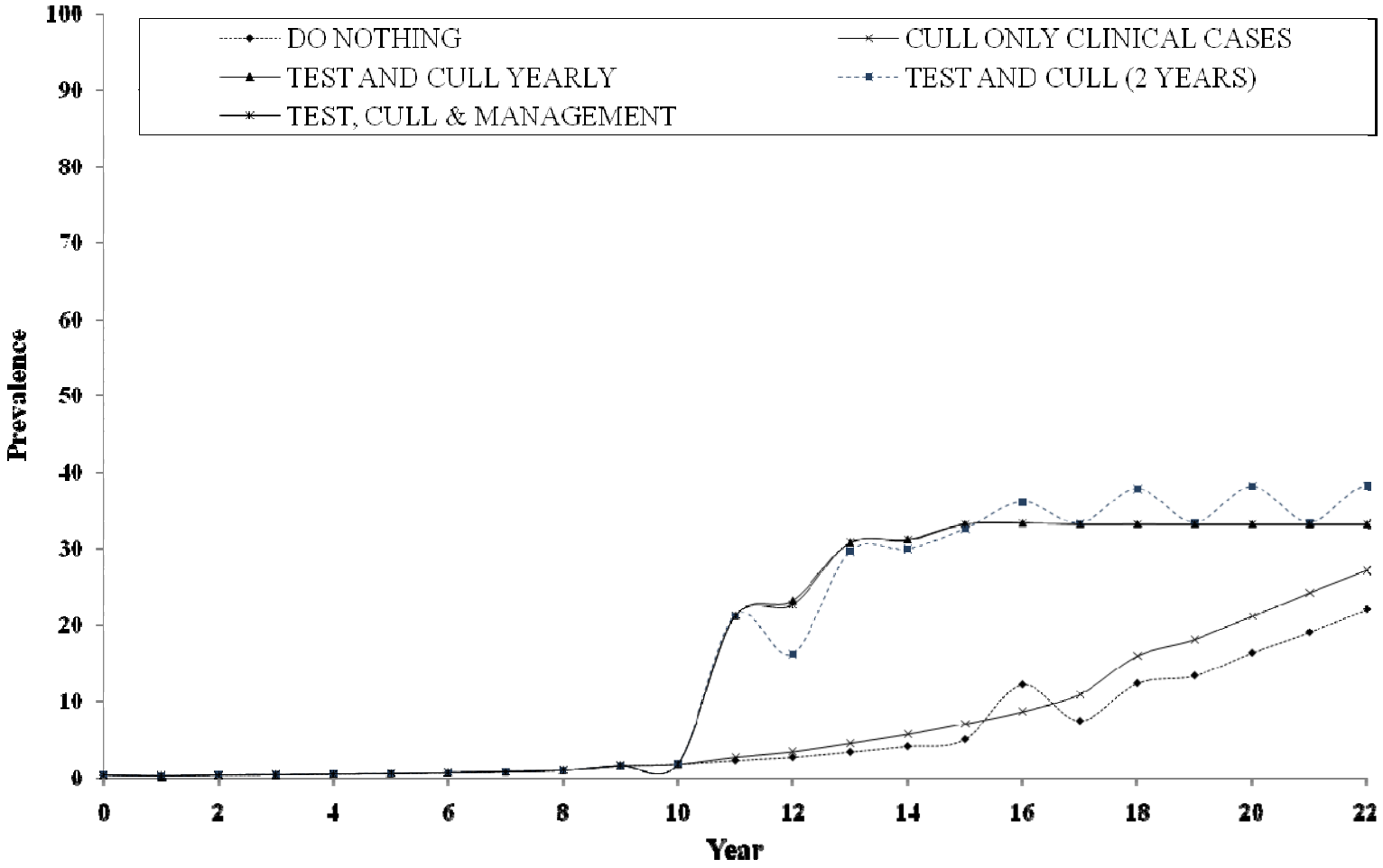

Figure 4.4. Prevalence of subclinical Johne's disease simulated by a deterministic, dynamic Markov chain model of a beef cattle herd with 150 head of cattle for replacement cattle acquired from herds with Johne's disease herd-level prevalence of 40%.

22 years. For all control strategies, interventions were introduced at year 7 when the first clinical case appears as a result of the initial introduction of an infected cow. At the selected probability of avoiding contact with units of Mptb in the environment, the outcome of the Test and cull strategy was similar to that of the Test and cull with management strategy. In addition, the prevalence of subclinical JD was 3 times more for the Do nothing strategy than for the Test and cull yearly and the Test and cull with management strategies. The highest prevalence reached was 16% for the Do nothing strategy in the 22^{nd} year. In Figures 4.3 and 4.4, the effect on prevalence of purchasing cattle from herds of unknown status and introducing them to the herd without initially testing for JD was to increase JD prevalence when the probability of introducing infected replacements was high, 90%.

Figures 4.5 and 4.6 are results from simulations of the control strategies using values of the quantity of Mptb shed daily in the feces of affected cattle that are 4×10^{12} and 6×10^{12}. The higher value of Mptb shed in the feces on a daily basis results in an approximate doubling of subclinical prevalence of JD under all control strategies.

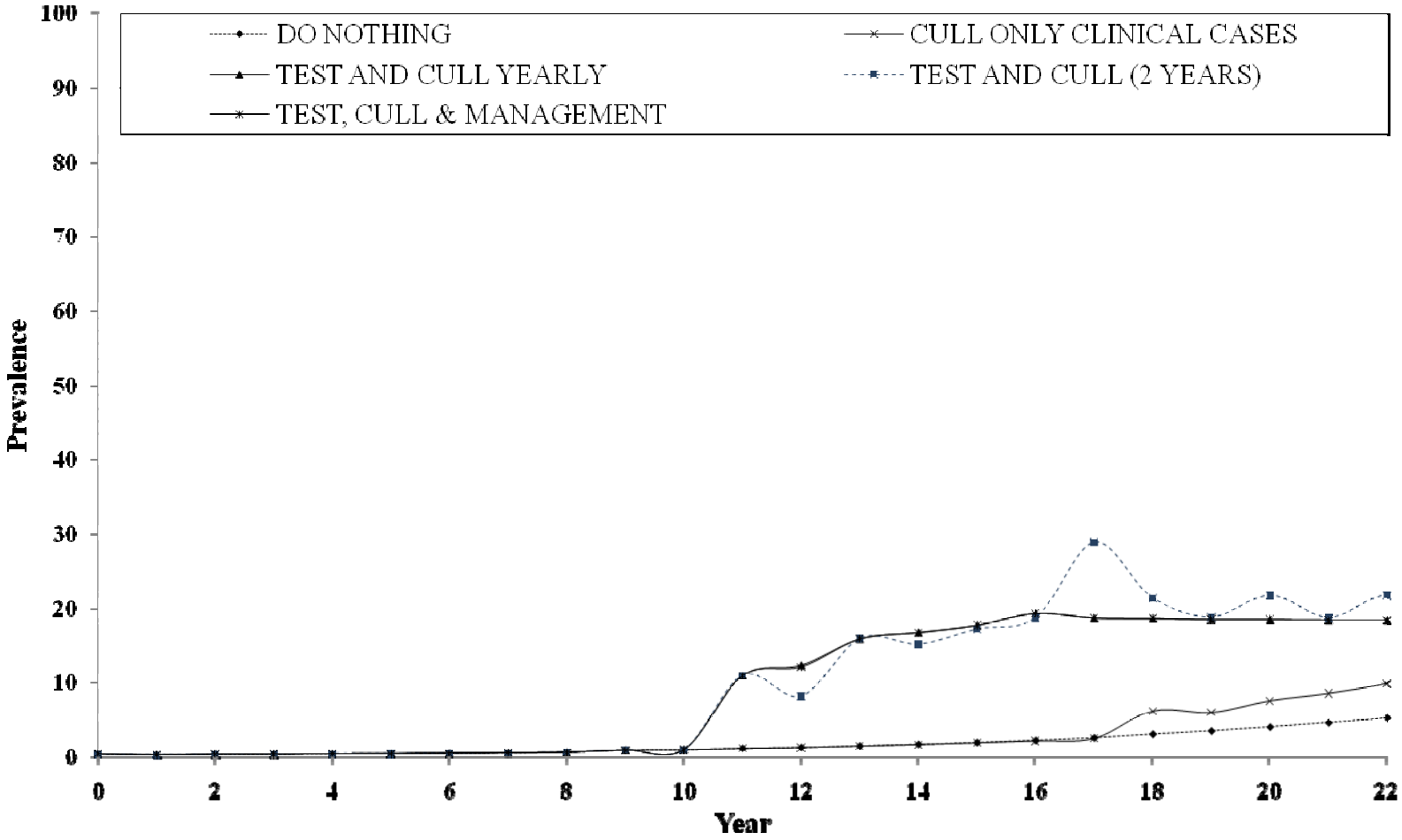

Figure 4.5. Prevalence of subclinical Johne's disease simulated by a deterministic, dynamic Markov chain model of a beef cattle herd with 150 head of cattle when the quantity of *Mycobacterium avium* subsp. *paratuberculosis* shed in the feces of clinically affected cattle was 4 x10^{12} per day.

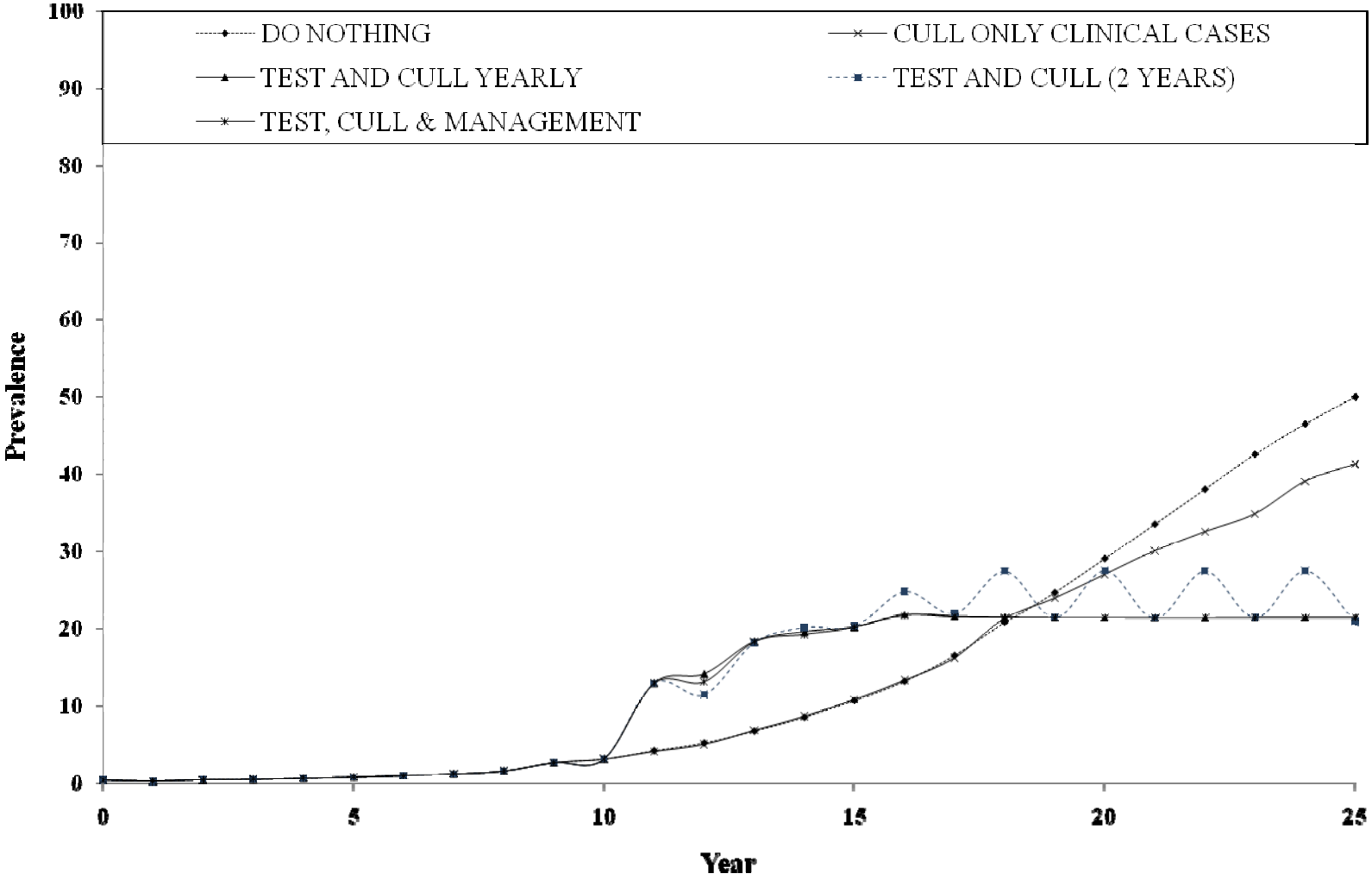

Figure 4.6. Prevalence of subclinical Johne's disease simulated by a deterministic, dynamic Markov chain model of a beef cattle herd with 150 head of cattle when the quantity of *Mycobacterium avium* subsp. *paratuberculosis* shed in the feces of clinically affected cattle was 6 $x10^{12}$ per day.

Outcomes of control herd subclinical prevalence were similar for the assumed range of sensitivity and specificity assessed for replacement cattle from a population having a herd prevalence of 50% and a within-herd prevalence of 0.09%.

The prevalence remained low for all levels of the Management strategy and the strategy which reduced the environmental load to 10% each year caused the lowest prevalence of subclinical infections over the 15 years of the analysis (Figure 4.7).

The assumed probability of Mptb surviving over a 1-year period had a substantial effect on the prevalence of subclinical JD. For the Do nothing strategy, with an initial annual probability of Mptb surviving of 1 $x10^{-5}$ the prevalence increased to 16% in the final year (Figure 4.2). If a probability of surviving 1 year of 1 x 10^{-6} is assumed, the prevalence was 0% for all 4 control strategies. In contrast, when probability of surviving 1 year was assumed to be 1 x 10^{-3} (Figure 4.8), prevalence ranged from 24 to 67% for all control strategies.

4.4. Discussion

Markov chain models can be helpful for the elucidation of the epidemiology of diseases. Probabilities in a deterministic model are single fixed values. In a dynamic

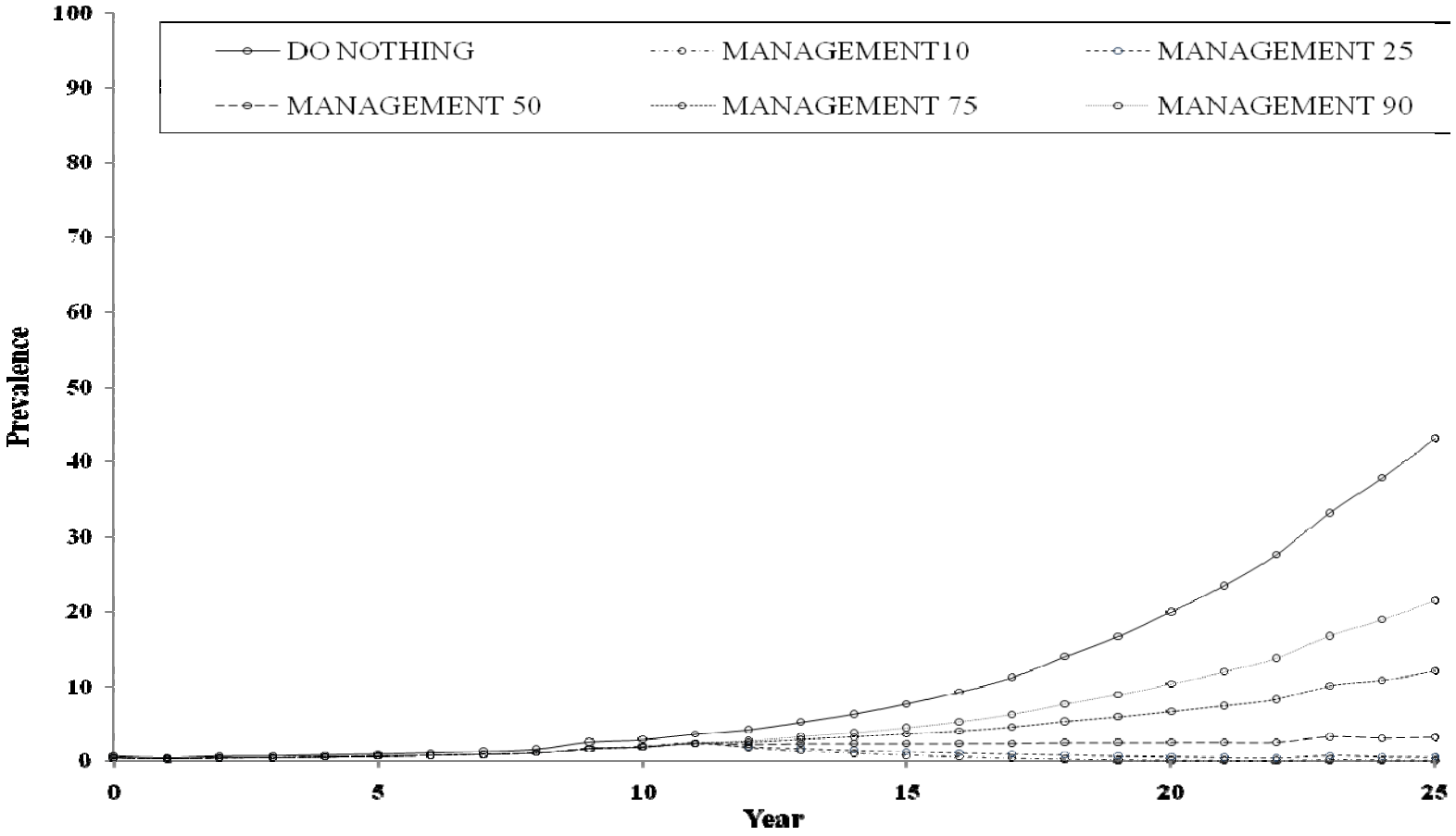

Figure 4.7. Prevalence of subclinical Johne's disease simulated by a deterministic, dynamic Markov chain model of a beef cattle herd with 150 head of cattle at different management levels (90%, 75%, 50%, 25% and 10%).

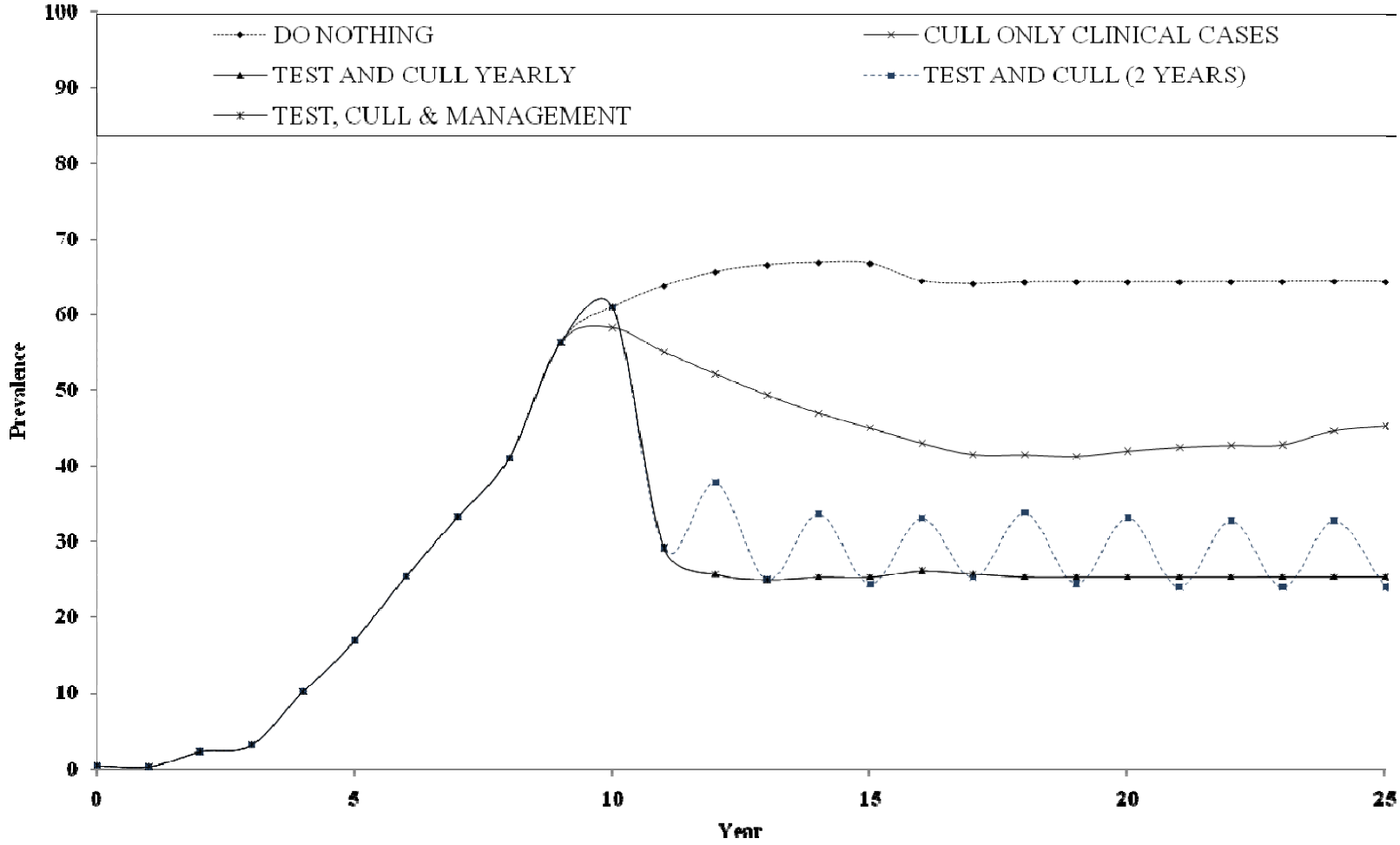

Figure 4.8. Prevalence of subclinical Johne's disease simulated by a deterministic, dynamic Markov chain model of a beef cattle herd with 150 head of cattle when the probability of Mptb survival over 1 year is 10^{-3}.

model the probabilities can be recalculated for each cycle. By determining the parameters to which the model is sensitive, the factors important to the control of disease can be identified for further study. In addition, the need to quantify parameters can reveal uncertainty in the estimates of parameters and indicate the need for research into related areas.

The attempt to explain differences between model output and observed outcomes can be beneficial to the epidemiologist because this can lead to a better understanding of the important parameters for the transmission of disease. Most other Markov chain analyses for JD have been conducted for dairy cattle (Walker et al., 1988; Collins and Morgan, 1992). One exception is a study conducted by Humphry et al. (2006) that employed a deterministic model specifically focused on the environmental route of transmission of Mptb in a Scottish beef suckler herd and explored, via sensitivity analysis, the impact of different levels of environmental contamination with Mptb on the success of different control measures. Similarly, the present model uses the environment as the major route of transmission. Rather than being predictive, this model is intended to gain a better understanding of the effect of control strategies for JD on beef farms.

A time step, cycle length, of 1 year was selected for this analysis because the relatively long period between infection with Mptb and the appearance of clinical signs of JD make it more practical for the time step to be on a scale of months or years. In addition, this 1-year period was convenient for analyzing the impact of the annual test and cull program strategy. The assumption that cattle died within the 1-year cycle after entering the clinical state is supported by the observation that within 3 to 4 months after

the appearance of clinical signs there is progression to advanced disease, characterized by profuse diarrhea and hypoproteinemia, and subsequent death within days in most cattle (Sweeney, 1996).

The time taken for the impact of the control program on the prevalence of disease in the herd to be observed is important because program participants are likely to become discouraged when the effect of the program is not apparent within a reasonable period of time. A time horizon of 15 years was selected for this study out of consideration for the desire of the producer to observe the impact of the control program within a period of time that is practical and the length of the incubation period of JD which can range from a few months to 14 years (Sweeney, 1996). However, an incubation period of 2 years was modeled in this study.

The development of infection and progression to clinical disease is highly dependent on cell-mediated response to infection. Differences may be observed in response to challenge with Mptb according to age and breed of cattle (Chandler, 1961; Rankin, 1962; Larsen et al., 1975; Elzo et al., 2006). From experimental studies it is believed that the dose of Mptb ingested is related to the incubation period, that is, higher doses are associated with shorter incubation periods (Hagan, 1938; Rankin, 1962).

In the present model, the R_0 remained less than 1 only when the control strategies were compared at the lowest survival of Mptb over the 1-year period. The model was sensitive to the probability of survival of Mptb over the 1-year cycle as prevalence increased to the highest levels for all control strategies, ranging from 24 to 67%. This suggests that the potential of the environment to support the survival of Mptb can be

important in explaining differences in prevalence of Mptb observed between farms with predictors of Mptb survival and those without.

