

**THREE SURVEILLANCE SYSTEMS FOR DESCRIBING THE SPATIAL
DISTRIBUTION OF JOHNE'S DISEASE SEROPOSITIVITY IN TEXAS
CATTLE**

A Thesis

by

BRIELLE H. PEARCE

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

MASTER OF SCIENCE

December 2007

Major Subject: Epidemiology

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Approved by:

Chair of Committee, Geoffrey T. Fosgate

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ABSTRACT

Three Surveillance Systems for Describing the Spatial Distribution of Johne's Disease
Seropositivity in Texas Cattle. (December 2007)

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Chair of Advisory Committee: Dr. Geoffrey T. Fosgate

Johne's disease is a chronic and debilitating disease of cattle caused by infection with *Mycobacterium avium* subsp. *paratuberculosis* (Mptb). This disease affects both dairy and beef cattle, though it is more commonly recognized in dairy cattle. Mptb is able to persist in the environment of cattle for extended periods of time; therefore the distribution of the disease depends on the presence of infected animals and environmental conditions. Three surveillance systems were used to describe the spatial distribution of Johne's disease seropositivity in Texas cattle. These three systems were hypothesized to describe different spatial patterns. These systems involved sampling, 1) herds throughout Texas, 2) market cattle from four markets each month (one each from northern, southern, eastern, and western regions of Texas) and 3) sick animals submitted by veterinarians throughout Texas. Samples were tested for Johne's disease at the Texas Veterinary Medical Diagnostic Laboratory using serum ELISA. Spatial distributions were estimated by kriging the sample-to-positive control ratios (S/P). Sera were evaluated for Mptb antibodies from 2358 cattle with 1084 animals in system 1, 1200 from system 2 and 74 from system 3. Total number of positive ELISA results was 51, with 25, 19 and 7 positive ELISA results for systems one, two and three, respectively.

Results showed an overall prevalence of 2.16%, and prevalence's of 2.31%, 1.58% and 9.46% for systems one, two and three, respectively. Differences in the spatial distribution of Johne's disease seropositivity, based on the three surveillance systems, confirmed our hypothesis that estimation of disease distribution is dependant upon the source of surveillance samples.

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CHAPTER I

INTRODUCTION

Epidemiology of Johne's Disease

Background

Johne's disease (JD) is caused by infection with *Mycobacterium avium* subsp. *paratuberculosis* (Mptb). JD was first recognized in the 1800s, but it wasn't until 1895 that Johne and Frothingham demonstrated the presence of mycobacteria in the intestines of affected cattle in Germany (Twort and Ingram, 1912). As reported by Naser (2007), Johne and Frothingham had received tissues of a cow thought to have succumbed to tuberculosis, but upon histological examination, they found an abundance of immune cells in abnormally thick intestinal mucosa and wall, including leukocytes and epithelioid-like cells. Acid fast staining and microscopic evaluation of the ruminant intestine showed colonies of acid-fast microorganisms similar to *Mycobacterium bovis*. In 1908, the first US occurrence was described by