Survival of Mptb is likely to be highly variable, depending on environmental predictors (Schroen et al., 2000; Whittington et al., 2004) and the location at which the contaminated fecal pat was deposited. Several factors, such as landscape, rest areas and water source locations, herd density and period of grazing (Peterson and Gerrish, 1995) will determine where feces are deposited on farms and the consequent exposure to Mptb contaminated feces. Mptb has been identified in the environment of beef herds around feeders and in cow-calf bonding-pens (Campbell et al., 2007). In addition, Shulaw (2008) cultured Mptb from the udders of cows on JD-affected farms. For farms participating in the National Johne's Disease Demonstration Herd Project in 2005, areas with preweaned calves on beef farms had the 2nd highest total risk scores (USDA, 2005b). These areas with susceptible calves can be targeted for reduction of environmental load of Mptb. Also, since the probability of Mptb surviving 1 year in the environment has a dramatic effect on the prevalence of subclinical disease in the herd, predictors which are associated with lower survival of Mptb (such as soil moisture and temperature) are likely to be important in determining the need for a control program.

Elevating the daily Mptb excreted in the feces by 20% above the upper level of the range derived from the literature also led to elevated prevalence, ranging from 4 to 16% for all evaluated control strategies. However, a lower range of prevalence for all control strategies was observed when the higher value was used for the Mptb excreted daily than was for a higher probability of survival. The increase in prevalence associated

with higher daily excreted Mptb can be explained by the presence of more units of Mptb for susceptible cattle to contact. An additional consideration related to the quantity of Mptb that is present in the environment is that the length of the latent period may be shorter when susceptible cattle are exposed to higher doses of Mptb (Hagan, 1938; Rankin, 1962) and this may lead to higher disease incidence. No association was assumed between the quantity of Mptb in the environment and the length of the latent period in the current model. A less pronounced effect of higher daily excretion on prevalence was observed because more Mptb was available to susceptible cattle within the cycle but the quantity carried over from one cycle to the next because of the higher survival probability.

Assuming that cattle culled as a result of a control program are replaced by disease-free cattle is a simplification of the real life situation where there will be a probability of introducing Mptb-infected cattle that can increase the environmental load of Mptb and subsequent exposure of susceptible home-grown calves. The probability of introducing infected replacement cattle needs to be considered because the NJDDHP (2005b) identified the purchase of infected animals as the major risk of introducing disease to beef ranches. In the present study, most control strategies were unable to prevent the subclinical cases due, in part, to the continuous re-introduction of JD subclinical cattle from the replacement herds in the general cattle population. The prevalence of JD in beef cattle is considered to be lower than in dairy cattle (Thoen, 1988), however, it will be beneficial to define a prevalence at which aggressive action

needs to be taken to prevent subclinical infection from rising above a critical prevalence level.

According to the NAHMS (1997a), 11.7% of replacement heifers and 24% of cows on cow-calf operations were purchased rather than home-grown, suggesting that there is a potential for replacement cattle to introduce JD to cow-calf operations in the US. In a survey of producers at Level 4 of the VBJDCP. (the lowest probability of infection) 35% reported that they had to close their herds or restrict purchases when they enrolled in the control program (Benjamin et al., 2009b). As a result, JD control is facilitated by positive attitudes of producers towards control program suggestions regarding the acquisition of replacement cattle as evidenced in the report that 98% (162/166) of beef producers in a recent survey agreed that getting replacements or additions from low-risk herds was useful for control of JD (Benjamin et al., 2009a). Any obstacles to the translation of this positive producer attitude into action should be identified and explored where they exist.

Husbandry changes and management may be used to decrease the probability of adequate contact (Martin et al., 1987). Using a stochastic simulation model, "JohneSSim," to compare control strategies for JD, Groenendaal et al. (2003) concluded that none of the evaluated strategies reduced the prevalence to 0 and that only the strategies based on separating calves and adults were able to significantly reduce disease prevalence. This finding presents a challenge because strategies based on separating calves and adult cows prior to weaning are not generally considered to be practical.

Different control strategies require different commitments of time, labor and financial resources. Researchers have identified the need to take into consideration the relevant general attitudes, risk attitudes and goals of the producer when determining which control option is optimal(Cher et al., 1997; Gunn et al., 2004; Humphry et al., 2005). Using a decision tree analysis of JD control costs and a Reed-Frost model to estimate the number of infected dairy cattle raised on the farm, Dorshorst et al. (2006) showed that the cost-effectiveness of improved herd hygiene can be greater than testing. In a survey of attitudes towards biosecurity practices, producers and veterinarians differed on their agreement with test and cull as the only means of controlling JD in a herd: 59% of beef producers agreeing, compared with only 35% of veterinarians agreeing. In addition, 94% of beef producers in the previously mentioned survey agreed that management changes were helpful for the control of JD (Benjamin et al., 2009a).

A major difference between the models reported in the literature (Collins and Morgan, 1992), primarily dairy, and the present one is that the contact rate in the models refer to contact between animals in dairy the herd. In these models there is the implication that the environment is a factor contributing to the success of the contact. However, in the present model, the contact rate refers explicitly to contact between the susceptible animal and Mptb that is present in the environment. All surviving Mptb were assumed to contribute towards the formation of units that could lead to infection in susceptible cattle. This is a simplification since it is expected that accessibility of susceptible cattle to bacteria on the farm will, in part, be dependent on the spatial distribution of Mptb on the farm. As a result, the assumption that the entire quantity of

Mptb deposited in the environment is accessible to susceptible calves represents the worst-case scenario.

Uncertain parameters used in this model included survival of Mptb in the environment and the contact rate of susceptible calves with Mptb. The susceptibility of calves was assumed to remain constant throughout the 1-year period. It has been proposed that cattle infected with Mptb when they are calves are more likely to progress to clinical disease than are those infected as adults (Hagan, 1938; Larsen et al., 1975) and experimental evidence suggests that when calves and adults are exposed to Mptb in the same environment, calves are more likely than adults to progress to clinical JD (Rankin, 1962).

It was assumed that cattle below the age of 2 years do not shed bacteria. However, fecal shedding of Mptb has been observed in cattle less than 2 years of age especially in heavily contaminated environments (van Roermund et al., 2002). Also, in the present study it was assumed that adult cattle are not susceptible to infection with

Mptb; however, it is known that adult cattle can be infected though progression to clinical disease is less often observed than infected calves in the same environment (Hagan, 1938).

The utility of this analysis relies on the closeness of the model to reality and the selection of appropriate parameter values (Taylor, 2003). A model is a simplified version of events which can be used to gain a better understanding of the important factors involved in the epidemiology of JD on a beef farm. Validation and appropriate alteration of parameter values used in this model are required. The model output can be compared with field data in the validation stage. Departures between model predictions and observed data may indicate the need to alter the model to better reflect the epidemiology of JD (Taylor, 2003). As a result, a better understanding of the disease transmission dynamics can be gained and more accurate predictions made about the impact of control strategies on the incidence and prevalence of JD. All parameters in the present study were point estimates. In the future, a stochastic model for JD transmission could be developed to reflect the uncertainty in parameter values by using ranges of

probability rather than single probability values. The stochastic model may better imitate the range of outcomes of JD on beef farms (Groenendaal et al., 2002; Groenendaal et al., 2003). Also, the interherd transmission of JD can be modeled to identify target areas for the control program. An advantage of Markov analysis for a chronic disease such as JD is that control strategies can be compared in a few minutes as opposed to the several years over which the progression of the disease will have to be followed in herds before the effect of the control program becomes apparent.

4.5. Conclusions

Increasing the probability of survival of Mptb in the environment over 1 year led to the most dramatic increase in the prevalence of subclinical JD over the period of the analysis. The quantity of Mptb excreted daily by cattle was another factor important for the prevalence of subclinical JD in this study. In addition, the herd prevalence of the population from which replacement cattle were purchased was important in determining the success of control programs in reducing the prevalence of subclinical JD in the modelled herd.

5. EFFECT OF TANGENTIAL FLOW FILTRATION ON THE SENSITIVITY OF NON-RADIOMETRIC MGIT CULTURE AND PCR FOR *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* IN POND WATER

5.1. Introduction

Mycobacterium avium subsp. *paratuberculosis* (Mptb), the etiologic agent for Johne's disease in ruminants (Twort and Ingram, 1912), has been reported to survive in water for long periods of time after being shed in the feces of some infected animals. There is experimental evidence for the prolonged survival of Mptb under different conditions. Larsen et al. (1956) recovered viable Mptb from tap water at 38ºC for up to 17-19 months. Positive Mptb samples were observed at day 407 and but not at days 632 or 841 from Mptb spiked sterile lake water cultured in mycobacterial growth indicator tube (MGIT) liquid medium (Pickup et al., 2005). Further evidence was provided by Lovell et al. (1944) who reported that Mptb survived for up 9 months in sterilized pond water at room temperature. Whittington et al. (2003) demonstrated the presence of Mptb in the environment from water and sediment of a dam on 1 of 6 Australian farms with Mptb infected sheep.

Environmental samples can be assayed for Mptb using MGIT culture and quantitative real-time polymerase chain reaction (QRT-PCR). The sensitivity of MGIT culture for environmental samples is affected by the concentration and distribution of Mptb in the soil. The time to detection in MGIT culture is quantitatively associated with

the concentration of Mptb in the inoculum, that is, growth in samples with higher concentrations of Mptb is detected sooner than in samples with lower concentrations. PCR can be inhibited by substances in the environment such as humic acids, magnesium ions, iron and potassium chloride causing false-negative results (Tsai and Olson, 1992; McPherson and Moller, 2006).

A major advantage of tangential flow filtration (TFF) is its ability to filter and concentrate microscopic organisms from large volumes of water (Giovannoni et al., 1990; Pickup et al., 1999). Particles retained in the TFF unit by a back pressure based on the pore size of the filter are flushed into a container when the back pressure in the TFF unit is released (Giovannoni et al., 1990; Pickup et al., 1999). This concentration of Mptb by filtration is important because the number of bacteria may be low and Mptb, like other mycobacteria may be unevenly distributed in the water as a result of clumping (Stinear et al., 2004b).

Pickup et al. (2005) successfully isolated Mptb from samples that were collected from the River Taff (UK) using TFF but, the effect of TFF on the sensitivities of MGIT culture and PCR for Mptb is unknown. The objectives of this study were to determine if tangential flow filtration increases the analytic sensitivity of MGIT culture and PCR assays for to detect Mptb in pond water.

5.2. Materials and methods

5.2.1. *Sample collection*

Pond water was collected in 20 L carboys (plastic containers) from a Mptb-free horse farm and autoclaved at 121°C for 30 minutes. Ninety milliliter samples of the autoclaved water were shipped on ice to the Johne's Testing Center, Wisconsin, for MGIT and to the Texas Veterinary Medical Diagnostic Laboratory (TVMDL) for real-time quantitative PCR.

Farm 1 was located in east Texas. The selected pasture was 25 acres and contained 14-15 heifers and a bull. Farm 2 was located in south-central Texas. The selected pasture was a 5 acre isolation pasture with a single pond. Farm 3 was located in central Texas. The selected pasture consisted of 150 acres and contained a single pond. There had been at least 1 suspected Mptb-infected animal on each sampled farm.

Twenty liters of pond water from farms 1 to 3 were collected in carboys and transported to the laboratory for tangential flow filtration. The pre- and post filtration samples were then transported to TVMDL for quantitative real-time PCR as previously described (Scott et al., 2007a). In addition, 50 ml water samples were shipped on ice to The Johne's Testing Center, Wisconsin for MGIT. Samples were recorded as Mptb positive or negative and tabulated.

5.2.2.*Experimental design*

Feces were collected from a serologically positive cow with clinical signs of JD. Duplicate fecal samples were shipped to the Johne's Testing Center, Wisconsin, for MGIT culture and the remaining feces were frozen at -80°C.

The aim of experiment 1 was to compare the analytic sensitivities of pre-filtration and post-filtration samples of Mptb spiked pond water using (1) fecal MGIT culture and, (2) direct PCR; The aim of experiment 2 was to compare the analytic sensitivities of pre-filtration and post-filtration samples of Mptb spiked pond water, to which Tween 80 to reduce clumping has been added, using only direct PCR; The aim of experiment 3 was to compare the analytic sensitivities of pre-filtration and post-filtration samples of Mptb spiked pond water using (1) fecal MGIT culture and, (2) direct PCR.

For experiment 1, 160g of feces were added to 20 L of autoclaved pond water. The mixture was slowly agitated for 5 minutes. Ten liters of the mixture were poured into a second empty autoclaved 20 L carboy. The first carboy with the 10 L of original mixture was stored at -4°C. Five hundred milliliters of sample was removed from the second carboy and the remaining 9.5 L were filtered using tangential flow filtration (Figure 7). In tangential flow filtration, the 9.5 L mixture formed the sample reservoir which was pumped (Masterflex I/P 77410-10, Cole-Parmer Instrument Co.) through a 5 μm pre-filter to remove the larger contaminants and to avoid fouling the subsequent 0.22 μm spiral filter. Initially, 1 L of permeate was collected in a sterile Nalgene bottle. Thereafter, the permeate or water filtered through the membrane was discarded. When the sample reservoir was empty, both hoses were placed in the retentate reservoir and the

pump was run until the retentate reservoir was reduced concentrating the sample. The permeate valve was closed and the 1 L of permeate collected at the beginning of the procedure was used to flush the retentate out of the TFF unit into a sterile Nalgene bottle. Duplicate 90 ml samples removed from the 0.5 L mixture stored at the beginning of the procedure (pre-filtration sample) and duplicate 90 ml samples removed from the flushed out retentate (post-filtration sample) were transported to the TVMDL for QRT-PCR. In a similar manner, duplicate 60 ml pre- and post-filtration samples were collected and shipped to the Johne's Testing Center, Wisconsin for MGIT culture. At the TVMDL 30 ml of the water sample were centrifuged at 3000 rpm. The supernatant was discarded and the pellet re-suspended in 2 ml of PBS. Samples were assayed using QRT-PCR as described by Scott et al. (2007a).

Doubling dilutions of the feces/pond water mixtures were then concentrated by TFF. The spiral filter was cleaned between successive dilutions with 4 L NaOH, 1 L 0.01 M sodium dodecyl, 2 L 10% ethanol and washed with 9 L autoclaved deionised water.

In experiment 2, 8 L of autoclaved pond water were mixed with 2 L of pond water spiked with 10g of Mptb contaminated feces. This initial mixture was diluted 4 times. A 0.5 L sample was collected before and a 9.5 L sample was collected after filtration for each dilution. Tween-80 at a concentration of 0.4 ml/100 ml was added to the mixture to reduce bacterial clumping. Samples were processed and transported to the TVMDL for QRT-PCR as described previously.

In experiment 3, 8 L of autoclaved pond water were mixed with 2 L of pond water spiked with 10g of Mptb contaminated feces. This initial mixture was diluted 4 times. A 0.5 L sample was collected before and a 9.5 L sample was collected after filtration for each dilution. The mixture was filtered and samples processed for MGIT culture and PCR as described previously.

5.3. Results

All water samples collected from farms were negative for Mptb by MGIT culture and QRT-PCR. Duplicate fecal samples from a serologically positive cow were positive for Mptb by MGIT culture.

The initial sample of pond water was negative for Mptb by MGIT culture. Results for MGIT and QRT-PCR in experiment 1 are detailed in Table 5.1. The initial sample of pond water was negative for Mptb by MGIT. QRT-PCRPCR results for experiment 2 are detailed in Table 5.2. Contamination of MGIT culture samples was reported as bacterial contamination. The initial sample of pond water was negative for Mptb by MGIT. Results for MGIT and QRT-PCR were all negative. In the present study, the time to detection for pre-filtration samples ranged from 2 to 5 weeks. Post-filtration samples were expected to contain higher concentrations of Mptb relative to the respective pre-filtration samples. The time to detection of bacterial growth for post-filtration samples ranged from 2 to 4 weeks.

Table 5.1. Culture and PCR results for Experiment 1 in a study of the effect of tangential flow filtration on the analytic sensitivity of non-radiometric MGIT culture and PCR for *Mycobacterium avium* subsp. *paratuberculosis* in pond water.

		MGIT			PCR		
Pre/Post	Dilution	Positive	Negative	Contaminated	Positive	Negative	Contaminated
Pre	1	2			2		
	2	1	1		2		
	3	2			2		
	4	2			2		
	5	2			2		
	6		1	1	1	1	
	7	2			1	1	
	8	1	1			2	
	9	1	1			2	
Post	1	2			2		
	2	2			2		
	3	2			2		
	4	2			2		
	5	1		1	2		
	6	2		2	2		
	7			2	1	1	
	8			2	1	1	
	9	1		1		2	

Table 5.2. PCR results for Experiment 2 in a study of the effect of tangential flow filtration on the analytic sensitivity of non-radiometric MGIT culture and PCR for *Mycobacterium avium* subsp. *paratuberculosis* in pond water.

		PCR		
Pre/Post	Dilution*	Positive	Negative	Contaminated
Pre	5	2		
	6	2		
	7	1	1	
	8	1	1	
Post	5	2		
	6	2		
	7	2		
	8	1	1	

*Dilutions 1 to 4 were not tested

5.4. Discussion

Environmental sampling is a cost-effective method of herd screening that may be used prior to culture of individual or pooled fecal samples. Levels of Mptb in the environment could be high enough for environmental screening to be feasible and may be more appropriate on intensively managed farms (Collins et al., 2006). Mptb was not detected in samples collected from ponds on sampled farms. It was possible that these ponds were not contaminated or that Mptb was present at levels too low for detection.

Analytic sensitivity refers to the lowest concentration of the target substance that can be detected by the test. Increasing the analytical sensitivity of MGIT culture and PCR will improve the ability to determine whether or not the farm environment is contaminated with Mptb.

Pre-filtration samples were dilutions of the initial mixture of Mptb contaminated feces and 20 L of water and post-filtration samples were TFF concentrations of these diluted samples. All post-filtration samples were expected to be positive if the pre-filtration sample were positive since post-filtration samples should contain higher quantities of Mptb per unit volume than the corresponding pre-filtration sample. In experiment 1, pre-filtration MGIT cultures for dilution numbers 1 to 5 were all positive, with the exception of 1 sample in dilution number 2. Also, both MGIT cultures for dilution number 7 were positive. At least one sample was MGIT culture negative for test numbers 6, 8 and 9. Similarly, 1 of the 2 QRT-PCR tests was negative for dilution numbers 6 and 7 in experiment 1. This suggests that if TFF is to increase the analytic sensitivity of PCR, it will be more useful at these higher dilutions. However, post-

filtration results were variable for dilution numbers 6 to 8. The analytic sensitivity of MGIT culture appeared higher than QRT-PCR because for dilution numbers 8 and 9 in experiment 1 at least one pre-filtration MGIT culture sample was positive but both samples were negative by QRT-PCR negative.

A sample with longer time to detection of growth is expected to have a relatively lower concentration of bacteria in the inoculum than one with a shorter time to detection of at the pre-determined growth index.

At dilution number 6, bacterial contamination was observed in the pre-filtration samples. For dilution numbers 6 to 9, at least 1 post-filtration MGIT culture sample was contaminated, suggesting that contaminants were concentrated by TFF. The effect of TFF on MGIT culture at the lowest concentrations of Mptb, dilution numbers 6 to 9, could not be interpreted because of this contamination. Contamination of Mptb culture samples, as observed in experiment 1, is considered to be a limitation of fecal culture techniques, especially in environmental samples where the relatively slow growing Mptb may be overgrown by the presence of other bacteria and fungi (Turcotte et al., 1986).

The direct detection of DNA from Mptb combined with PCR for amplification of DNA sequences is an alternative to MGIT culture. An advantage of PCR over MGIT culture with respect to decision-making is the shorter time period required for return of results (average 3 days) versus (average 8 weeks) for culture. However, MGIT culture and PCR results are not expected to completely agree because live bacteria are required for culture whereas DNA of either dead or living cells can yield positive PCR results. Positive MGIT cultures indicate the presence of viable bacteria currently in the

environment whereas direct PCR simply indicates the presence of the Mptb genome sequence in the environment that may or may not be from viable Mptb capable of causing infection. Information related to the quantity and presence of Mptb can inform decision making for environmental management to reduce cattle exposure. In addition, the distribution and quantification of Mptb in the environment can be used in mathematical models comparing alternative disease control options.

It is believed that Mptb exhibits dormancy under stressful environmental conditions (Whittington et al., 2004) and it may not be culturable even if living cells are present in the sample. The failure of dormant bacteria to grow may lead to underestimates of the hazard posed by Mptb in the environment. Similarly, underestimates may occur when PCR is inhibited by substances present in the environment that cause false-negative results. DNA extraction procedures should not lead to extensive loss of Mptb. Immunomagnetic bead separation, used in this experiment, involves lysing Mptb in the sample prior to assay. This bead separation has been reported to increase the analytic sensitivity of PCR (Khare et al., 2004). Binding of Mptb to soil particles in water present in carboys may have occurred, leading to decreased analytic sensitivity. The effect of this binding may have been more severe at lower concentrations (higher dilutions). Another possible source of error is related to the clumping of bacteria in samples leading to inter sample variation. In experiment 2, Tween 80 was used in attempt to reduce mycobacterial clumping. At the concentration of Tween 80 used in experiment 2, the analytic sensitivity of PCR did not appear to be greater for spiked pond water with Tween 80 than without it in experiment 1.

Increasing the sensitivity of detection for Mptb in the environment can define the environmental load to which susceptible cattle are exposed. The Johne's Disease Integrated Program (JDIP) Animal Model Standardization Committee (AMSC) proposed that 10^9 CFU/dose (100 mg wet wt) on 2 successive days will reliably produce disease in calves less than 8 weeks of age (Hines et al., 2007).

The number of samples assayed at each dilution was small and it will be beneficial to increase the number of replicates at each dilution. The effect of tangential flow filtration on the analytic sensitivities of MGIT culture and PCR were inconclusive, but if TFF is to increase the sensitivity it is more likely to be of importance at lower concentrations of Mptb as in tests dilutions 5 to 9 of experiment 1. Analytic sensitivity of MGIT culture may be greater than for PCR, but interpretation of MGIT culture results was complicated by the growth of contaminants.

In addition, the pre-filter was added to the TFF system in order to prevent fouling of the spiral filter and consequent restriction of flow during filtration. It was not likely that some bacteria were trapped in the 5 μm pre-filter because the size of Mptb is 0.5 by 1.5 μm. If bacteria were trapped in the filter, however, this would explain the inconsistent results at low concentrations. This loss of bacteria at low concentrations may have led to the quantity of Mptb falling below detection levels.

It is a limitation of this study that the concentration of Mptb was not standardized by spiking Mptb negative feces with pure bacterial cultures because assumed dilutions are not necessarily as expected. In addition, the repeatability of MGIT culture and PCR

for detection of Mptb from feces is important because feces from a cow shedding Mptb were used in these experiments.

5.5. Conclusions

The effect of tangential flow filtration (TFF) on the analytic sensitivities of MGIT culture and PCR were inconclusive. Tangential flow filtration is more likely to have an effect on the sensitivity of MGIT culture and PCR at higher dilutions of Mptb, as in tests 5 to 9 of

Experiment 1. The analytic sensitivity of MGIT culture might be greater than for PCR, but the interpretation of MGIT culture results was complicated by the growth of contaminants.

6. INTERPOLATION OF ENVIRONMENTAL PREDICTORS FOR *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* IN BEEF CATTLE PASTURES

6.1. Introduction

Mycobacterium avium subsp. *paratuberculosis* (Mptb), the etiologic agent for Johne's disease (JD) (Twort and Ingram, 1912), has been isolated from the environment of farms with JD (Raizman et al., 2004; Campbell et al., 2007) and there is evidence that Mptb can survive in the environment over long time periods (Lovell et al., 1944; Larsen et al., 1956; Whittington et al., 2004). The presence of Mptb in the environment is a source of infection for susceptible cattle in the herd (Sweeney, 1996). The implementation of successful JD control programs is challenging because infected cattle are capable of shedding Mptb into the environment before clinical signs of disease become apparent (Armstrong, 1956; Whitlock and Buergelt, 1996).

Environmental predictors act at multiple levels (scales) to determine the spatial distribution of micoorganisms (Franklin and Mills, 2003; Pickup et al., 2003; Singer et al., 2006). Several experimental and observational studies have examined the association between environmental predictors and Mptb or JD (Lovell et al., 1944; Johnson-Ifearulundu and Kaneene, 1999; Schroen et al., 2000). Observational studies are generally conducted at the farm level, however, survival and distribution of bacteria in the environment is related to factors that are operational at finer levels (Ettema and Wardle, 2002; Franklin and Mills, 2003; Martiny et al., 2006). Increased survival of

Mptb is likely due to the probability of the deposition of Mptb contaminated feces in a favorable location and the heterogeneity of the farm environment. Suspected environmental predictors for the survival of Mptb include soil type (Kopecky, 1977), iron concentration (Johnson-Ifearulundu and Kaneene, 1999), pH (Richards, 1989), calcium concentration (Jansen, 1948), moisture (Schroen et al., 2000), shade (Katayama et al., 2004; Whittington et al., 2004) and water in rivers or ponds (Whittington et al., 2005). Some of the factors that determine the distribution of fecal pats (potentially contaminated with Mptb) on farms include depressions in the landscape, location of rest areas, source of water, stocking density, and grazing period (Peterson and Gerrish, 1995).

Several reports suggest an association between acid soil type and the occurrence of JD (Reviriego et al., 2000; Ward and Perez, 2004). In a recent study in Alberta it was reported that beef cattle herds located in regions with a combination of alkaline pH, southern latitude and dry climate had moderately decreased seropositivity (Scott et al., 2007b). Johnson-Ifearulundu and Kaneene (1999) reported a 5% decrease in the number of ELISA-positive cattle with each 0.1 increase in soil pH and a 4% increase in the number of ELISA-positive cattle with each 10 ppm increase in soil iron content (Johnson-Ifearulundu and Kaneene, 1999). The optimal pH for growth of Mptb in the laboratory is 5.5 (Morrison, 1965). In addition, it has been reported that the D-values, time for a $\log_{10}$ reduction in viable bacteria, of Mptb doubled for each unit increase in pH (Sung and Collins, 1998). Soil pH is believed to mediate its effect by increasing the iron availability in acidic soils compared with more alkaline soils (Johnson-Ifearulundu

and Kaneene, 1999). The solubility of iron is at a minimum between a pH range of 7.4 to 8.5.

Mycobacterium avium subsp. *paratuberculosis* does not normally grow in the environment or in vitro since it lacks the iron transport carrier known as mycobactin (Morrison, 1965; Snow, 1970). However, growth of Mptb can occur when laboratory media are supplemented with iron or mycobactin at an acid pH (5.0) (Lambrecht et al., 1988). Due to the presence of hydroxide or carbonate ions, high calcium soils are negatively correlated with low pH and this is a possible explanation why calcium content of the soil has been reported to be associated with decreasing seroprevalence of JD (Johnson-Ifearulundu and Kaneene, 1999) and clinical JD (Lugton, 2004). Calcium is hypothesized to act by altering soil pH when added to the soil in the form of lime.

Shade can increase the survival of Mptb (Larsen et al., 1956; Whittington et al., 2004) and the mechanism could be by preventing ultra-violet light from reaching the bacteria (Katayama et al., 2004). Relatively lower temperatures also occur in shaded areas and this is another possibility to explain the higher survival of Mptb (Schroen et al., 2000; Whittington et al., 2004). Also, shaded areas could have relatively higher moisture levels. Factors that affect the distribution of fecal pats on farms will ultimately determine whether or not Mptb contaminated feces are deposited in particular locations. Cattle often choose to lie on North-eastern slopes because of the higher soil moisture and this could cause a higher deposition of fecal pats in these areas (Lovell et al., 1944; Pfost et al., 2000; Schroen et al., 2000).

Mycobacterium avium subsp. *paratuberculosis* can survive in water for even longer periods than it can survive in soil. According to Whittington et al. (2005) Mptb survived for 48 weeks in a shaded water trough, but only 36 weeks in soil under similar conditions. Earlier reports of Mptb survival in water ranged from 9 months in sterilized pond water at room temperature (Lovell et al., 1944) to 17-19 months in tap water at 38ºC (Larsen et al., 1956).

Geographic Information Systems (GIS) and spatial analysis have been used to study the distribution of soil properties and of bacteria in the environment on farms (Goovaerts, 1998; Brown et al., 2004; French et al., 2005). Interpolation can be used to produce maps of environmental predictors at sampled and unsampled locations within the study area (Goovaerts, 1999). The distribution of Mptb in the environment on beef ranches is largely unknown and this information could be used to inform JD transmission models and to develop targeted JD control strategies. The objective of this study was to describe the distribution of environmental predictors for Johne's disease and Mptb survival on beef ranches in Texas.

6.2. Materials and methods

6.2.1. *Study location*

Three study farms that had a history of at least one JD suspect animal during the previous 3 years were selected for sampling. The pasture evaluated on Farm 1 previously housed a test-positive animal with clinical signs of disease, including

diarrhea. Farm 2 had Mptb culture positive cattle in the previous year. Test positive cattle had been identified among cattle in the pasture sampled on Farm 3.

Farm 1 was a Brangus registered herd in east Texas with a total of 250 head of cattle grazed over 607 hectares. Cattle were housed on separate pastures depending on the stage of production. Heifers were housed in the sampled pasture from the age of 14 months and bred for the first time. There were 30 head of cattle in the sampled pastureFarm 2 was a Longhorn with 50 head of cattle on 83 hectares, located in central Texas. The herd on Farm 2 was run as a single unit although there were different pastures on the property. The isolation pasture on Farm 2 was sampled. Farm 3 was Texas was a Brahman breeding ranch in south-eastern. The sampled pasture housed 40 head of cattle on 45 hectares.

6.2.2. *Sampling*

Fixed and random points on study farms were selected and sampled. Fixed points included feeders, drinking locations and shade/rest areas. On Farm 1, 119 points were randomly selected. Seventeen random and 1 fixed points on Farm 2 were selected. Thirty-eight random and 2 fixed points on Farm 3 were selected. Two areas were sampled on Farm 3. The first consisted of a defined 10 acres area which included the feeder. The other area was 7 acres adjacent to a pond to which cattle had access.

The longitudes and latitudes of sample points were selected using a random number generator. Pasture boundaries were mapped and sample points located using a Global Positioning Systems (GPS) Unit (GARMIN International Inc., 1200 East 151st

Street, Kansas 66062). Wide Area Augmentation System (WAAS) was used increase the accuracy of point detection. Points located in the pasture using the GPS unit were downloaded to a computer using an available software program (G7ToWin – C.R. Henderson, 1997-2009).

6.2.3. *Environmental testing*

Soil was collected to a depth of 6 inches from the soil surface subsequent to the removal of non-decomposed plant material. Fecal pat samples were collected within a 7 feet radius of the GPS located point. Fecal pats were classified as, fresh (1), signs of aging but intact (2) and aged, degenerated, fibrous and dehydrated with growing grass (3). The scale was modified based on the report by Brown et al. (2004). The fecal pat nearest to the selected point was collected for testing. Composite fecal samples were also collected from shaded rest areas. One side of each feeder and drinking water source was selected for soil sample collection by randomly selecting a piece of paper with a compass direction corresponding to a side of the structure. Fecal pat samples were collected from the same side as the soil sample. Composite fecal samples were collected within a 180º sector with a 7 feet adius of the sampled side. The diameter of the sampled fecal pats was recorded and the number of fecal pats within each area was counted. The fecal pat density was measured by estimating the number of fecal pats within a circle of radius 7 feet. The unit area was calculated as πr^2, where r = 7 feet.

Soil and fecal samples were placed in Whirl Pak bags and transported to the College of Veterinary Medicine and Biomedical Sciences. Soil samples were submitted

to the Soil, Water and Forage testing laboratory at Texas A&M University for routine (pH, nitrate, phosphorus, potassium, calcium, sodium, sulphur and electrical conductivity), micronutrient (zinc, iron, copper and manganese) and texture analysis. Electrical conductivity, the ability of the soil to conduct an electrical current, and pH measurements were performed at the Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University using a portable unit (SM 802 Economy pH/EC Meter, Spectrum Technologies, Plainfield, Illinois). Two parts of soil were mixed with 1 part of distilled water for pH and conductivity measurements (μS/cm). Soil and fecal composite samples from each sampled point were mixed in a 1:1 ratio. Duplicate soil/fecal mixtures were transported to the Texas Veterinary Diagnostic Laboratory for quantitative real-time PCR (Scott et al., 2007) and to The Johne's Testing Center in Wisconsin for MGIT culture in sealed containers with cold packs.

Evaluated predictors also included the following: shade, landscape (depression, slope or flat), night/rest area, available moisture (Soil Moisture Meter, Spectrum Technologies, Inc., Plainfield, Il, USA), vegetative cover and soil surface condition (loose or compact).

6.2.4. *Data analysis*

Kriging was used to create spatial risk maps (ArcMap version 9, Environmental Systems Research Institute Inc., Redlands, CA, 2005). First, a semivariogram was created using VARIOWIN 2.2 (1995). Then, values for the following parameters were estimated: lag, nugget, sill and range. Next, an ordinary kriging model was fit to the empirical semivariogram estimated from the data and a map was produced. Cross validation was performed to test the kriging estimation method.

A composite environmental risk score (CERS) was calculated for predictors suspected to increase survival of Mptb `based on the literature:

CERS = ST + SpH + SoI + SM + TMP + SE + FPD

All predictors were dichotomous and ST was soil type (Clay = 0; Sand = 1) (Kopecky, 1977; Reviriego et al., 2000), SpH was the soil pH (pH > 5.5 = 0; pH < less than 5.5 = 1) (Morrison, 1965), SoI was the iron (SoI low = 0; SoI high = 1) (Lambrecht and Collins, 1992; Dhand et al., 2009) SM was the soil moisture or electrical conductivity (SM low = 0; SM high = 1) (Schroen et al., 2000), TMP was the environmental temperature (TMP ≤ 10°C = 0; TMP > 10°C = 1) (Schroen et al., 2000), SE was shade (Yes = 0; No = 1) (Whittington, 2001; Whittington et al., 2004) and FPD

was fecal pat density or dense scattered feces (Low = 0; High = 1). The maximum possible risk score was 7 and the minimum possible was 0. The probability of the CERS being above the median value (3) in the pasture was kriged using the indicator kriging technique (ArcMap version 9, Environmental Systems Research Institute Inc., Redlands, CA, 2005).

6.3. Results

6.3.1. *Farm 1*

The evaluated pasture on Farm 1 was 25 acres and the soil type was loam/sandy loam. Sixty locations were evaluated for pH and conductivity only. In addition, 59 random and 4 fixed points were evaluated for fecal pat density, pH, soil texture and soil nutrient. PCR and radiometric culture samples were negative for Mptb. Soil moisture measurements were all 0.

Figures E-1 and E-2 in the Appendix are the semivariogram and the spatial prediction map for soil iron. The median soil iron in the pasture was 80.8 ppm (range 15.20 to 377.80 ppm). Shade trees were located in an area with 122 to 168 ppm iron. One feeder was located at 72 to 81 ppm and the 2 others at 42 to 57 ppm. E-3 and E-4 are the semivariogram and the spatial prediction map for soil pH, respectively. The median soil pH in the pasture was 5.4 (range 4.3 to 7.0). The lowest pH values were in

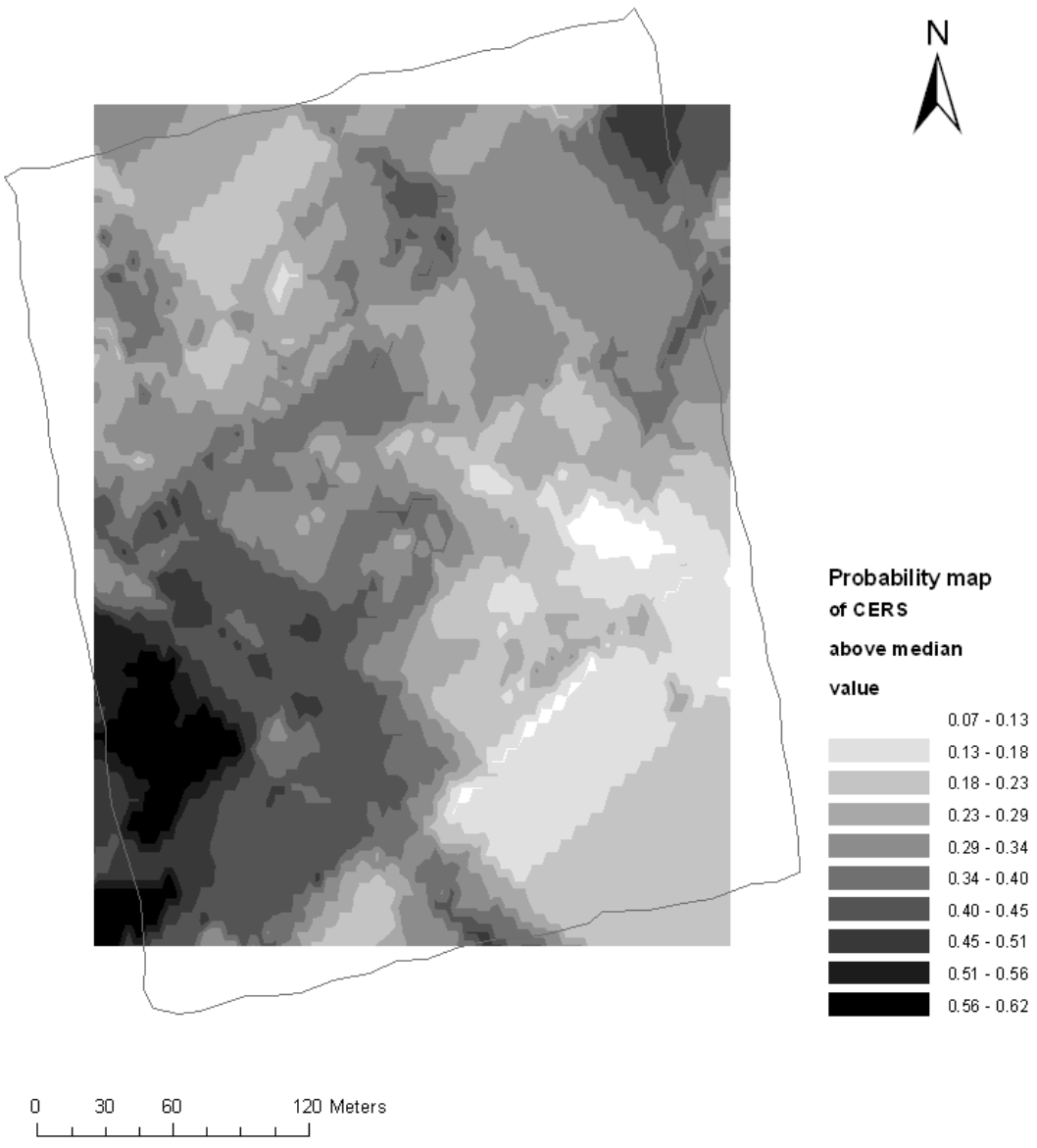

Fig. 6.1. Spatial prediction map of CERS for Farm 1.

the south-western and in the north-eastern areas of the pasture. The shade trees were located in the area of the pasture with low pH 5.2 to 5.3. One feeder was located in an area of pH 5.5, the optimal for growth of Mptb. E-5 and E-6 are the semivariogram and spatial prediction map for soil calcium. The median soil calcium in the pasture was 677 ppm (range 234 to 1231). Shade trees were located at 405 to 681 ppm calcium and feeders at 962 to 1250 and 701 to 811 ppm calcium. E-7 and E-8 are the semivariogram and spatial prediction map for fecal pat density. Fecal pat density ranged from 0.00 to 1.71 fecal pats/square foot. Higher fecal pat densities were encountered in the south-eastern end of the pasture and the lowest in the north-eastern end. Fecal pats under the shade tree were too numerous to be counted. CERS ranged from 1 to 5 and Figure 6.1 is the CERS spatial prediction map.

Figure 6.2 is a map of cokriging of iron and pH and Figure 6.3 is a map of cokriging of calcium and pH on Farm 1.

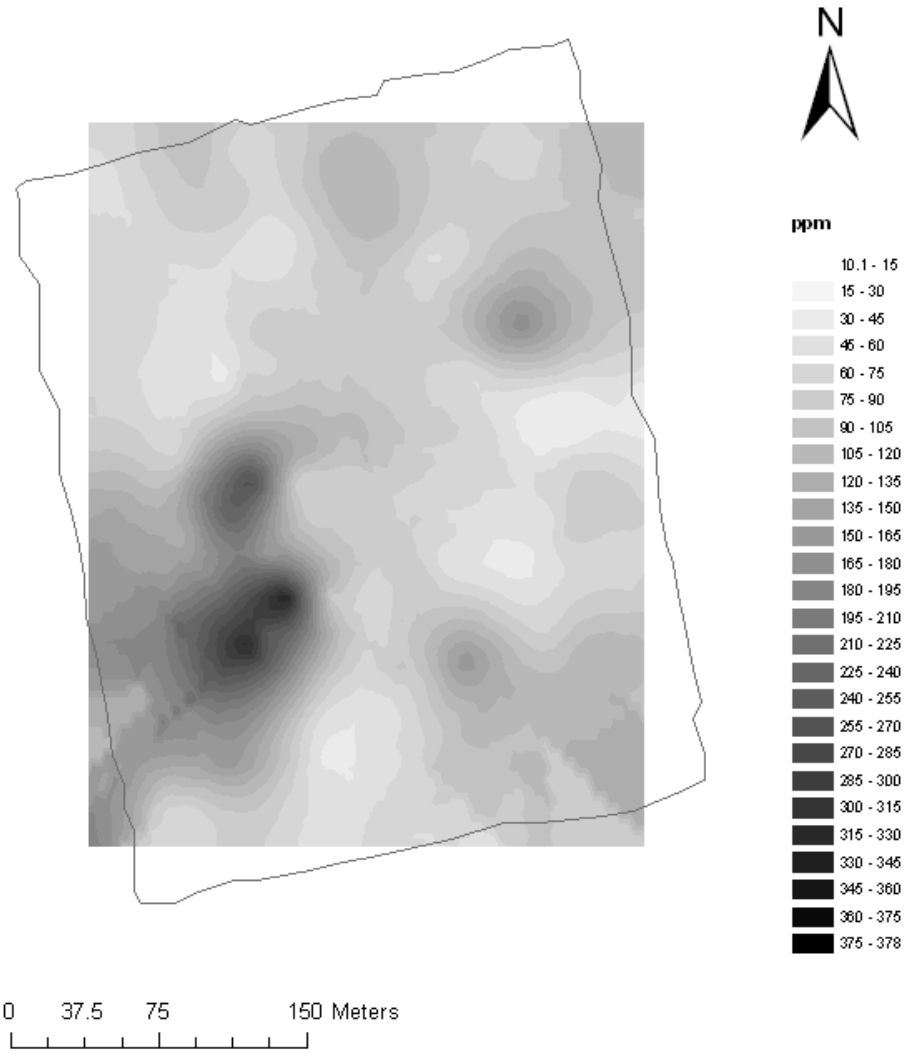

Fig. 6.2. Cokriging of iron and pH for Farm 1.

Fig. 6.3. Cokriging of calcium and pH for Farm 1.

6.3.2. *Farm 2*

The sampled pasture on Farm 2 was 5 acres and the soil type on Farm 2 was loam/silt loam. Nine points were evaluated for pH and conductivity only. In addition, eight points were evaluated for fecal pat density, pH, soil texture and soil nutrients. PCR and radiometric culture were negative for Mptb.

E9 and E-10 are the semivariogram and spatial prediction map for soil iron. The median soil iron in the pasture was 134 ppm (range 63.9 to 162.8). The lowest soil iron concentration was in the south eastern area. E-11 and E-12 are the semivariogram and spatial prediction map for soil pH. The median soil pH in the pasture was 4.6 (range 4.1 to 5.6). The lowest pH values were at the center of the pasture and the highest in the south western area. E-13 and E-14 are the semivariogram and spatial prediction map for soil calcium. The median concentration of calcium was 470 ppm (range 235 to 1194). The lowest calcium concentration was at the center of the pasture. The median CERS was 2 (range 2 to 5) in this pasture.

6.3.3. *Farm 3*

The areas in the sampled pasture on Farm 3 were 7 and 10 acres, and the soil type was sandy loam. Fifteen points were evaluated for pH and conductivity only. Seventeen points were evaluated for fecal pat density, pH, soil texture and soil nutrients. PCR and radiometric culture were negative for Mptb.

E-15-1, E-15-2 are the semivariograms for the trough and pond areas of the sample pasture. E-16 is the soil iron spatial prediction map. The median soil iron was 29.5 ppm (range 19.6 to 45.3). The highest concentrations of iron were in the south eastern area of the pasture where the surface was heavily shaded by vegetation. E-17-1 and E-17-2 are the semivariograms for pH. E-18 is the spatial prediction map for pH. The median soil pH in the sampled pond area was 7.4 (range 7.2 to 7.5). The lowest pH values were at the southern end of the pasture. The median soil calcium in the pasture was 10472 ppm (range 8293 to 11576). Lowest areas of calcium were in the east, south west and north. The highest areas of calcium were in the south east and north central. E-19-1 and E-19-2 are semivariograms for calcium in the trough and pond areas. E-20 is the spatial prediction map for calcium.

The pond was located in the western region of the sampled area. Cattle had direct access to the pond. The CERS was either 1 or 2 for the 6 sample points with complete data in the area which contained the feeder and drinking water.

Table 6.1: Correlation of pH vs iron and calcium vs iron for Farms 1, 2 and 3

	iron and pH		iron and calcium		calcium and pH	
Farm number	Correlation coefficient	p value	Correlation coefficient	p value	Correlation coefficient	p value
1	-0.590	<0.001*	0.677	<0.001*	0.702	<0.001*
2	-0.336	0.349	0.293	0.413	0.656	0.039*
3	-0.425	0.079	-0.271	0.276	0.405	0.100

6.4. Discussion

Overall the CERS in this study ranged from 0 to 5, indicating different levels of risk in the environment of sampled pastures. Probability maps of CERS on Farm 1 were based on the probability that CERS would be above the median value in the pasture. Heterogeneity with respect to the distribution of suspected environmental predictors for Mptb was apparent at the scale in which the study was conducted. These results are supported by recent reports that have explored the heterogeneity of the environment at different scales and the relationships between environmental predictors that act at these scales to determine the distribution of microorganisms (Franklin et al., 2002; Ritz et al., 2004; Becker et al., 2006; Singer et al., 2006). Mptb can survive in the environment for prolonged periods. For example, using radiometric culture, Cook et al. (2007) detected Mptb in spiked pasture soil for up to 210 days. Samples for the present study were collected in pastures of farms and predictive maps were developed using kriging.

Several reports suggest an association between soil type and JD seropositivity (Johnson-Ifearulundu and Kaneene, 1999; Reviriego et al., 2000). However, since these studies are at regional and national levels there is a danger of ecological fallacy when these results are applied to individual farms. The range of pH in the section of Farm 2 in which the feed trough was located was the narrowest for all farms sampled**.** The soil pH in Farms 1 and 3 ranged from acid to slightly alkaline. Therefore bacteria deposited in

the environment of Farms 1 and 3 could be exposed to any of a range of pH values despite the pH associated with the general soil type of the area. This finding is of interest since Mptb was recently isolated in and around feeders on beef farms in Saskatchewan, Canada (Campbell et al., 2007).

Since shade has been shown to increase survival of the bacteria, it is expected that there will be higher survival of Mptb in fecal pats located in shaded areas of farms, that is, the effect of environmental predictors on Mptb survival could depend on the specific combinations of predictors present. For example, high soil iron content and low pH might represent a greater hazard than either alone.

The environment of the pre-weaning area can be targeted for sampling since this area was identified as the second greatest hazard for beef farms enrolled in the National Johne's Disease Herd Demonstration Project In addition, it has been observed that calves are more likely to develop infection and progress to clinical JD than are adult cattle when exposed to similar levels of Mptb (Chiodini et al., 1984; Nelli et al., 2008). The CERS is a rapid means of assessing the ability of a particular environment to support increased survival of Mptb and can be used in the targeted sampling of pastures. All factors are weighted equally in the CERS presented in this paper, but it might be more appropriate to weigh some factors more heavily than others depending on the relative strength of their impact on the survival of Mptb.

The ability to accurately represent the heterogeneous distribution of Mptb on farms is expected to improve the predictive ability of models of the intra-herd dynamics of JD and lead to improved recommendations for disease control (Humphry et al., 2006). Using this method of sampling, a permanent record of problem areas on the farm can be produced and monitored over time. This introduces a measure of continuity in addressing the problem of environmental contamination with Mptb and is useful because of the high turnover of veterinarians and farm managers that might be experienced by beef farms.

The correlations between pH with iron, calcium with iron and calcium with pH were assessed using Spearman's rho. Variables with significant correlations at the 5% level were co-kriged (ArcMap version 9, Environmental Systems Research Institute Inc., Redlands, CA, 2005).

Samples were collected in this study using random sampling. A disadvantage of random sampling is that, just by chance, all of the samples can be located in the same area. This possibility was addressed in this study in some measure by dividing the study area into quadrants and sampling within each quadrant. However, an alternative approach to sample collection would be to divide the pasture into a grid as was done in a previous study of *Campylobacter* (Brown et al., 2004). Another limitation of the methods used in this study is related to the relatively small area interpolated. Also, a

larger study, similar to that conducted in Canada by Campbell et al (2007) which involved 27 herds on farms with different landscapes and with different JD prevalences, should yield a more comprehensive picture of the spatial distribution of environmental predictors for Mptb.

This study is also limited by its crosssectional design. It is expected that there will be temporal variation of moisture and temperature and as a result, a longitudinal rather than a crosssectional study is more appropriate for measurement of these variables. Positive correlations between electrical conductivity and moisture have been reported (Harstock et al., 2000), but might have been unique for the surveyed area (Johnson et al., 2001). In addition, one study reported a relatively weak correlation between electrical conductivity and moisture in a field located in a dry area with relatively low rainfall. Average historical temperatures were used for the sampled farms in the present study.

Geographical Information Systems can be used to represent the distribution of environmental predictors at the farm level and can be a useful tool in the multiscale monitoring of environmental predictors for on farm bacterial populations with known or suspected zoonotic implications, such as Mptb. Researchers can also use the data acquired at the individual farm level to investigate associations between the occurrence of Mptb in the environment and JD in ruminants. These data could then be used at the regional or national level. The assessment of the environment's potential to support

survival of Mptb can be improved by further research on the relationship between single as well as different combinations of environmental predictors on the survival of Mptb.

The effect of environmental predictors on Mptb survival may well depend on the specific combinations of factors present, for example high soil iron content and low pH may represent higher risk than either alone. Potential applications of the CERS include targeted sampling of farms and in environmental research programs. In addition, the CERS can be used by farm managers to strategically place feeders, waters and artificial shade.

6.5. Conclusions

The environment of a farm is heterogeneous with respect to risk predictors suspected of influencing the survival of Mptb. The CERS provides a rapid assessment of the potential of Mptb to survive in the heterogeneous farm environment. The success of applying the CERS may depend on the size of the area under consideration and the sample size

7. SUMMARY AND CONCLUSIONS

7.1. Summary and conclusions

Biosecurity practices aim to prevent the entry of JD and to prevent the intraherd spread of disease. Uniform program standards developed for the Voluntary Johne's Disease Control Program (VBJDCP) in the US (USDA, 2002) include biosecurity practices and requires the veterinarian to perform a JD risk assessment on farms based on these practices. States, such as Texas, which choose to participate in the program, must adopt standards that are equivalent to or more stringent than the program standards. The program standards consist of 3 elements: education, management and, herd testing and classification.

Beef producers in Section 2 were surveyed to determine their perceptions of the benefits that they received by participating in the VBJDCP with test-negative Level 4 status, that is, with the lowest probability of having JD. According to the Innovation-Decision model, the perception of benefits to be gained from adopting a new idea will determine how quickly it is adopted (Rogers, 2003). A more rapid adoption of the VBJDCP would be possible if producers participating in the VBJDCP were able to benefit from their JD-low risk classification including the marketing of their cattle at a premium. One-quarter of the test-negative Level 4 producers in this survey reported that they received benefits (financial and non-financial) as a result of their participation in the VBJDCP. In addition, most producers suggested that the program could be improved by increased marketing. One possible outcome of increased marketing of the VBJDCP is the enrollment of more herds at all levels of the program and a consequent higher

demand for JD low-risk replacements. Another possibility is that purchasers of cattle who are not enrolled in the VBJDCP will be aware of the availability of JD low-risk cattle and of the advantages of acquiring these cattle. This should translate to greater marketability of cattle from program herds and an improvement in the perception of financial benefits to be derived from participation in the VBJDCP.

In addition to financial benefits, most producers reported that their major reason for joining the VBJDCP was because of concerns about herd health and to increase their confidence that they were selling healthy cattle. It is encouraging that there are reasons for producer enrollment in the VBJDCP apart from perceived benefits because it is conceivable that unrelated market forces which drive demand and sales of cattle can act as an obstacle to the consistency of the financial benefit (Combs, 1996).

There may be greater implications for registered producers who sell JD-affected cattle than for commercial-only producers who do the same. This difference may in part explain the relatively high proportion of these registered producers, 63%, identified in this survey at Level 4 in the VBJDCP compared with the proportion in the US, 5.8% (National Animal Health Monitoring System, 1998a). Overall, Section 2 lent insight into the characteristics of the producers who remained participants of the program through Levels 1 to 4. This information can be used to successfully target new participants. In addition, the reported challenges faced by these producers, such as high cost of tests, and their recommendations for improvement can be used to improve the perceptions of the benefits to be gained by participating in the VBJDCP.

Researchers have become increasingly aware of the need to identify attitudes of producers towards biosecurity practices and their behavioral practices (Hoe and Ruegg, 2006; Gunn et al., 2008). The relationship of attitudes to participation in a control program, such as the VBJDCP, can be viewed as an innovation-decision process as described by Rogers (2003). The progressive basic stages of a innovation-decision process are Knowledge, Persuasion, Decision and Confirmation. Initially, in the Knowledge stage, the producer learns that the program exists and how it works. In the VBJDCP (and TVJDP) the veterinarian introduces the program to the producer and educates the producer about how the program works. In the Persuasion stage, a favorable or unfavorable attitude is formed about the idea (program). Next, in the Decision stage, the individual acts to accept or reject participation in the program. If participation is selected, the producer then puts the program recommendations into place in the Implementation stage. Subsequently, in the Confirmation stage, the producer will seek more information to confirm the initial decision to participate. The producer may decide to discontinue participation in the program at the confirmation stage.

Overall, only 1/5 of producers agreed that they knew how to control and prevent JD, indicating that there is a need for more information about this disease among beef producers. This information can be presented in the form of articles in trade magazines and seminars because the majority of these producers expressed interest in receiving information in this form. Options for control of JD include test and cull as well as management changes.

Only 14% of producers in Section 3 disagreed that testing was helpful for JD control. One practical concern is the length of time required for the return of fecal culture results as evidenced by responses in Section 3 where the proportion of veterinarians who felt that the rapidity of return of fecal PCR and serum ELISA results were useful for decision-making was 50% more than the proportion of veterinarians who reported the same about fecal culture. This difference in belief is because traditional (conventional) fecal culture results are typically available 16 weeks after sampling, whereas serum ELISA and fecal PCR results can be available from the laboratory within 3 days. Newer liquid culture techniques are more rapid and capable of providing results within a shorter period of time (8 weeks). The test and cull control strategy reduces but does not eliminate environmental contamination with Mptb in the herd because subclinical shedders could be present (Whitlock and Buergelt, 1996). This means that it is still possible for susceptible cattle to be infected because this strategy does not completely prevent exposure to Mptb. More producers than veterinarians agreed that JD can be controlled by test and cull only. Two possible reasons for this disparity between the beliefs of producers and veterinarians were greater familiarity of veterinarians with control and prevention of JD and producers' beliefs about the practicality and feasibility of management changes on their operations. The importance of the utility of tests and testing protocols was reflected in Section 2 where producers listed tests and testing as a problem.

Management changes are used separately or in combination with test and cull. Management changes are designed to reduce the probability of susceptible cattle coming

into contact with Mptb. In Section 3, most producers agreed that management changes were helpful for the control of JD. Also, the only belief significantly associated with the producers reporting "I have no JD problem on my ranch" was separating cows with clinical JD from youngstock is helpful for control of JD. This belief towards JD control is supported by the findings of Groenendaal et al. (2003) who reported that although none of the 5 combinations of management and test and cull options in a simulation of alternative options for the control of JD on Dutch beef cow farms were able to eliminate the disease, only options based on separating calves and adult animals significantly reduced disease prevalence.

Most producers in Section 2 reported that the major change that they had to make was to restrict purchases/close their herds. This is not surprising because, according to the NAHMS (1997b) report on beef cow-calf health and health management practices, 39% of cow-calf operations introduced new animals to their herds. In addition, in Section 3 only 9% of producers reported that they added (purchased) no new cattle during the preceding 5 years. The recommendation that cattle replacements be restricted to JD low-risk replacements is supported by the finding that the purchase of infected animals was the major risk for introducing JD to beef farms in the National Johne's Disease Demonstration Herd Project (USDA, 2005b). It is encouraging that most producers in Section 3 agreed that sourcing replacements from herds at low-risk for JD is helpful for control of JD in their own herds. However, the significantly lower proportion of veterinarians agreeing that producers will source low-risk replacements for their herds is of concern and the reasons for this disparity should be examined further.

Two initial considerations for facilitating the acquisition of replacements from JD low-risk herds are that these low-risk replacements should be available in the first instance and that producers should know how to acquire these animals. Purchasers of cattle should also be aware of the advantages of having these low-risk cattle in their herds. A challenge to this objective is that producers who do not see JD as an important problem in their region, who do not have a positive attitude towards the required control measures and who do not perceive that there will be a financial benefit associated with participation will not be encouraged to join the control program. Familiarity with JD and the TVJDP can only further this objective.

An additional potential challenge to biosecurity is the high percentage of producers who reported that cattle from neighboring herds often or sometimes gained access to their herds. The possibility of JD entering the herd as a result of contact with other herds should be considered in areas in which there is a high probability that neighboring herds are infected. The introduction of Mptb contaminated feces to farms may also inadvertently occur, for example, one producer in Section 2 reported that he had an agreement to accept manure from a neighboring dairy farm that had experienced JD in the past. However, it is expected that attitudes may be influenced by customs and practices unique to different regions.

Although most veterinarians had heard of the TVJDP, far fewer had become certified to participate in the program. In fact, 16% of the non-certified veterinarians had considered becoming certified. Certified veterinarians had enrolled a median of 0 (range 0-25) herds. The reasons why more veterinarians had not become JD-certified

and the challenges to veterinarians in enrolling producers should be further explored. Possible ways in which veterinarians may be assisted include marketing of the program amongst producers, availability of producer-friendly packages for educating producers and real-time assistance with interpreting test results. The findings of studies of beliefs of producers and veterinarians can be ultimately used to improve communication between stakeholders in the disease control decision-making process and serve to identify obstacles (Gunn et al., 2008; Heffernan et al., 2008). Consequently, recommendations that producers are likely to have positive attitudes towards may be implemented where possible. It is expected that consideration of the attitudes of producers and veterinarians will help to reveal the utility of proposed control measures that can then be used to develop more tailored recommendations for JD control. Although, it should be kept in mind that the control strategies considered optimal can vary; the attitudes of producers and veterinarians towards control program measures will help to determine which control strategies are considered optimal under different circumstances.

Another factor related to the perceived advantage of adopting a new idea (the control program) and consequently the rate of adoption of the control program is any uncertainty surrounding the new idea (Rogers, 2003). One area of uncertainty relevant to the control of JD is the role played by environmental predictors in the intra-herd dynamics of the disease.

In Section 4, a Markov model was used to analyze alternative options for the control of JD in beef cattle. This model assumed that the environment was the major

source of new infections. The Test, cull and management B option (Q=0.993) was the only control option for which the prevalence of JD fell below that of the Do nothing option within the time horizon of the analysis, 15 years (Q=0.995). In the present model, the only options for which prevalence remained 0 was the lowest probability of survival of Mptb in the environment and those at the highest management level (Q=0.998). A Markov model is only a crude representation of the true disease process and development of the model is a continuing process which requires feedback and field data concerning parameter values. Accurate assessments of the quantity of Mptb present in the environment of beef farms and the JD incidence in associated herds are needed. Modeling of intra-herd dynamics and environmental studies will be more useful in intensive production systems. Modeling the transmission of Mptb within the beef herd is useful because we can use the basic model to determine the effect that the introduction of a control program will have on the prevalence and incidence of disease under certain assumptions. In addition, comparison of possible control strategies using a Markov model is a useful tool because the relatively low prevalence of JD in beef cattle presents fewer opportunities to observe the impact of control programs on the course of this disease. The relatively long period between infection with Mptb and the appearance of clinical signs of disease means that studies assessing the impact of control strategies must encompass an extended period of time and translate to high cost and labor.

Sections 5 and 6 dealt with techniques for isolating Mptb from environmental samples and with factors for predicting the distribution of Mptb on beef farms. The ability to isolate and quantify Mptb from environmental samples and to describe its

presence in the environment is useful for the development of Makov and other models of the dynamics of Mptb within beef herds.

In Section 5 the effect of tangential flow filtration on MGIT culture was inconclusive and it did not increase the analytic sensitivity of PCR for Mptb in pond water. The possibility of contamination of MGIT cultures of samples collected for environmental experiments is expected and is, in part, due to the presence of bacteria and fungi that are faster growing than Mptb. Clumping of mycobacteria is a common phenomenon (Stinear et al., 2004a) and pre-experimental fine tuning of methods is often necessary to improve results.

In Section 6 spatial risk maps revealed heterogeneity of the pasture environments sampled and a composite risk score was proposed. Description of environmental predictors is affected by size of pasture sampled, organization of farm, method of sample collection and number of samples collected. A more intimate knowledge of the role played by environmental predictors in the intra-herd dynamics will give greater support to recommendations of JD control strategies on farms. *Mycobacterium avium* subsp. *paratuberculosis* has been isolated from the environment of beef farms (Campbell et al., 2007). The environment may serve as a reservoir of Mptb since these bacteria can survive there for prolonged periods (Lovell et al., 1944; Whittington, 2001; Whittington et al., 2004). Understanding the role of the environment as a source of Mptb is crucial since the fecal-oral route of transmission is the most important for this disease (Sweeney, 1996). In addition, the accessibility of susceptible cattle to Mptb is determined by its location in the environment of farms. Use of a cross-sectional survey

to collect information on benefits of program participation is limited by the reliability and extent of records kept or the ability of the producer to recall expenses and receipts.

In questionnaire investigations such as in Sections 2 and 3 non-response bias, a type of selection bias, is of concern. Non-response bias is present when the association between exposure and disease differs between respondents and those who did not respond. For example, if those producers who had JD on their farms and did not respond had different attitudes or beliefs from those producers who had JD on their farms did respond then this should result in bias. Non-response of producers who have JD positive herds is likely, despite the assurance of the researchers that the survey was anonymous, since producers may have been reluctant to reveal their familiarity with the disease due to the negative images associated with JD (Hood and Seedsman, 2004). Also of concern is recall bias, a form of differential misclassification bias which can result in prevalence ratios that may be biased away from or towards 1. Since most questions regarding exposures on the survey are phrased in such a manner that they will elicit responses based on current beliefs and practices, it is believed that recall bias was not an important factor.

Awareness of the perceptions of producer benefits of the JD control program and of attitudes towards program recommendations helps those in control of the program to develop target groups and to make changes to the program that will eventually lead to increased enrollment of producers. Information regarding attitudes towards general biosecurity practices in Section 3 can also be applied to multiple diseases that require similar practices for control. Markov chain models can be used to compare outcomes,

identify parameters that should be further researched due to uncertainty surrounding them or because of the effect that they may have on the outcome of disease.

7.2. Proposed future study

A more complete picture of the advantages of the VBJDCP can be gained from a longitudinal study on--farm study of the financial benefits received by seedstock beef producers enrolled in the at all levels of the program. Willingness to pay studies could be conducted in order to determine the value of control strategies to producers. Entry and exit surveys for producers and veterinarians in the VBJDCP should allow program administrators to keep current on the experiences of participants with the program and to identify major difficulties. Further research on tests is necessary since producers in Section 2 identified this as a major problem. Attention to the utility of tests for decision making will be beneficial. Also, further studies can be designed around reducing uncertainties in parameters in the Markov model. The ability to accurately model JD will lead to a more complete understanding of the epidemiology of JD on beef farms and will help optimize the use of resources. More research is needed on the distribution of environmental predictors for survival of Mptb in the environment of farms, which will aid in the identification and targeting of areas on farms that are at high risk. Finally, a case-control study of environmental and management predictors on beef farms with JD and those without will generate useful information.

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APPENDIX A

Survey of Level 4 Test Negative Beef Producers in the Voluntary Bovine Johne's Disease Control Program

Thank you in advance for taking part in this survey of Level 4 producers in the Voluntary Bovine Johne's Disease Control (VBJDC) Program. This questionnaire is designed to examine your experiences as Level 4 producers with the VBJDC Program and will be of value to all producers. An extra sheet of paper is provided at the end of the questionnaire for any additions to answers or relevant remarks that you wish to make.

The VBJDC Program

1. How long have you been involved in the VBJDC Program?

_____ years

2. Do other producers approach you for information about your experience with the VBJDC program?

a. Often
b. Sometimes
c. Never

3. Do you own another herd(s) that is not enrolled in the VBJDC Program at level 4?

a. Yes
b. No

4. What is your main source of new information about Johne's disease?

a. Veterinarians
b. Other ranchers
c. Agricultural magazines
d. Journals
e. Extension officers
f. Other source. (*Please state*)

_____

_____

__

Herd information

5. How long has this herd existed?

_____

6. How many acres do you use for your cattle?

_____ acres

7. What is the total number of "test eligible" cattle in your herd?

_____

8. Is your cattle operation your major source of income?

a. Yes
b. No

9. What type of operation is this herd?

a. Commercial cow-calf herd
b. Registered
c. Seed stock herd (not registered
d. Mixed
e. Other (*Please state*)

_____

_____

—

10. Which of the following do you sell? (*Please circle all that apply*)

a.	Feeder calves ….	Yes	No
b.	Young animals for breeding……..…	Yes	No
c.	Mature animals for breeding……..…	Yes	No
d.	Embryos…….	Yes	No
e.	Cull cows ……...	Yes	No
f.	Other. (*Please state*)		

_____

_____

11. What breeds of cattle do you have in your program herd?

Number	Breed

Benefits

12. What was your major reason for entering the VBJDC Program?

_____

_____

_____

_____

13. What are the three most important benefits of the VBJDC program?

_____

_____

_____

_____

14. In your opinion, what should be the added value per head of cattle sold because of your level 4 status in the VBJDC program?

Bulls

_____ dollars/head

Heifers/cows

_____ dollars/head

Other

_____ dollars/head

15. What is your best estimate of the value added premium that you get per head of cattle sold because of your level 4 status in the VBJDC program?

Bulls

_____ dollars/head

Heifers/cows

_____ dollars/head

Other

_____ dollars/head

16. Please estimate the annual gross benefit of being on the VBJDC program.

_____ dollars/year

17. After your herd reached level 4 status, did it increase your opportunities for marketing cattle?

a. Yes

b. No

If "Yes," please specify,

_____

_____

_____

_____

_____

18. Were there any management factors that you had to address when you introduced the VBJDC program on your operation?

a. _____

_____

b. _____

_____

c. _____

_____

19. Approximately how much additional time do you spend working on your operation because of this program?

_____ hrs/year

20. Please estimate the annual additional cost of being on the VBJDC.

_____ dollars/year

21. At this time, to what extent do the benefits of being involved in this program compare with the costs? (monetary and non-monetary)

a. Significant benefits
b. Marginal benefits
c. No difference
d. Marginal Losses
e. Significant losses

22. Do you feel that there are any problems with the VBTDC program in its current form?

a. Yes
b. No

If "Yes," please specify

a. _____

_____

b. _____

_____

c. _____

_____

23. Please list up to three incentives that you believe would make the VBJDC program more attractive to other producers?

a. _____

_____

b. _____

_____

c. _____

_____

24. Did you experience any decrease in disease on your operation that may be related to the changes that you made for the VBJDC program?

a. Yes
b. No

If "Yes," please specify

a. _____

_____

b. _____

_____

c. _____

_____

25. Did you experience any other indirect benefits on your operation from being involved in the VBJDC program?

a. Yes
b. No

If "Yes," please specify

a. _____

_____

b. _____

_____

c. _____

_____

26. When you decided to put the VBJDC into action, did any requirements of this program conflict with your usual management practices at the time?

a. Yes
b. No

If "Yes," please specify

a. _____

_____

b. _____

_____

c. _____

_____

Please use this sheet to any complete any questions from the preceding pages or to make any relevant comments.

Thank you for your time.

BEST WISHES IN THE FUTURE!

Please return your completed questionnaire in the enclosed Self Addresses Stamped Envelope to us at:

Department of Veterinary Integrative Biosciences
Texas A & M University
College Station, Texas 77843-4458

APPENDIX B

INFORMATION SHEET
Epidemiologic investigations of Johne's disease in beef cattle: Attitudes towards participation in the voluntary control program

You have been asked to participate in this research project because you are a cattle producer in Texas. The purpose of this study is to determine factors associated with the transmission of Johne's disease (Paratuberculosis) in beef cattle and to improve participation in voluntary control programs. If you agree to participate in the study you need only to complete the enclosed questionnaire and return it in the prepaid self-addressed envelope.

This study is confidential and the records of this study will be kept private. All data will be stored in locked file cabinets and on password protected computers. No identifiers linking you to the study will be included in any sort of report that might be published. Research records will be stored securely and only Dr. Geoff Fosgate and other researchers directly associated with this study will have access to the records. Your decision whether or not to participate will not affect your current or future relations with Texas A&M University and the Texas Voluntary Johne's Disease Program administered through the Texas Animal Health Commission. If you decide to participate, you are free to refuse to answer any of the questions that might make you uncomfortable. You can withdraw at any time without your relations with the university, job, benefits, etc., being affected. If you have further questions, you can contact me, Dr. Geoff Fosgate, Assistant Professor of Epidemiology at (979) 845-3203 (gfosgate@cvm.tamu.edu). In the event that I am not available, Dr. Evelyn Tiffany-Castiglioni the Department Head of Veterinary Integrative Biosciences can be contacted at (979) 845-0733 (ecastiglioni@cvm.tamu.edu).

This research study has been reviewed by the Institutional Review Board - Human Subjects in Research, Texas A&M University. For research-related problems or questions regarding subjects' rights, you can contact the Institutional Review Board through Ms. Angelia M. Raines, Director of Research Compliance, Office of the Vice President for Research at (979)458-4067 (araines@vprmail.tamu.edu).

A SURVEY OF PRODUCERS' ATTITUDES TOWARDS THE

TEXAS VOLUNTARY JOHNE'S DISEASE PROGRAM IN CATTLE

Please complete the questions that pertain to you even if you do not currently raise cattle

1. Do you raise any of the following animals? (*Please check all that apply*)

   ☐ Beef cattle ☐ Dairy cattle ☐ Sheep

   ☐ Goats ☐ White-tailed deer ☐ Other

2. What is the total number of cattle in your herd? _____ head

3. How many cattle do you have that are 2 years or older?

   ☐ Cows _____ ☐ Bulls _____

   ☐ Steers _____

4. What type of cattle do you raise? (*Please check all that apply*)

   ☐ Commercial cow-calf (Registered) ☐ Commercial cow-calf (Not-registered)

   ☐ Seed stock herd (Registered) ☐ Seed stock (Not-registered)

   ☐ Feedlot ☐ Mixed

   ☐ Other (*Please state*) _____

5. Which of the following do you sell? (*Please check all that apply*)

☐ Feeder calves ☐ Young animals for breeding

☐ Mature animals for breeding ☐ Embryos/semen

☐ Cull cows

☐ Other (*Please state*) _____

6. Do you have other employment independent of your cattle herd?

☐ Yes ☐ No

7. What is the age of the major decision maker for the ranch? _____ years

8. What was the highest educational level completed by the major decision maker?

☐ Less than a high school diploma ☐ High school diploma

☐ 2 year college degree ☐ Bachelor's degree

☐ Graduate degree ☐ Professional degree

9. How much longer do you and/or your family members plan to raise cattle?

☐ 0-1 years ☐ 1-5 years ☐ 5-10 years

☐ 10-15 years ☐ More than 15 years Unsure ☐ Indefinitely

☐ Unsure

10. I am interested in receiving additional education or information about herd health from trade magazines.

☐ Yes ☐ No

11. I am interested in receiving additional education or information about herd health from seminars.

☐ Yes ☐ No

12. Is your ranch enrolled in the Brucellosis and/or Tuberculosis programs for cattle?

☐ Yes ☐ No

For questions 13-15, please <u>circle</u> a single response that best describes your level of agreement with each statement.

13. I manage my herd to maximize profit.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

14. I manage my herd to have a stable income.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

15. I manage my herd to produce quality stock.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

16. Do you test purchased cattle for diseases before adding them to the herd?

☐ Often ☐ Sometimes ☐ Never

If you do test, please list the tests that are performed.

_____

_____

17. Over the past 5 years, on average, how many new animals have you added to your herd each year? _____head/year

18. Which of the following are sources of purchased cattle on your ranch? (*Please check all that apply)*

☐ Private sales ☐ Production sales

☐ Public auction markets ☐ Not applicable

19. Do you sell cattle to other states of the US? (*Please check one response)*

☐ Often ☐ Sometimes ☐ Never

20. Do you export cattle to other countries? (*Please check one response)*

☐ Often ☐ Sometimes ☐ Never

21. Do cattle from neighboring ranches gain access to your premises?
(*Please check one response)*

☐ Often ☐ Sometimes ☐ Never

For questions 22 and 23, please <u>circle</u> a single response that best describes your level of agreement with each statement.

22. I believe that there is a financial benefit in operating a closed herd.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

23. I believe that a closed herd benefits disease control.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

---

24. I have heard of Johne's disease (Paratuberculosis).

☐ Yes ☐ No

25. Please rank the following sources of information in terms of their usefulness to you in understanding Johne's disease. (*Rank from 1-4 with 1 being the most useful*)

Trade magazines _____

Scientific journals _____

The internet _____

Other source(s) _____

☐ I am not familiar with Johne's disease

26. Please rank the following in terms of how useful they are to you as sources of information about cattle diseases? (*Rank from 1-8 with 1 being the most useful*)

Veterinarians _____
Other ranchers _____
Trade magazines _____
Scientific journals _____
Extension personnel _____
The internet _____
Producer's organizations _____
Other source(s) _____

☐ I am not familiar with Johne's disease

For questions 27 – 41, please <u>circle</u> a single response that best describes your level of agreement with each statement.

27. I am quite familiar with Johne's disease in general.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

28. I am quite familiar with factors associated with transmission of the organism that causes Johne's disease.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

29. I do not have a problem with Johne's disease on my ranch.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

30. I am familiar with the Texas Voluntary Johne's Disease Program.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

31. I have considered participation in the Texas Voluntary Johne's Disease Program.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

32. I know how to control and prevent Johne's disease from becoming a problem on my ranch.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

33. Ranch managers should include Johne's disease on the list of diseases to be tested for purchased cattle.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

34. Newly purchased cattle should be quarantined until Johne's disease test results are received.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

35. Johne's disease is responsible for significant production losses in beef cattle in Texas.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

36. Testing is not helpful for the control of the Johne's disease.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

37. Johne's disease in beef cattle can be controlled through testing and culling clinical suspects alone.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

38. Serum ELISA is a good test for confirming clinical suspects with Johne's disease.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

39. Fecal culture is good test for confirming clinical suspects with Johne's disease.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

40. Management changes are helpful for Johne's disease control.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

41. Water contamination with feces significantly increases the risk of transmission of Johne's disease on beef ranches.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

42. Which of the two following options is better for controlling Johne's disease in beef herds?

☐ A regulatory control program

☐ A voluntary subsidized control program

Please complete the questions (43-53) on the following pages if you are a beef commercial cow/calf or seedstock producer

For question 43, please indicate your level of agreement with each proposed management practice for the control of Johne's disease on beef ranches by <u>circling</u> a single response.

43. Concerning the following management practices for the control of Johne's disease on beef ranches, do you feel that:

a. Separating Johne's disease clinicals or suspects from calves or heifers is useful for control.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

b. Separating weaned heifers and bull calves from mature cows is useful for control.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

c. Separating bred heifers and yearling bulls from mature cows is useful for control.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

d. Getting replacements or additions from herds at low risk for Johne's disease is useful for control.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

e. Testing cattle for Johne's disease every 10-14 months is useful for control.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

f. Decreasing cow-calf pair density is useful for control.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

g. Improving sanitation of the calving area (reducing manure contamination and limiting moisture) is useful for control.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

44. How many acres do you have for your herd? _____ acres

45. Over the previous 12 months, how did you manage grazing?

☐ Rotational ☐ Continuous (single pasture)

☐ Management- intensive ☐ Other (*Please state*)

_____

46. What times of the year do you work your cattle? (*Please state*)

_____

47. How long is your calving season? _____ months

48. Do cows and weaned calves have separate feeding and drinking areas or are you willing to provide separate feeding and drinking areas for cows and weaned calves as part of a disease control program?

☐ Yes ☐ No

49. Do you have or are you willing to provide a separate pasture or pen for newly purchased cattle as part of a disease control program?

☐ Yes ☐ No

50. Do you have or are you willing to provide a separate pen or pasture for sick animals as part of a disease control program?

☐ Yes ☐ No

51. Do you normally use or are you willing to use artificial insemination as part of a disease control program?

☐ Yes ☐ No

52. If your cattle participate in shows or other events, would you be willing to quarantine and re-test them for Johne's disease after each show as a part of a control program?

☐ Yes ☐ No

53. Do you spread cattle manure on your pastures? (*Please check one response)*

☐ Often ☐ Sometimes ☐ Never

If you do spread manure on your pastures, what is the source of manure? (*Please check all that apply*)

☐ Your own herd ☐ A dairy herd ☐ A beef herd

☐ Other (*Please specify*) _____

Do you know the Johne's disease status of the herd that is the source of the manure?

☐ Yes ☐ No

Please return the completed survey in the postage-paid envelope.

Thank you for taking the time to complete this survey!

APPENDIX C

INFORMATION SHEET
Epidemiologic investigations of Johne's disease in beef cattle: Attitudes towards participation in the voluntary control program

You have been asked to participate in this research project because you are a veterinarian working in bovine or mixed large animal practice and a member of the Texas Veterinary Medical Association (TVMA). The purpose of this study is to determine factors associated with the transmission of Johne's disease in beef cattle and to improve participation in voluntary control programs. If you agree to participate in the study you need only to complete the enclosed questionnaire and return it in the prepaid self-addressed envelope.

This study is confidential and the records of this study will be kept private. All data will be stored in locked file cabinets and on password protected computers. No identifiers linking you to the study will be included in any sort of report that might be published. Research records will be stored securely and only Dr. Geoff Fosgate and other researchers directly associated with this study will have access to the records. Your decision whether or not to participate will not affect your current or future relations with Texas A&M University and the Texas Voluntary Johne's Disease Program administered through the Texas Animal Health Commission. If you decide to participate, you are free to refuse to answer any of the questions that might make you uncomfortable. You can withdraw at any time without your relations with the university, job, benefits, etc., being affected. If you have further questions, you can contact me, Dr. Geoff Fosgate, Assistant Professor of Epidemiology at (979) 845-3203 (gfosgate@cvm.tamu.edu). In the event that I am not available, Dr. Evelyn Tiffany-Castiglioni the Department Head of Veterinary Integrative Biosciences can be contacted at (979) 845-0733 (ecastiglioni@cvm.tamu.edu).

This research study has been reviewed by the Institutional Review Board - Human Subjects in Research, Texas A&M University. For research-related problems or questions regarding subjects' rights, you can contact the Institutional Review Board through Ms. Angelia M. Raines, Director of Research Compliance, Office of the Vice President for Research at (979)458-4067 (araines@vprmail.tamu.edu).

A SURVEY OF VETERINARIANS' ATTITUDES TOWARDS THE TEXAS VOLUNTARY JOHNE'S DISEASE PROGRAM FOR BEEF HERDS

Please complete the questionnaire even if you do not have many beef clients

1. What type of practice do you operate?

   ☐ Retired ☐ Bovine exclusive

   ☐ Large animal – mixed species ☐ Other (*specify*) _____

2. How frequently do you develop herd health programs for beef producers?

   ☐ Often ☐ Sometimes ☐ Never

3. Are you a Johne's disease certified veterinarian?

   ☐ Yes ☐ No

   If "Yes," how many herds have you enrolled in the Texas Voluntary Johne's Disease Program? _____ herds

   If "No," Have you thought about becoming a Johne's disease certified veterinarian?

   ☐ Yes ☐ No

4. Approximately, how many beef clients do you have? _____

5. Among your beef clients, approximately what percentages are represented by the following types of operations? (*Responses should sum to 100%*)

Seed stock herd	_____ %
Cow/calf commercial	_____ %
Feedlot	_____ %
Mixed	_____ %
Total	100 %

6. What percentage of your clients raise registered cattle? _____%

7. Please rank the following sources of information in terms of their usefulness to you in explaining Johne's disease to your beef clients.
(*Rank from 1-4 with 1 being the most useful*)

Trade magazines	_____
Scientific journals	_____
The internet	_____
Other source(s)	_____

8. Please rank the following in terms of how popular they are with your beef clients as sources of information about cattle diseases?
(*Rank from 1-8 with 1 being the most popular*)

Veterinarians _____
Other ranchers _____
Trade magazines _____
Scientific journals _____
Extension personnel _____
The internet _____
Producer's organizations _____
Other source(s) _____

9. During the past 3 years:

What percentage of client-owned ranches with whom you work had cattle 2 years of age and older that showed signs of chronic diarrhea and/or weight loss?
_____%

What percentage of client-owned ranches with whom you work was affected with Johne's disease? _____%

***Please indicate your opinion of the statements listed as questions 10 – 14,* by *circling* a single response that best describes your level of agreement.**

10. Ranch managers should include Johne's disease on the list of diseases to be tested for purchased cattle.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

11. Newly purchased cattle should be quarantined until Johne's disease test results are received.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

12. Johne's disease is responsible for significant losses in beef cattle production in Texas.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

13. Johne's disease in beef cattle can be controlled through testing and culling clinical suspects alone.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

14. The producer's goal should be elimination of the disease agent:

If Johne's disease is present in a commercial cow/calf herd.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

If Johne's disease is present in a registered cow/calf herd.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

---

15. Has Johne's disease been eliminated from any of your clients' herds following the implementation of a control program?

☐ Yes ☐ No

16. Have you educated one or more beef producers in any of the following areas of Johne's disease during the past 3 years?

The basic cause of Johne's disease	☐ Yes	☐ No
Stages of disease	☐ Yes	☐ No
Transmission of the etiologic agent	☐ Yes	☐ No

17. Have you discussed management strategies to control or eliminate Johne's disease with one or more of your beef clients during the past 3 years?

☐ Yes ☐ No

18. Have you discussed available tests for Johne's disease with one or more of your beef clients during the past 3 years?

Fecal culture	☐ Yes	☐ No
Fecal PCR	☐ Yes	☐ No
ELISA	☐ Yes	☐ No
Interferon-gamma	☐ Yes	☐ No

19. Have you discussed the interpretation of tests for Johne's disease with one or more of your beef clients during the past 3 years?

☐ Yes ☐ No

For questions 20-28, please <u>circle</u> a single response that best describes your level of agreement with each statement.

20. I feel that my beef clients would be willing to separate cattle with clinical or suspected Johne's disease from calves and heifers.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

21. I feel that my beef clients would be willing to separate weaned heifers and bullcalves from mature cows.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

22. I feel that my beef clients would be willing to separate bred heifers and yearling bulls from mature cows.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

23. I feel that my beef clients would be willing to obtain replacements or additions from herds that are at low risk for having Johne's disease.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

24. I feel that my beef clients would be willing to test cattle for Johne's disease every 10-14 months.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

25. In my opinion, the following management conditions increase the risk of transmission of Johne's disease in <u>beef</u> cattle:

Feeding cattle on the ground in calving areas.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

Having drinking water that is contaminated with feces.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

Allowing cattle to drink from ponds and streams.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

Having increased cow-calf pair density.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

26. Concerning the fecal culture test for Johne's disease:

I feel that this test is useful for informing producers about the presence and extent of Johne's disease on their ranches.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

I feel that it is cost-effective for beef producers.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

I feel that the rapidity with which results are returned makes it useful for decision-making about Johne's disease on ranches.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

27. Concerning fecal PCR for Johne's disease:

I feel that this test is useful for informing producers about the presence and extent of Johne's disease on their ranches.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

I feel that it is cost-effective for beef producers.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

I feel that the rapidity with which results are returned makes it useful for decision- making about Johne's disease on ranches.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

28. Concerning the serum ELISA for Johne's disease:

I feel that this test is useful for informing producers about the presence and extent of Johne's disease on their ranches.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

I feel that it is cost-effective for beef producers.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

I feel that the rapidity with which results are returned makes them useful for decision- making about Johne's disease on ranches.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

29. Have you submitted any of the following samples for Johne's disease testing on behalf of your beef clients during the past 3 years?

Fecal culture	☐ Yes	☐ No
Fecal PCR	☐ Yes	☐ No
ELISA	☐ Yes	☐ No
Other (*specify*) _____	☐ Yes	☐ No

30. Have you heard of the Texas Voluntary Johne's Disease Program?

☐ Yes ☐ No

31. Have you recommended the Texas Voluntary Johne's Disease Program to beef producers during the past 3 years?

☐ Yes ☐ No

If "yes," duing the past 3 years approximately how many beef producers did you recommend this program to? _____

32. What percentages of the beef producers to whom you recommended the Texas Voluntary Johne's Disease Program fell within each of the following classes?

☐ Confirmed infected _____%

☐ Suspected to be infected _____ %

☐ Known uninfected _____%

☐ Suspected to be uninfected _____%

☐ Not applicable

33. What percentage of your beef clients have heard of Johne's disease when you discussed it with them?

☐ _____% ☐ Don't know ☐ Have not discussed

34. What percentage of the beef producers that you have asked to join the Texas Voluntary Johne's Disease Program have enrolled?_____ %

35. Were any of the following reasons given by beef producers for declining enrollment in the Texas Voluntary Johne's Disease Program? (*Check all that apply and rank the checked responses in terms of frequency with 1 being the most frequent*)

☐ Program was too expensive _____

☐ Required management changes were not feasible _____

☐ Time commitment was too high _____

☐ Labor commitment was too high _____

☐ Considered inconvenient to round-up animals for annual testing_____

☐ Tests not reliable for making decisions about Johne's disease _____

☐ Not convinced that Johne's disease was an important problem _____

☐ Other (*specify)* _____ _____

36. Have you participated in the Texas Voluntary Johne's Disease Program's training program?

☐ Yes ☐ No

37. The rules governing participation in the Texas Voluntary Johne's Disease Program are clearly explained in the training program. (***Please circle the <u>single</u> response that best describes your level of agreement with this statement.***)

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

☐ Not applicable

38. Have you read the document which describes the Texas Voluntary Johne's Disease Program for Cattle?

☐ Yes ☐ No

39. Are the requirements of the Texas Voluntary Johne's Disease Program clearly explained in the program document?

☐ Yes ☐ No

☐ Don't know

40. Have you performed a risk assessment for the Texas Voluntary Johne's Disease Program for one or more of your beef clients?

☐ Yes ☐ No

41. In your opinion, how many of the risk assessment components are helpful for the control of Johne's disease in beef cattle?

☐ All ☐ Many

☐ Few ☐ None

☐ Don't know

42. Have you discussed with one or more clients the classification levels for herds in the Texas Voluntary Johne's Disease Program during the past 3 years?

☐ Yes ☐ No

For questions 43 and 44, please <u>circle</u> a single response that best describes your level of agreement with each statement.

43. The existence of a Texas Voluntary Johne's Disease Program should be promoted more widely among veterinarians.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

44. The existence of a Texas Voluntary Johne's Disease Program should be promoted more widely among producers.

Strongly Agree *Agree* *Undecided* *Disagree* *Strongly Disagree*

45. Do you feel that the veterinarian's compensation for working with the Texas Voluntary Johne's disease Program is adequate?

☐ Yes ☐ No

46. What is the current veterinarian compensation from the Texas Voluntary Johne's Disease Program?

$ _____ per risk assessment

$ _____ per herd tested

$ _____ per animal sampled

☐ Unsure

Please return the completed survey in the postage-paid envelope

Thank you for taking the time to complete this survey!

APPENDIX D

Calculation of P_a

Total contamination at the end of the year - based on assumed excretion rates by clinical and subclinical cattle

Total contamination units at the end of the year (C_t) = (Total contamination at end of the year)/(2×10^9)

Contamination units – capable of causing infection in susceptible cattle if ingested

$P_a = 1 - Q^C_t$

Table D-1. Transition matrices for intra herd spread of Johne's disease in beef cows from ages 2 to 10 years

Age (years)	Transition probabilities						
2				To			
			NOTSUSCEP	SUBCLIN	CLIN	DEATH/CULLS	Total
		NOTSUSCEP	0.807	0	0	0.193	1
	From	SUBCLIN	0	0.657	0.15	0.193	1
		CLIN	0	0	0	1	1
		DEATH/CULLS	0	0	0	1	1
3				To			
			NOTSUSCEP	SUBCLIN	CLIN	DEATH/CULLS	Total
		NOTSUSCEP	0.847	0	0	0.153	1
	From	SUBCLIN	0	0.697	0.15	0.153	1
		CLIN	0	0	0	1	1
		DEATH/CULLS	0	0	0	1	1
4				To			
			NOTSUSCEP	SUBCLIN	CLIN	DEATH/CULLS	Total
		NOTSUSCEP	0.840	0	0	0.160	1
	From	SUBCLIN	0	0.690	0.15	0.160	1
		CLIN	0	0	0	1	1
		DEATH/CULLS	0	0	0	1	1

Table D-1 (continued). Transition matrices for intra herd spread of JD in beef cows from ages 2 to 10 years

Age (years)		Transition probabilities				
5			To			
		NOTSUSCEP	SUBCLIN	CLIN	DEATH/CULLS	Total
	NOTSUSCEP	0.826	0	0	0.174	1
From	SUBCLIN	0	0.676	0.15	0.174	1
	CLIN	0	0	0	1	1
	DEATH/CULLS	0	0	0	1	1
6			To			
		NOTSUSCEP	SUBCLIN	CLIN	DEATH/CULLS	Total
	NOTSUSCEP	0.839	0	0	0.161	1
From	SUBCLIN	0	0.689	0.15	0.161	1
	CLIN	0	0	0	1	1
	DEATH/CULLS	0	0	0	1	1
7			To			
		NOTSUSCEP	SUBCLIN	CLIN	DEATH/CULLS	Total
	NOTSUSCEP	0.824	0	0	0.176	1
From	SUBCLIN	0	0.674	0.15	0.176	1
	CLIN	0	0	0	1	1
	DEATH/CULLS	0	0	0	1	1

Table D-1 (continued). Transition matrices for intra herd spread of Johne's disease in beef cows from ages 2 to 10 years

Age (years)	Transition probabilities						
8				To			
			NOTSUSCEP	SUBCLIN	CLIN	DEATH/CULLS	Total
		NOTSUSCEP	0.801	0	0	0.199	1
	From	SUBCLIN	0	0.651	0.15	0.199	1
		CLIN	0	0	0	1	1
		DEATH/CULLS	0	0	0	1	1
				To			
9			NOTSUSCEP	SUBCLIN	CLIN	DEATH/CULLS	Total
		NOTSUSCEP	0.721	0	0	0.279	1
	From	SUBCLIN	0	0.571	0.15	0.279	1
		CLIN	0	0	0	1	1
		DEATH/CULLS	0	0	0	1	1
10				To			
			NOTSUSCEP	SUBCLIN	CLIN	DEATH/CULLS	Total
		NOTSUSCEP	0	0	0	1	1
	From	SUBCLIN	0	0	0	1	1
		CLIN	0	0	0	1	1
		DEATH/CULLS	0	0	0	1	1

APPENDIX E

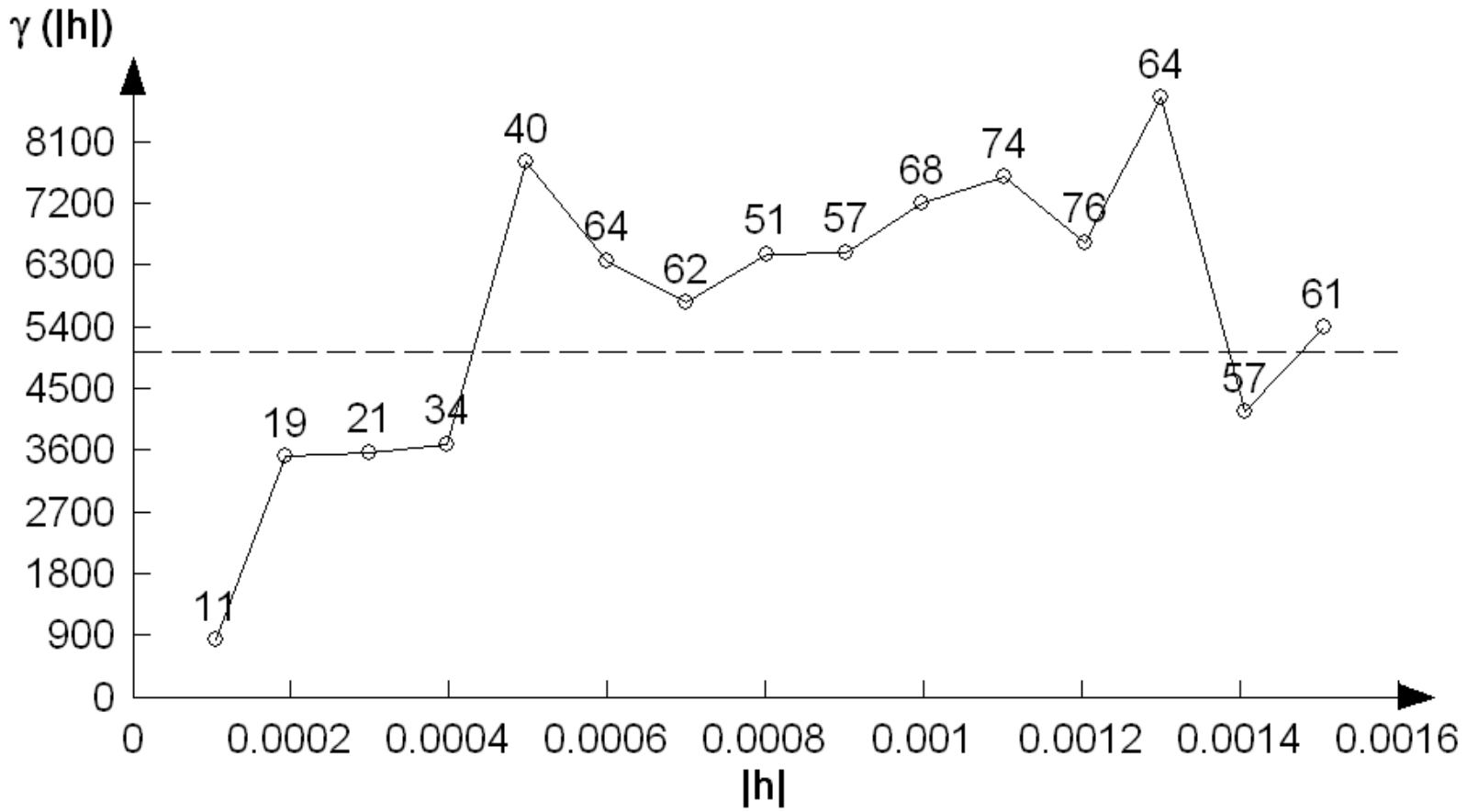


Figure E-1. Semivariogram for iron for Farm 1.

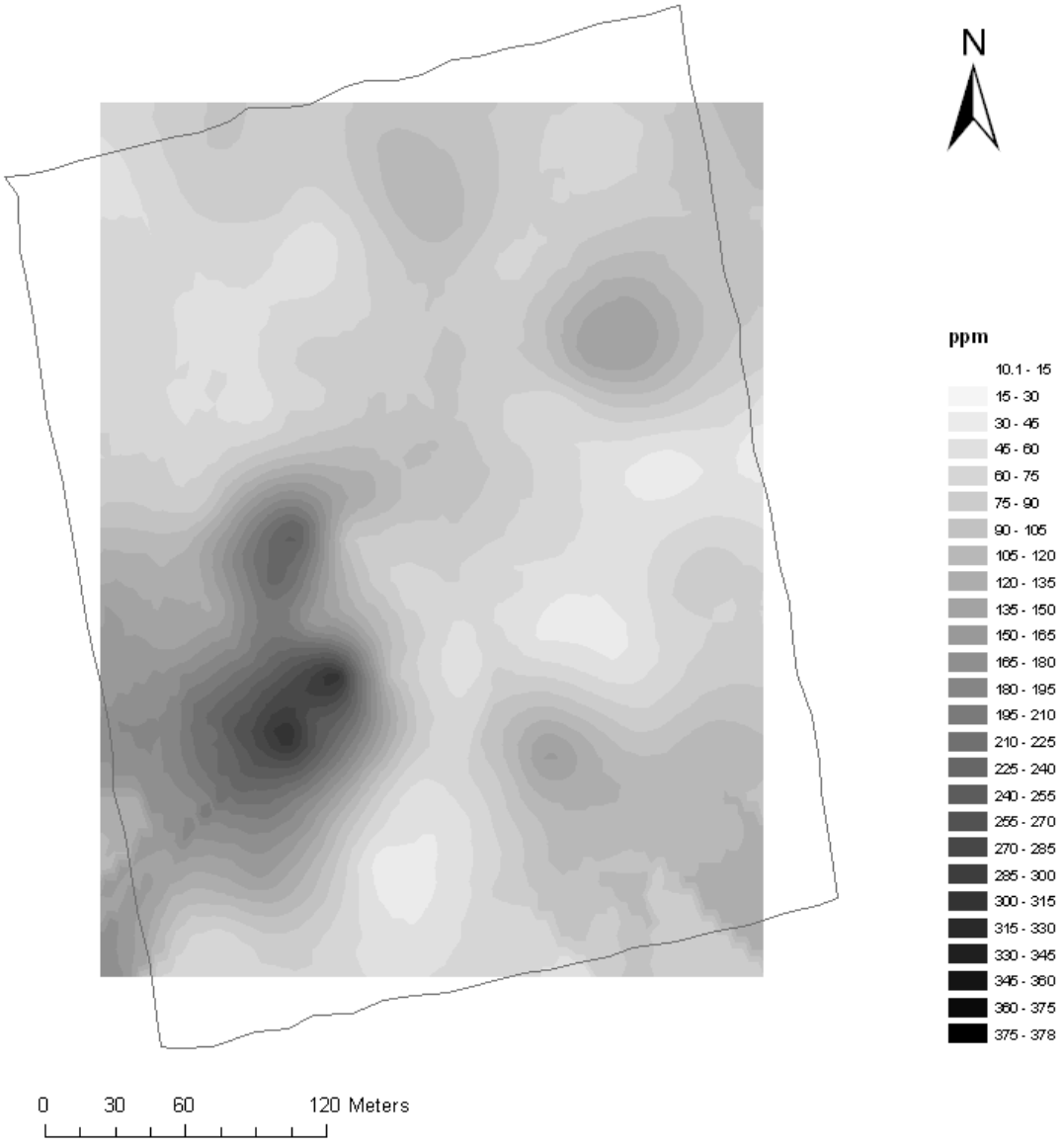


Figure E-2. Soil iron spatial prediction map for Farm 1.

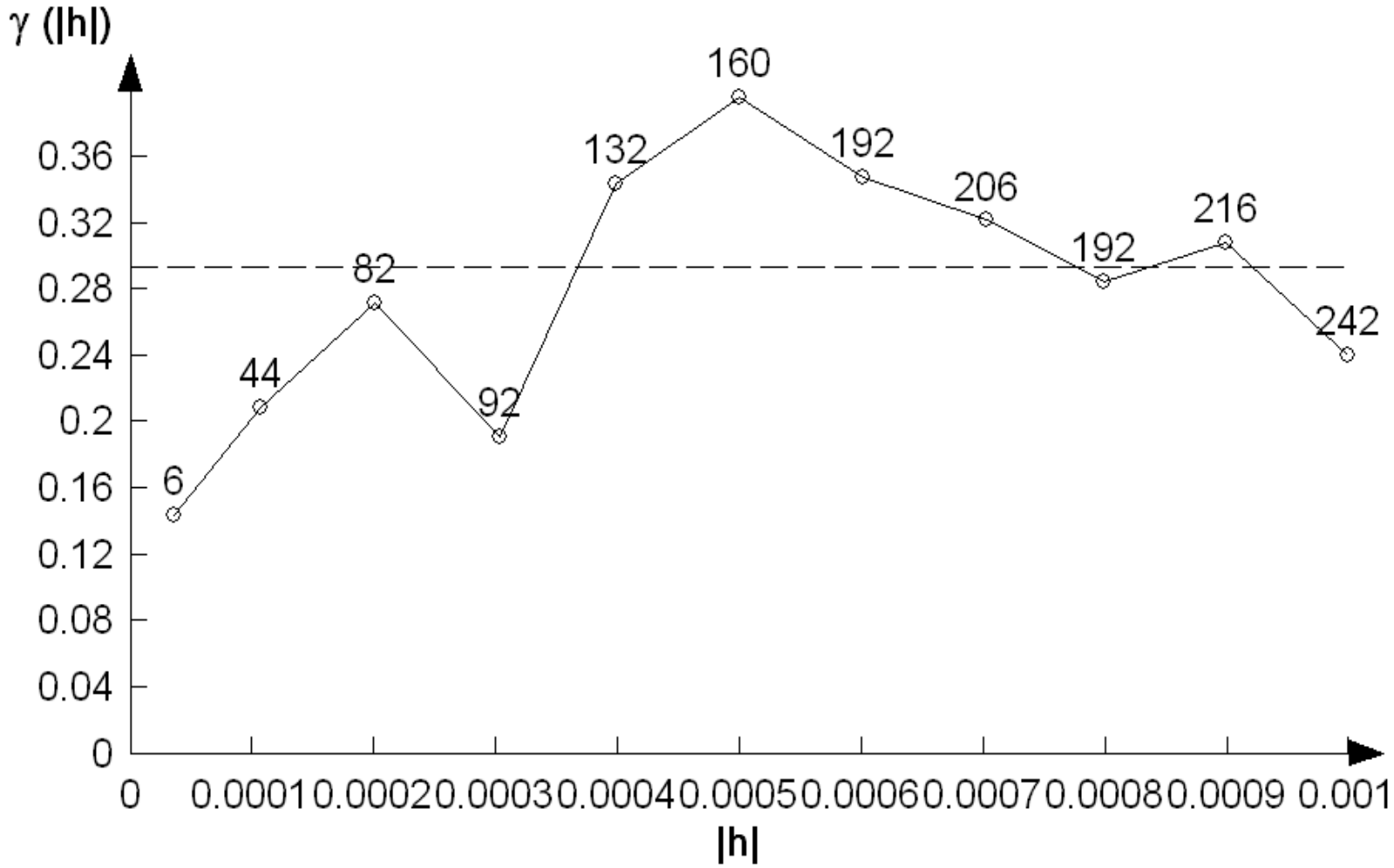


Figure E-3. Semivariogram for pH for Farm 1.

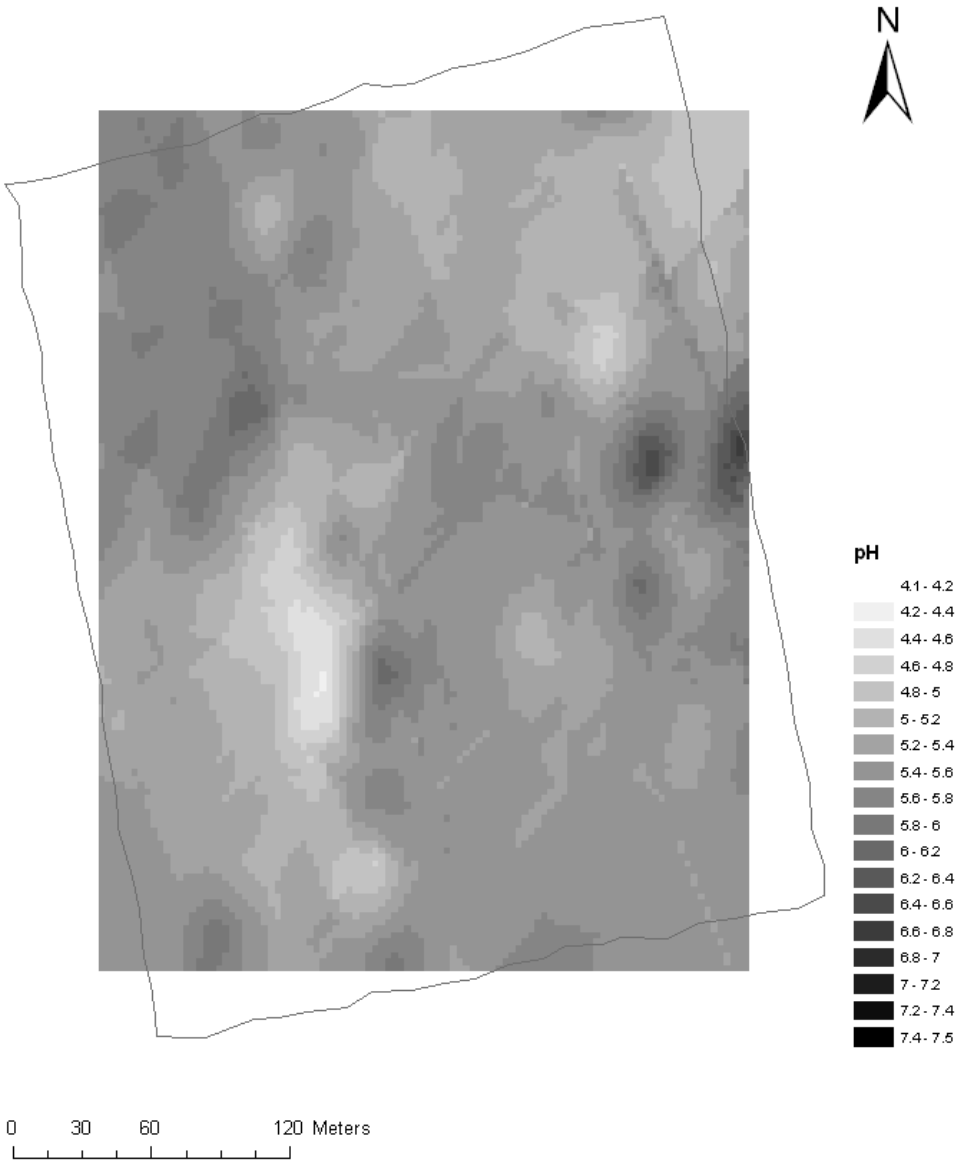
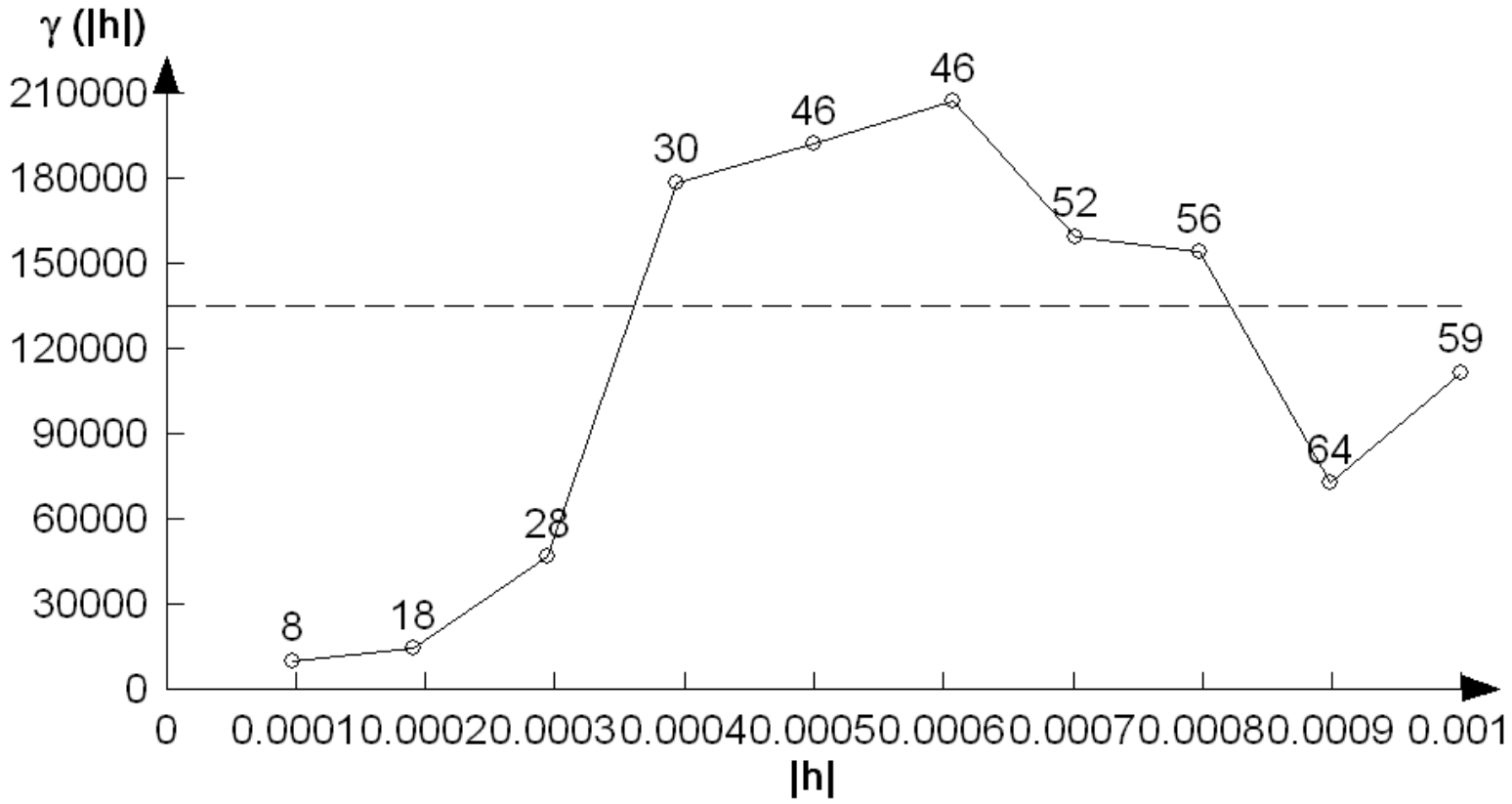


Figure E-4. Soil pH spatial prediction map for Farm 1.



E-5. Semivariogram for calcium for Farm 1.

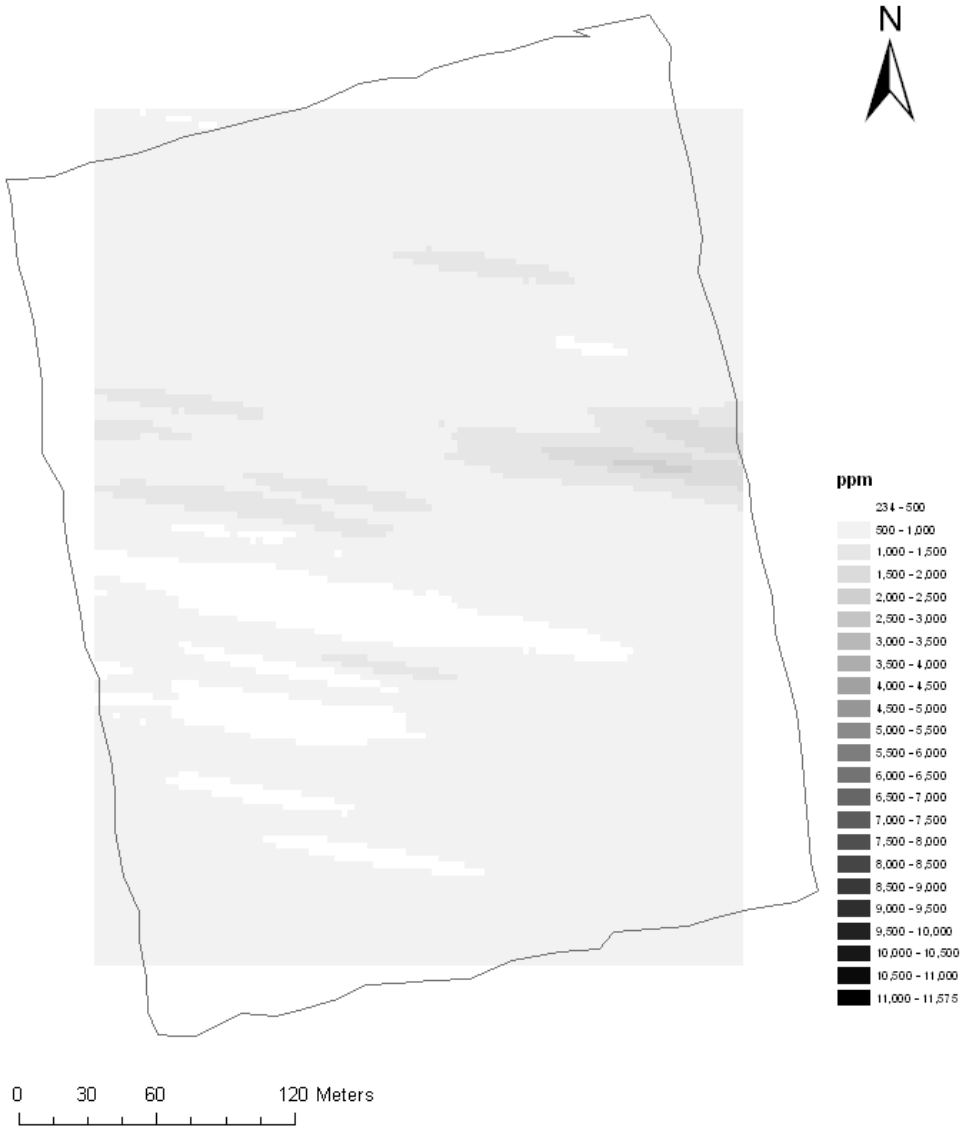


Figure E-6. Soil calcium spatial prediction map for Farm 1.

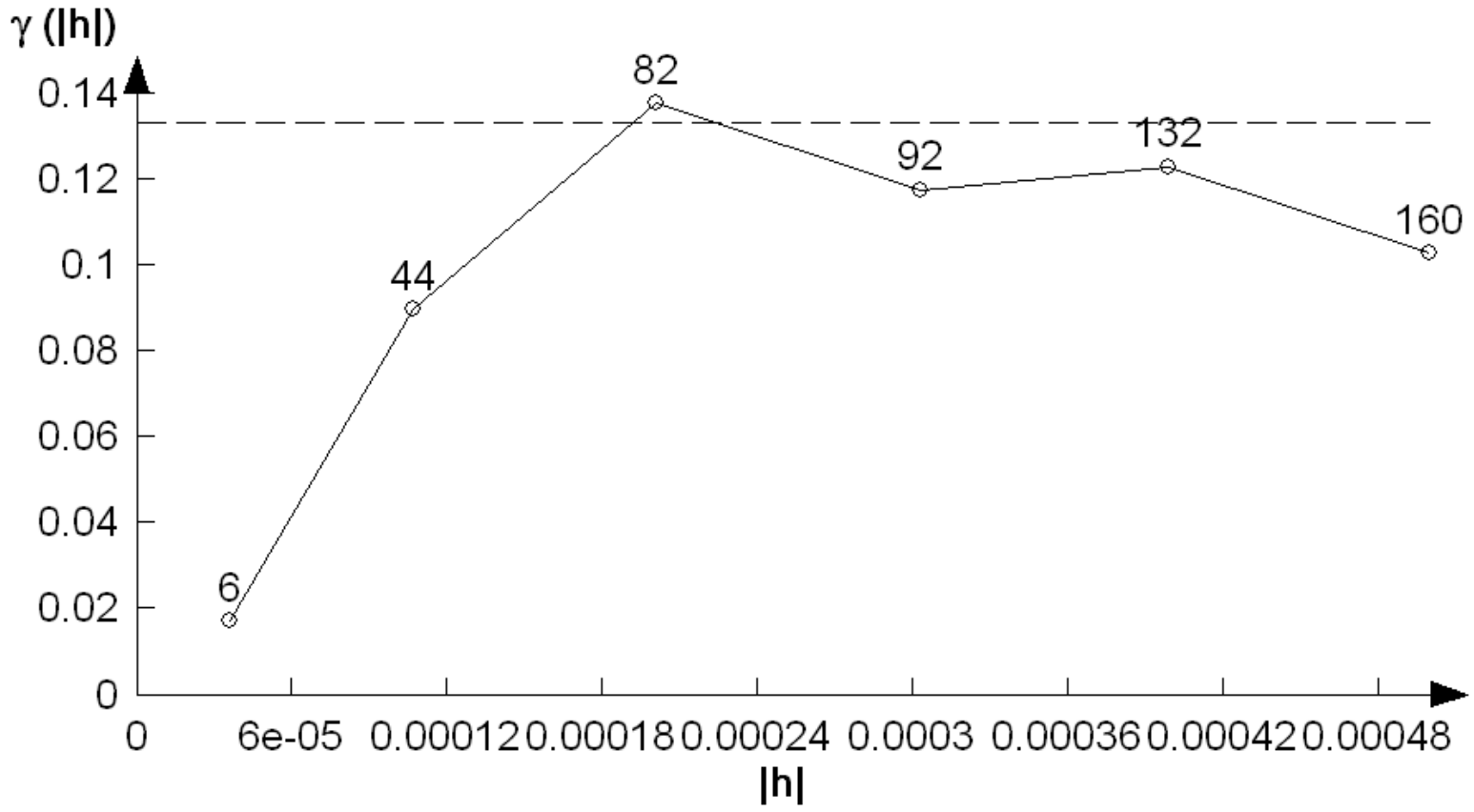


Figure E-7. Semivariogram for iron for Fecal Pat Density.

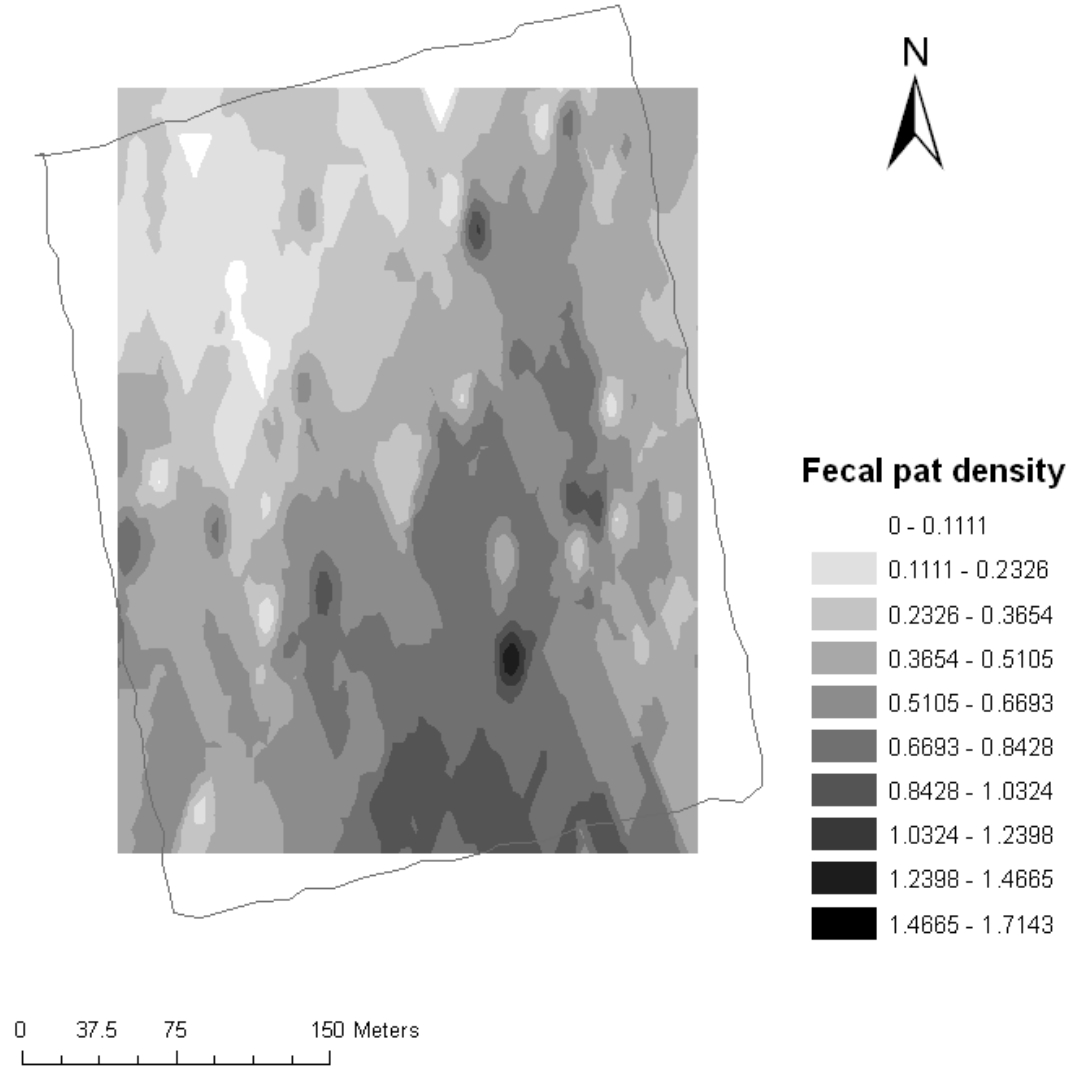
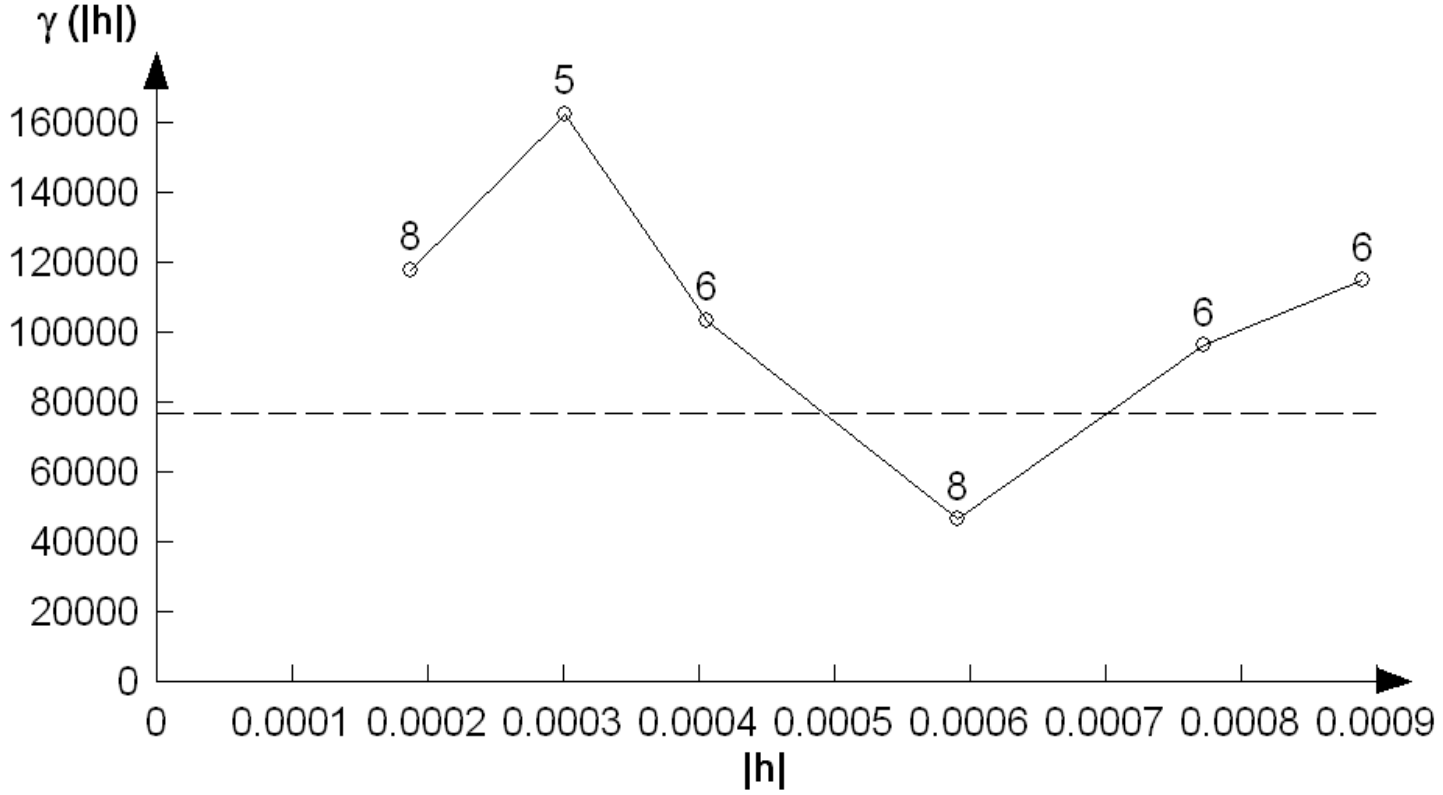


Figure E-8. Fecal pat density spatial prediction map for Farm 1.



FigureE-9. Semivariogram for iron for Farm 2.

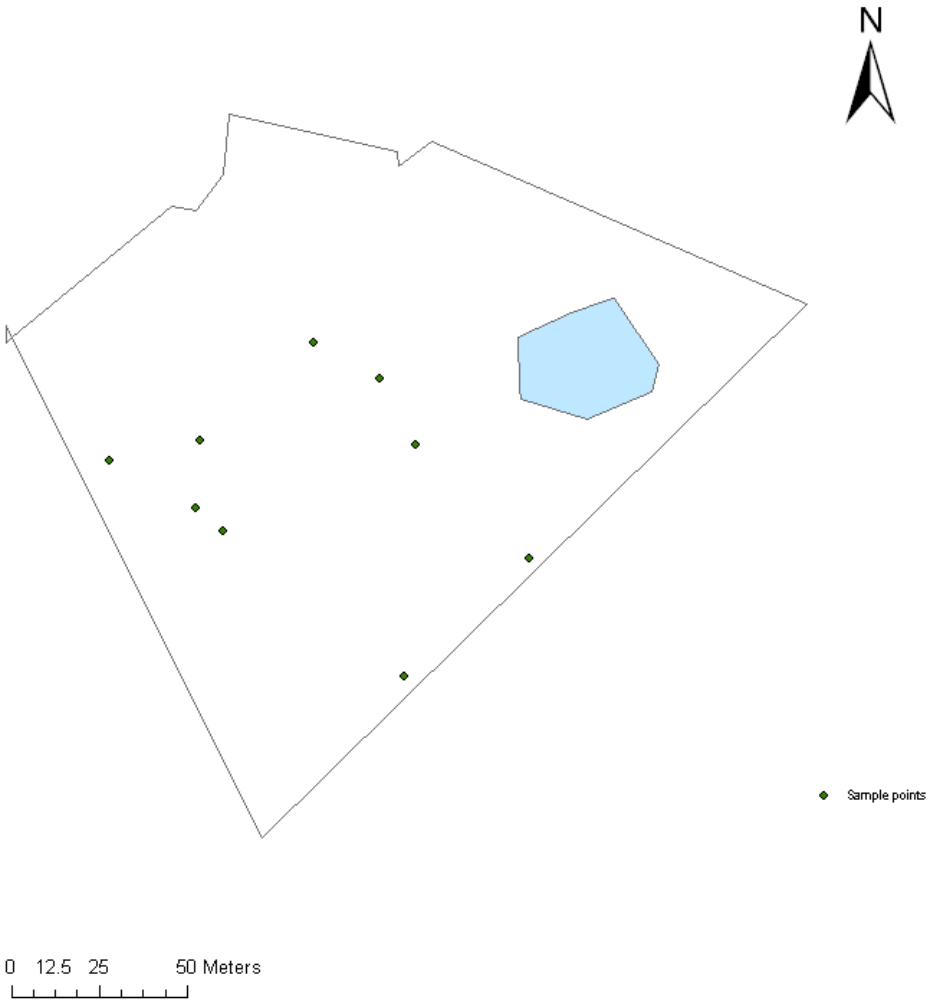


Figure E-10. Soil iron spatial prediction map for Farm 2.

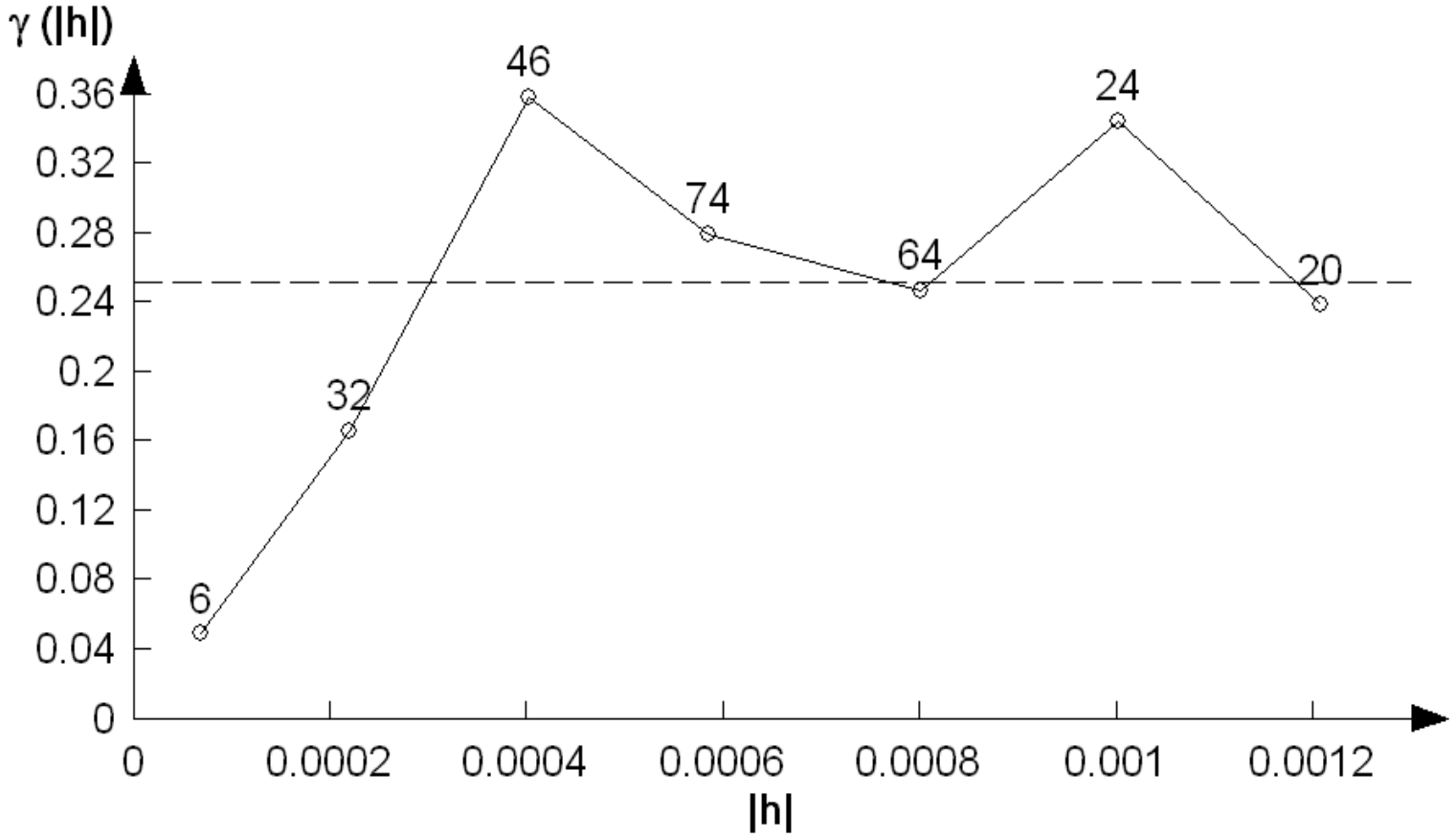


Figure E-11. Semivariogram for pH for Farm 2.

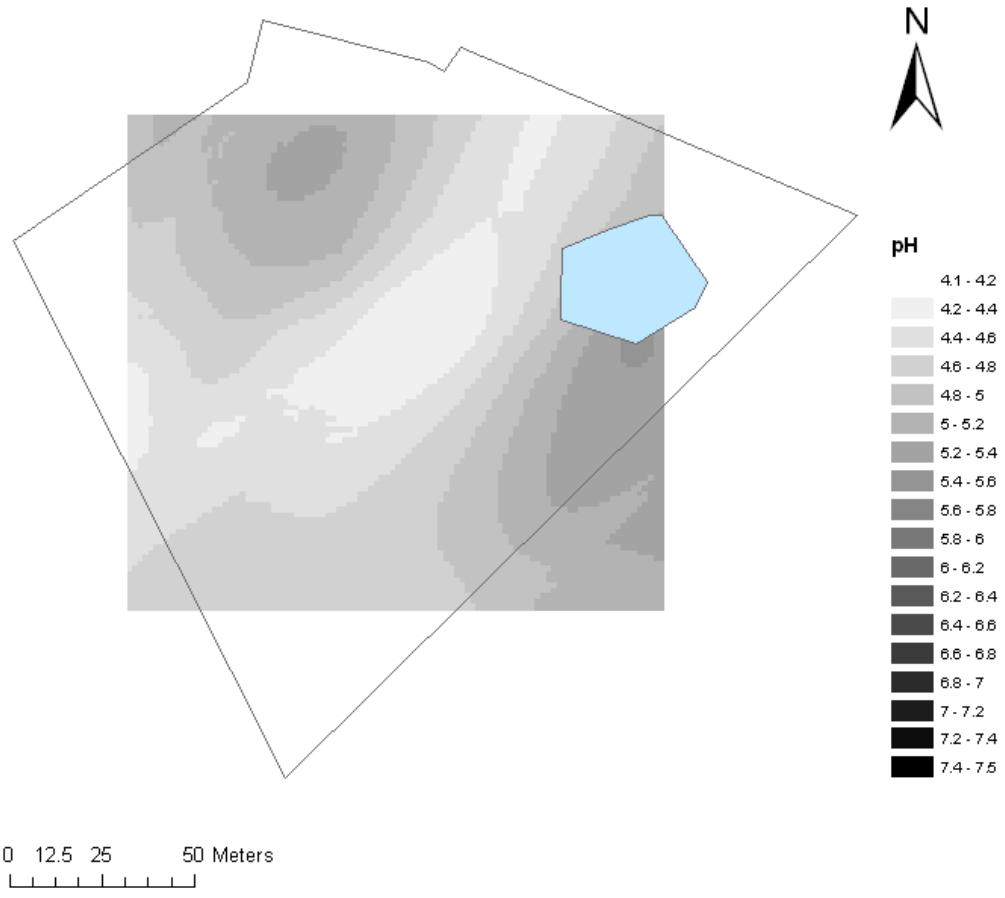


Figure E-12. Soil pH spatial prediction map for Farm 2.

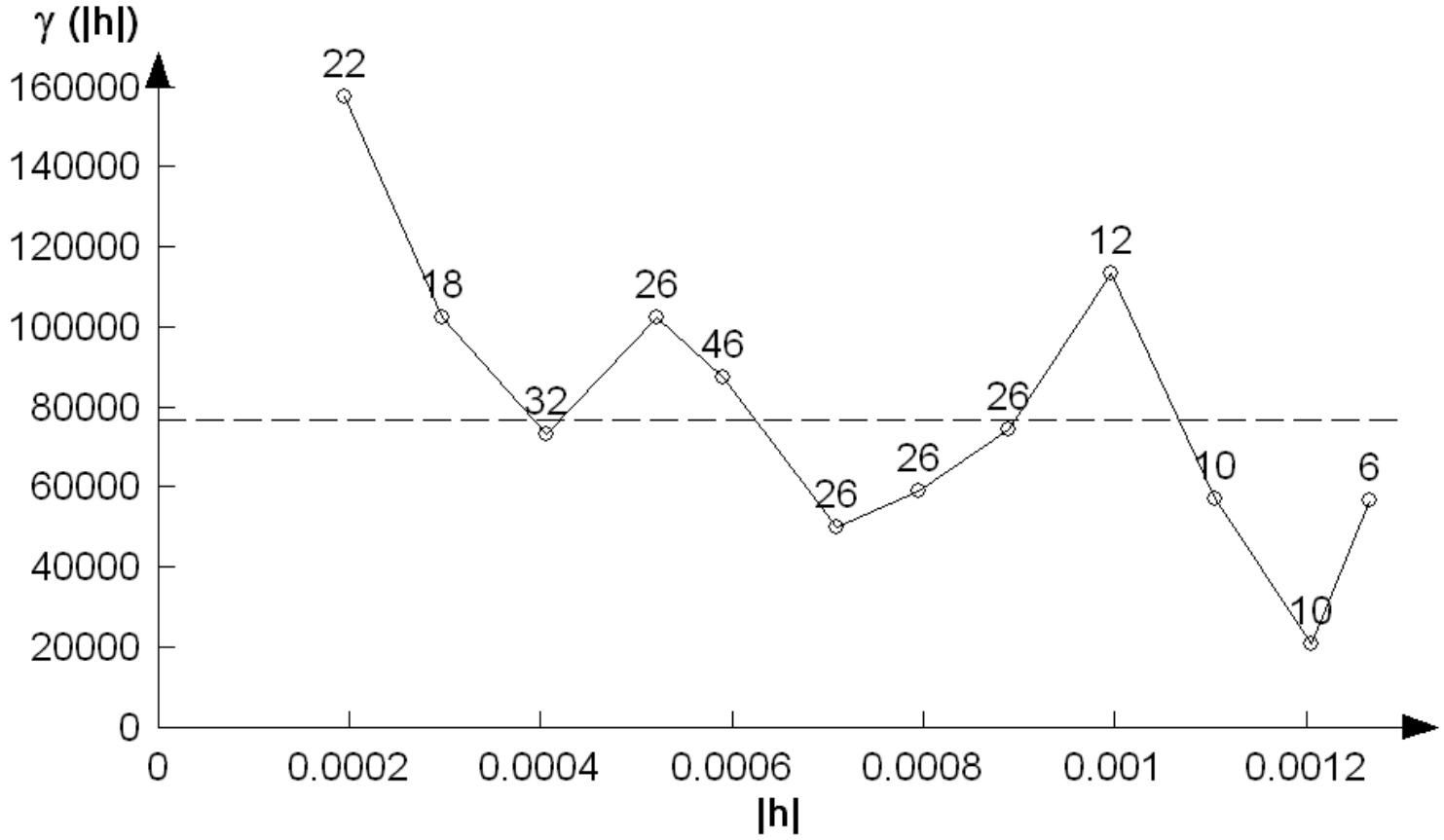


Figure E-13. Semivariogram for calcium for Farm 2.

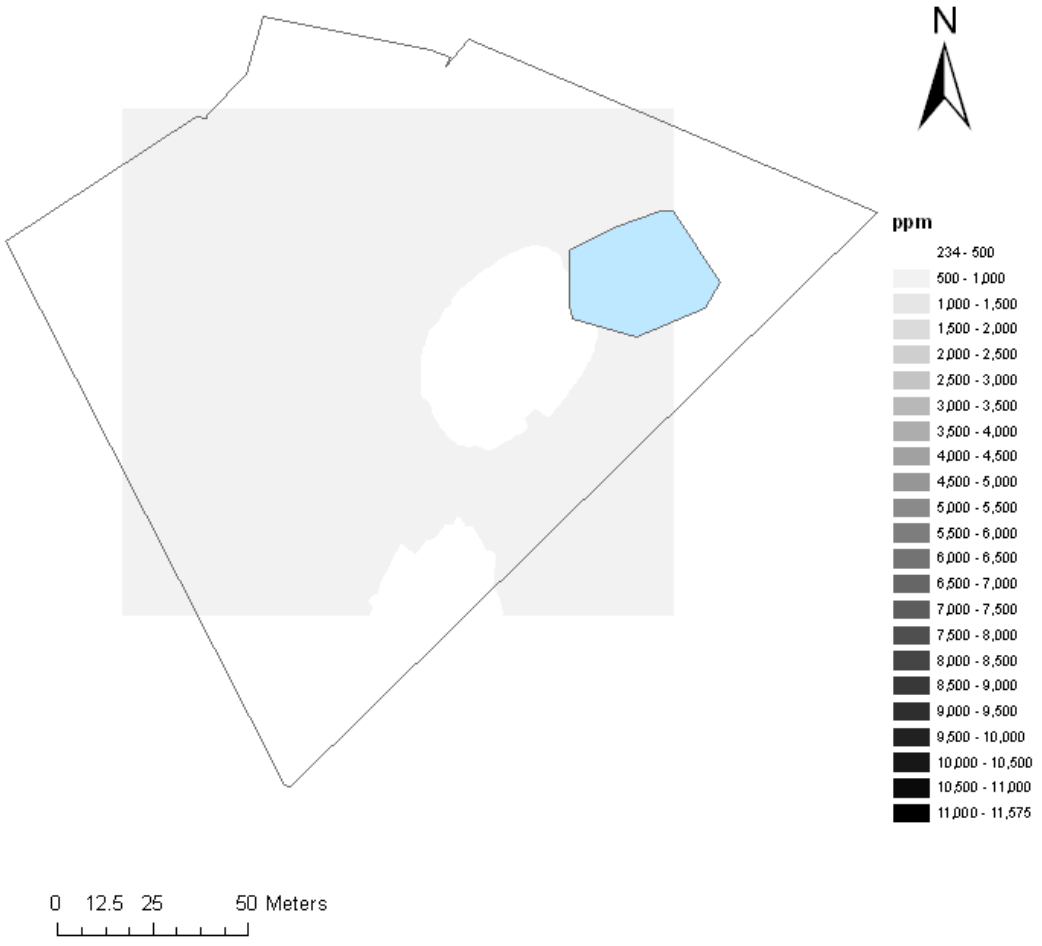


Figure E-14. Soil calcium spatial prediction map for Farm 2.

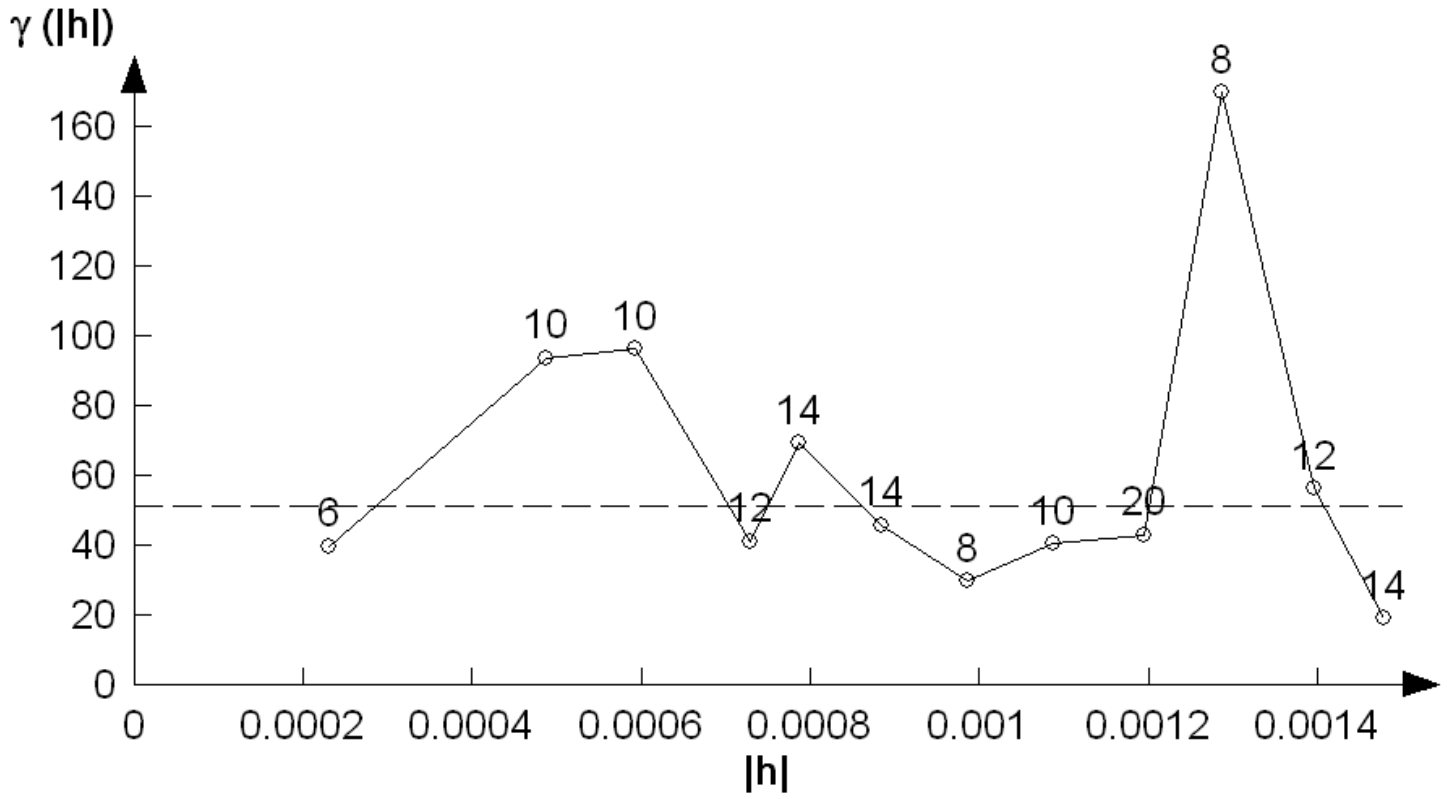


Figure E-15-1. Semivariogram for iron for Farm 3 (Feeding area).

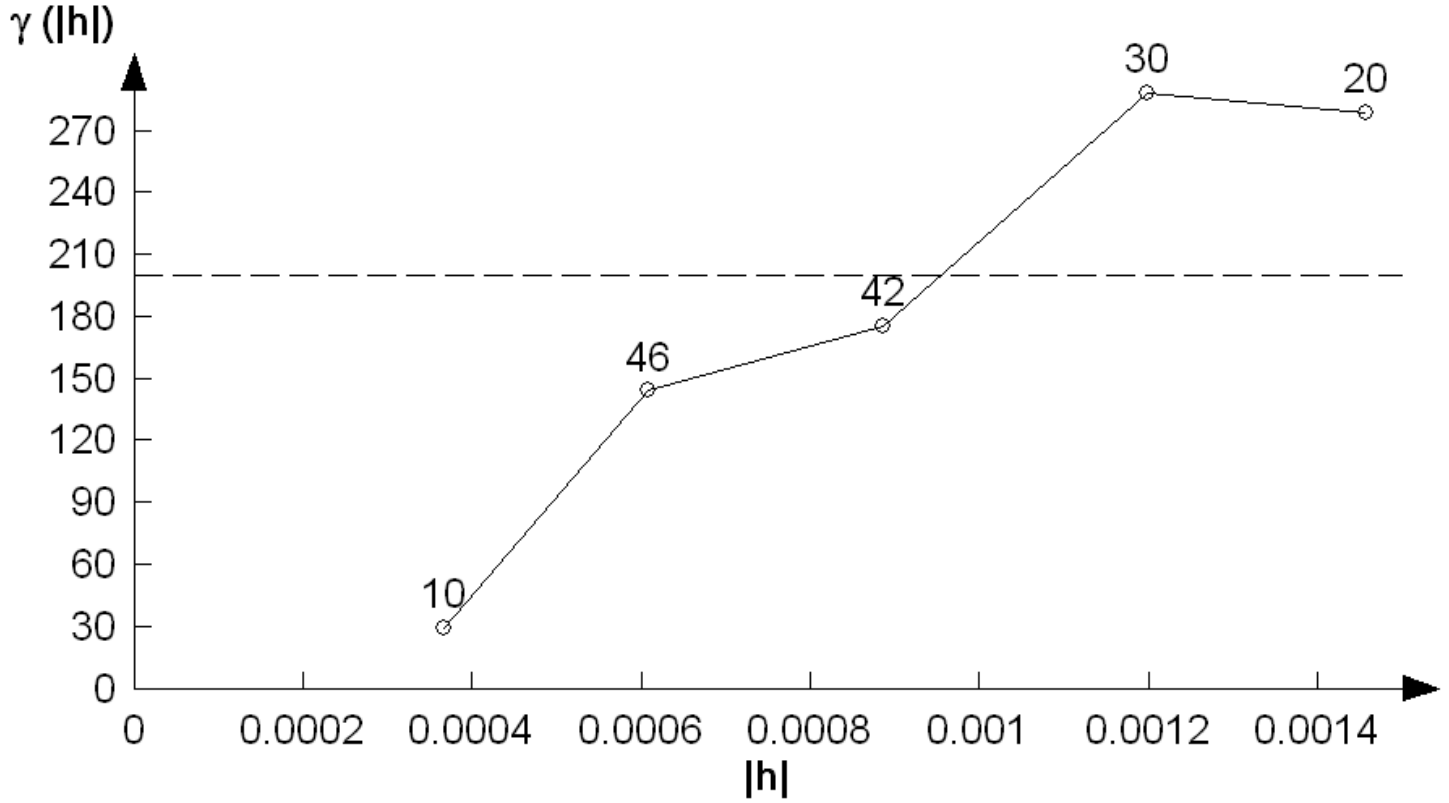


Figure E-15-2. Semivariogram for iron for Farm 3 (Pond area).

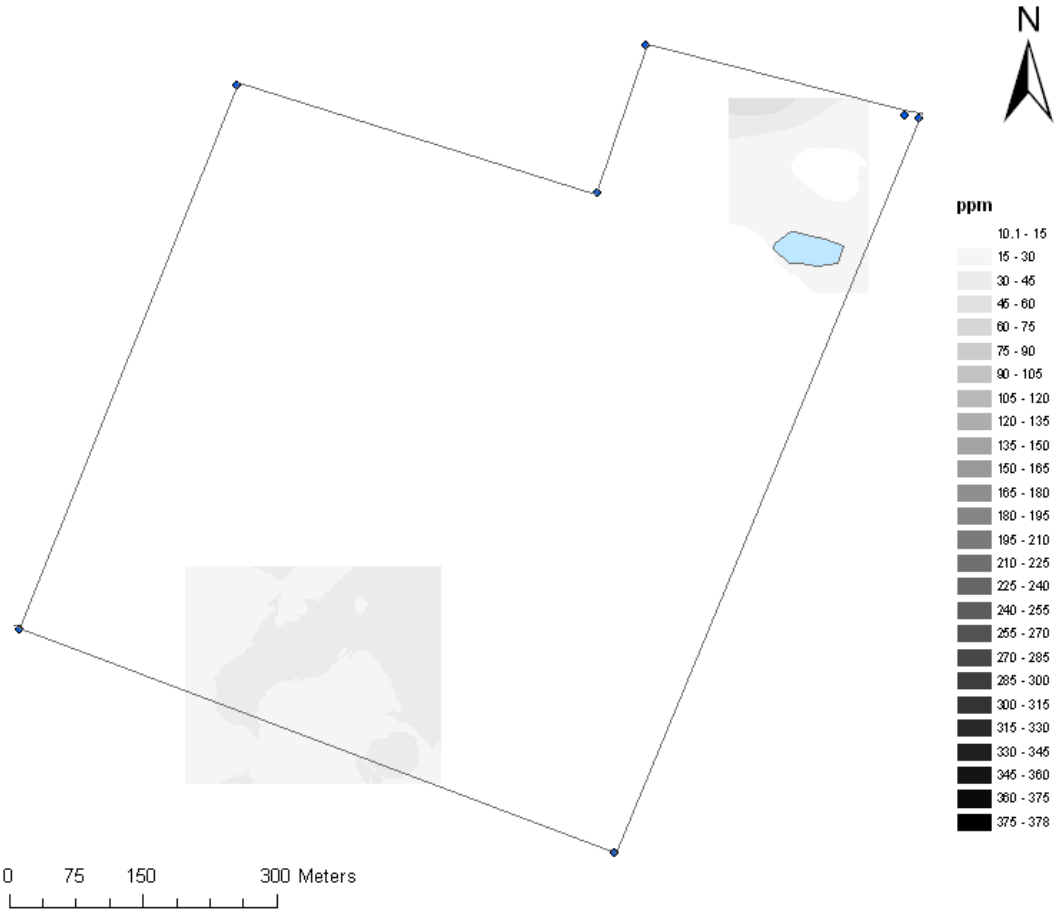


Figure E-16: Soil iron spatial prediction map for Farm 3.

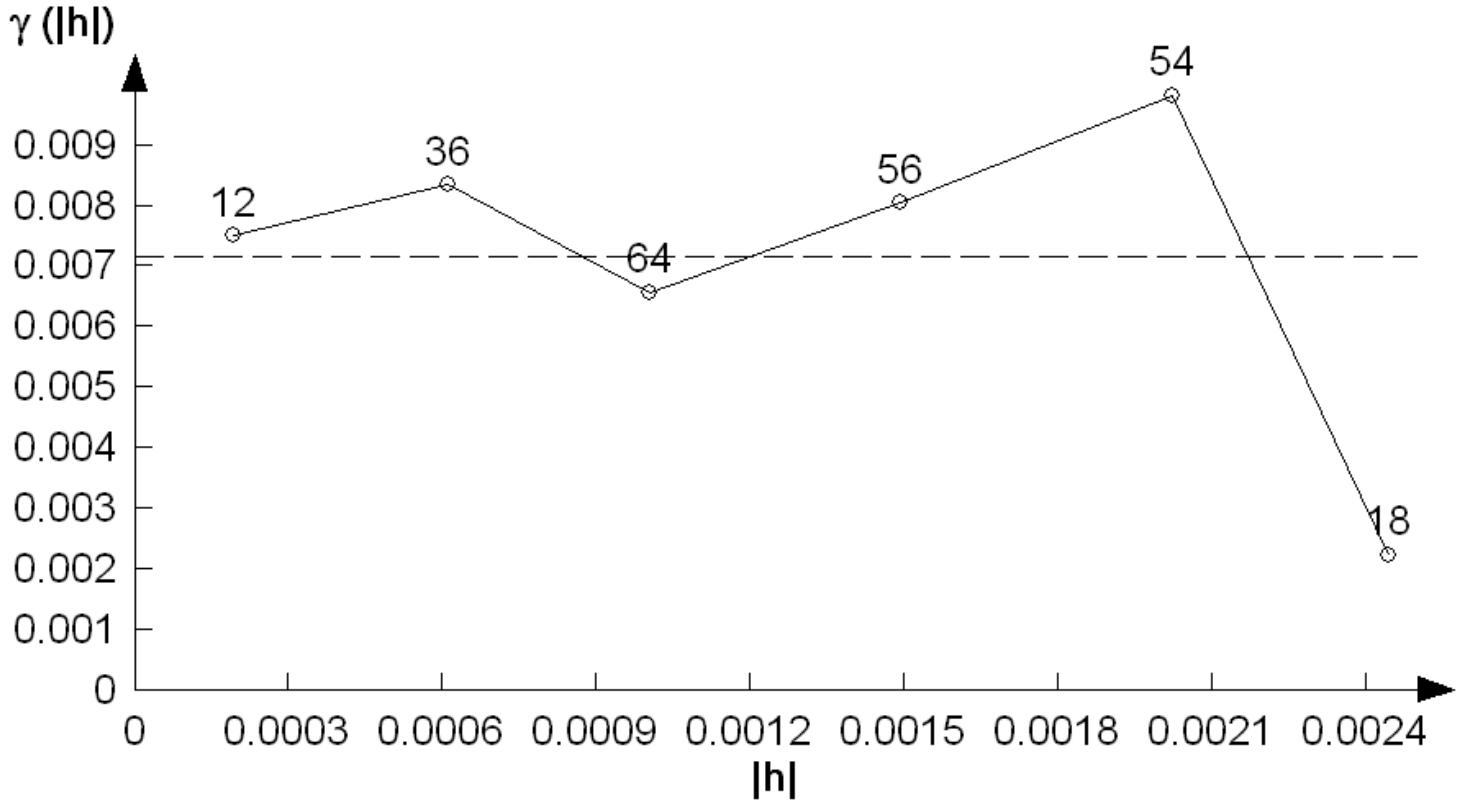


Figure E-17-1. Semivariogram for pH for Farm 3 (Feeder area).

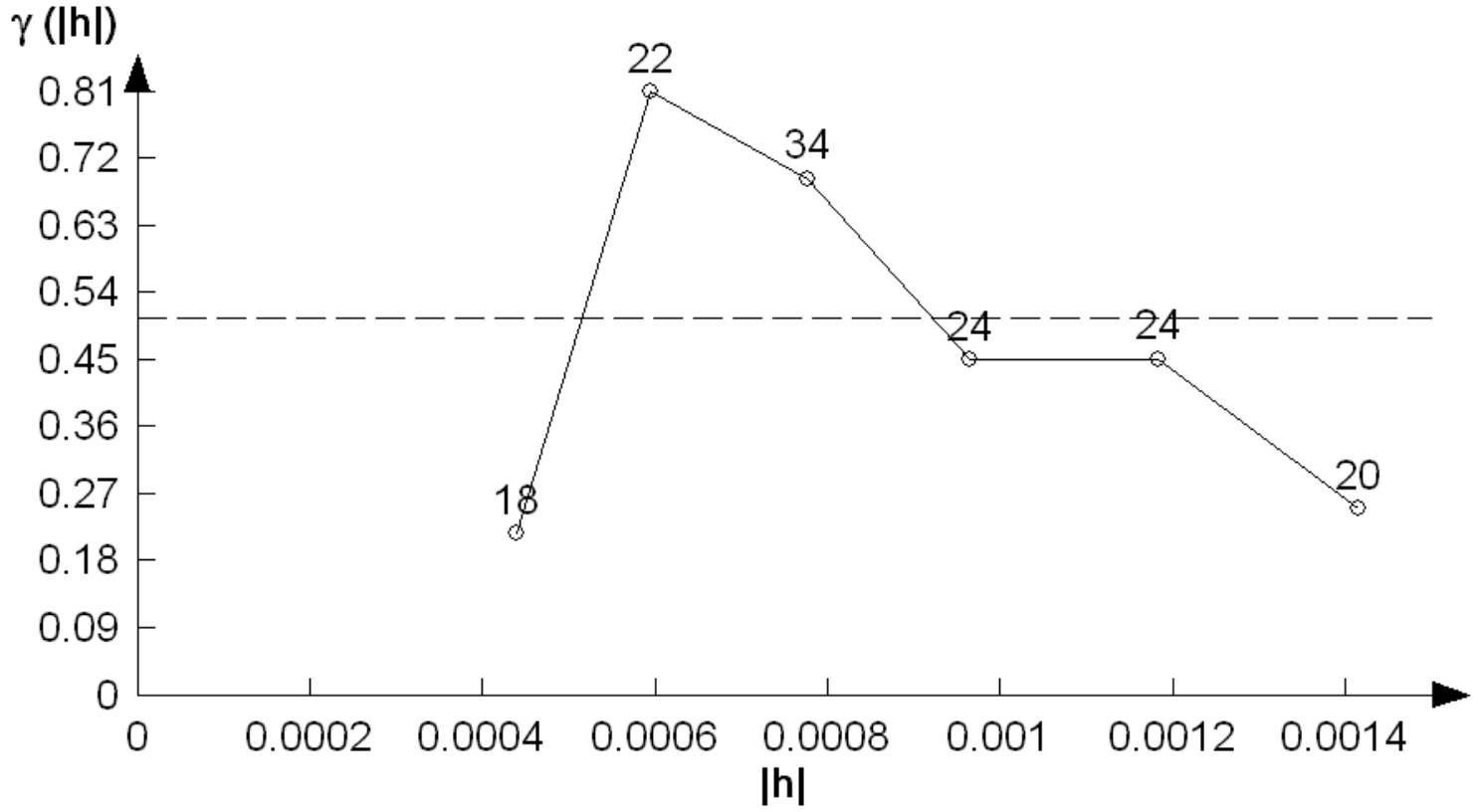


Figure E-17-2. Semivariogram for pH for Farm 3 (Pond area).

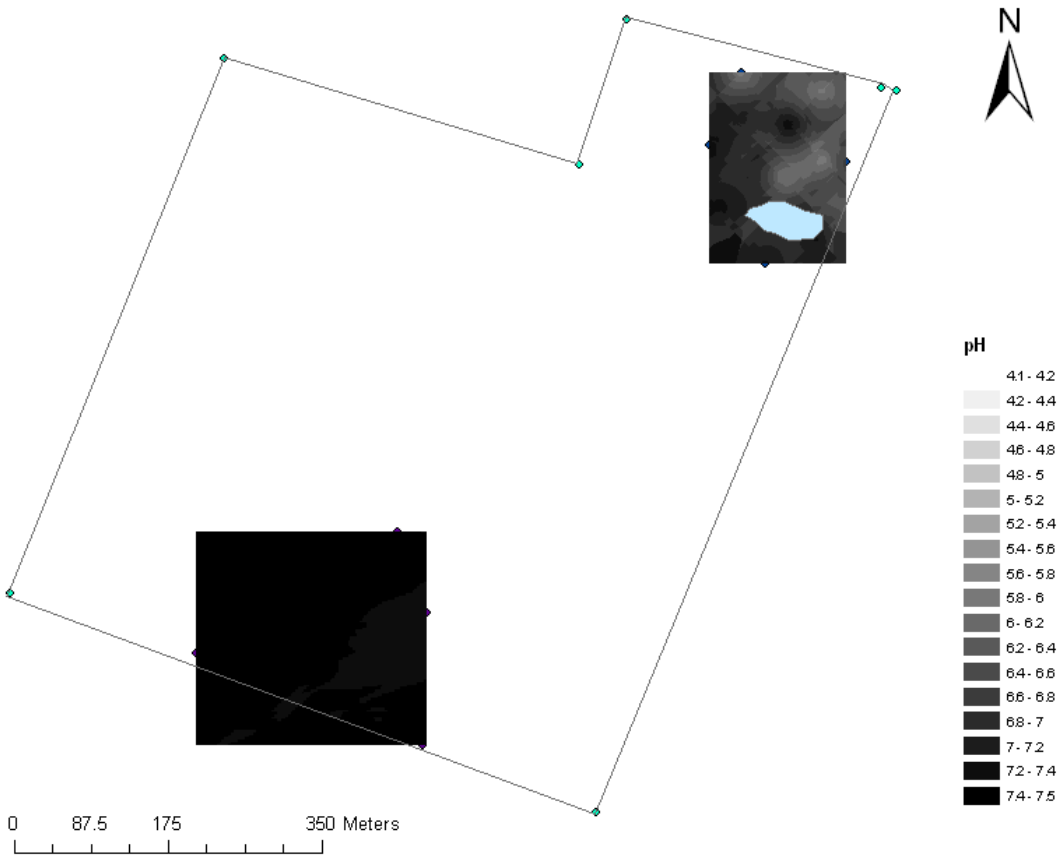


Figure E-18: Soil pH spatial prediction map for Farm 3.

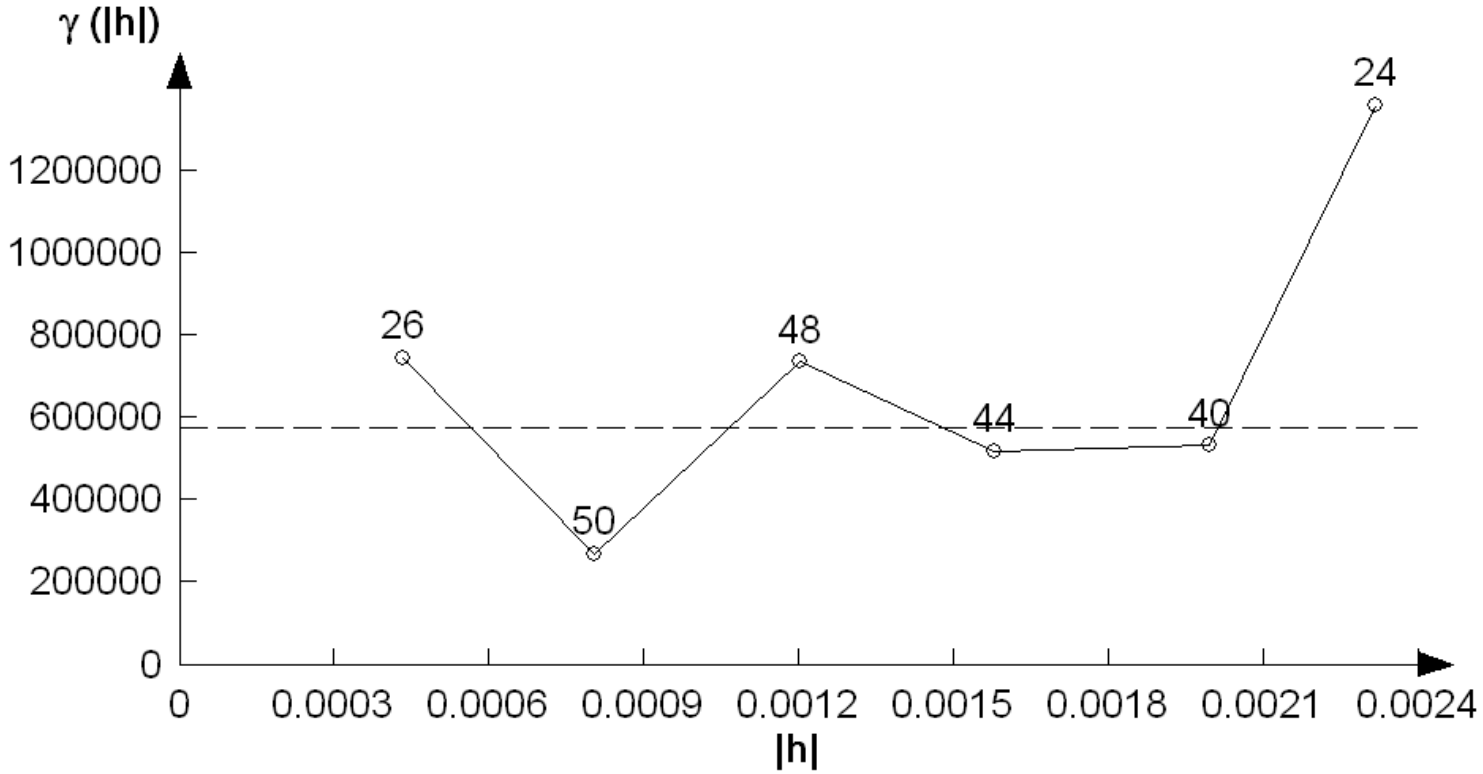


Figure E-19-1. Semivariogram for calcium for Farm 3 (Feeding area).

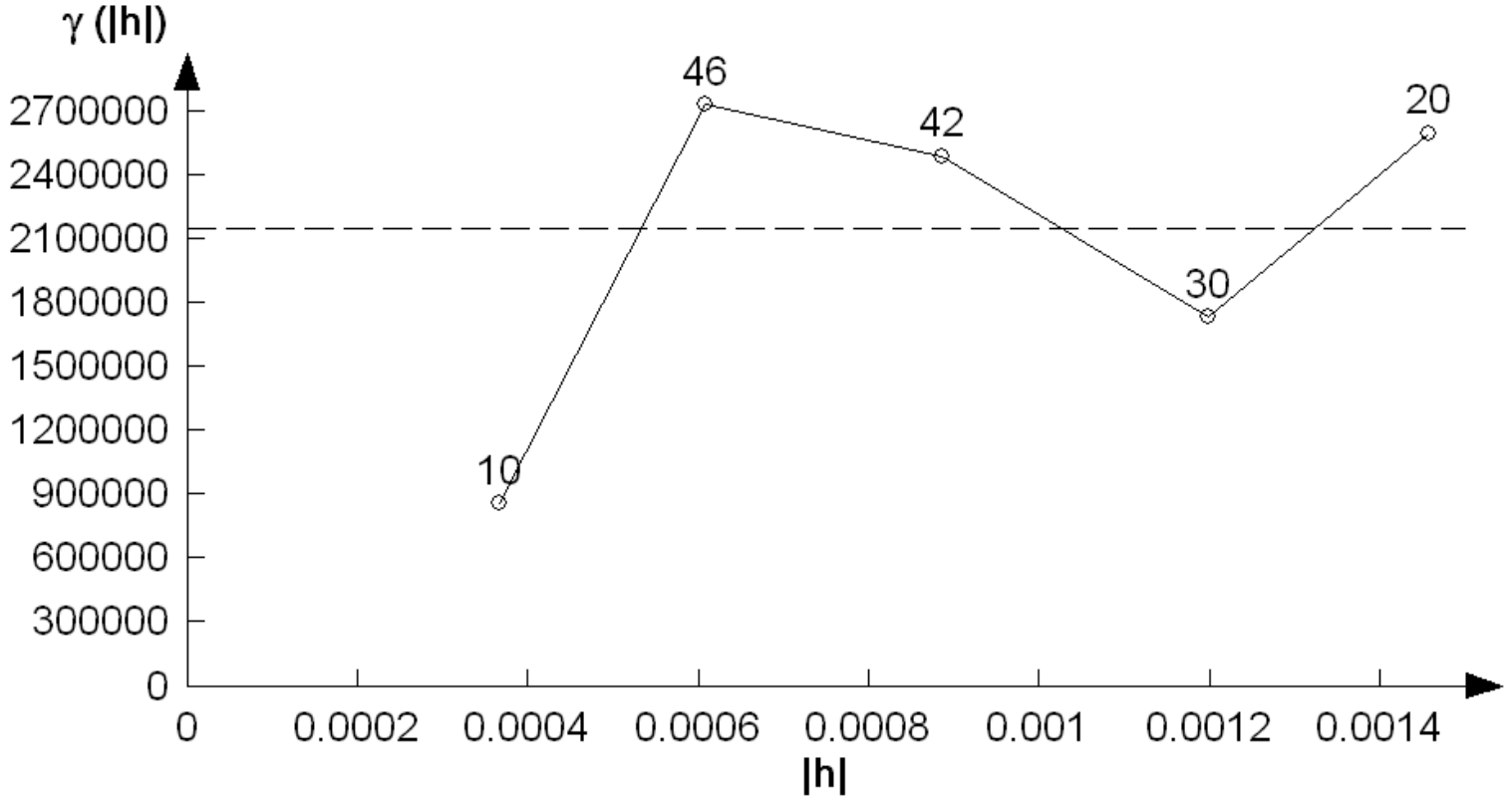


Figure E-19-2. Semivariogram for calcium for Farm 3 (Pond area).

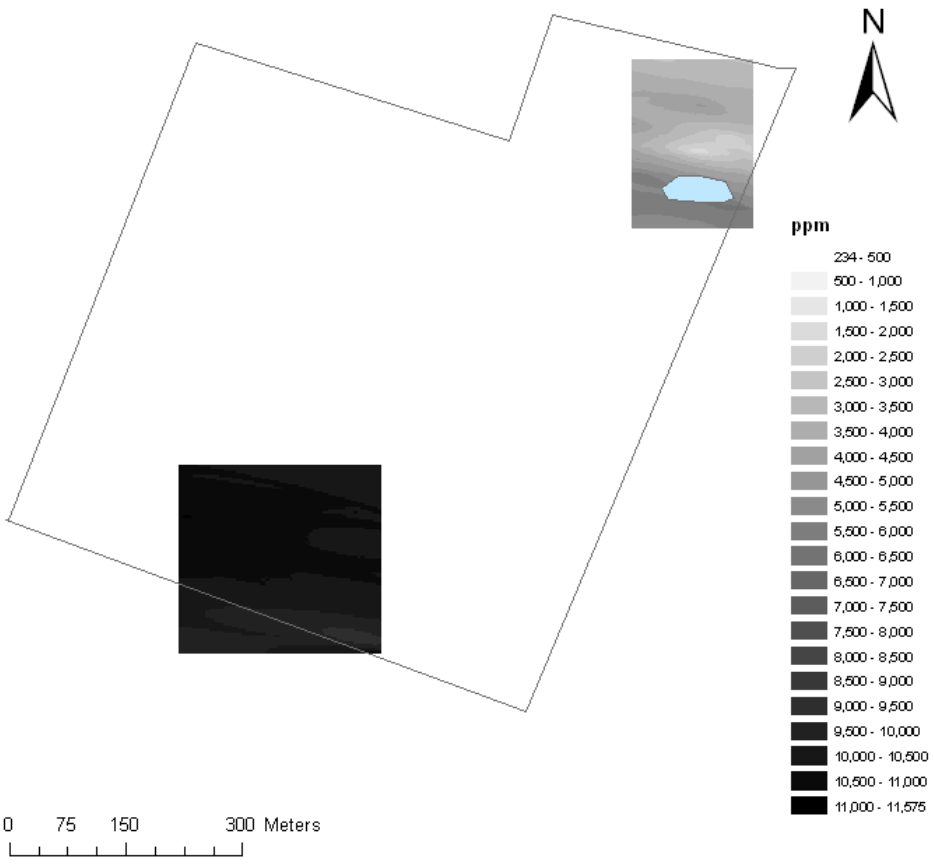


Figure E-20. Soil calcium spatial prediction map for Farm 3.

Table E-1. Model parameters for spatial prediction maps

		Model parameters					
Farm ID	Variable	Model type	Lag size (decimal degrees)	Number of lags	Range	Nugget	Sill/Partial sill
Farm 1	iron	Spherical	0.0001	15	0.0006	918	5100
	pH	Spherical	0.0001	7	0.0004	0.054	0.261
	calcium	Spherical	0.0001	10	0.001	27819	116100
	fecal pat density	Spherical	0.0001	10	0.0001	0.008	0.114
Farm 2	iron	Exponential	0.00009	10	0.0008	213.6	774.3
	pH	Spherical	0.0002	15	0.001	0.078	0.264
	calcium	Spherical	0.0001	15	0.0012	52360	43890
Farm 3	iron	Spherical	0.0001	15	0.0007	14.56	48.86
	pH	Spherical	0.0005	15	0.0015	0.006	0.002
	calcium	Exponential	0.0004	10	0.0006	174000	417600

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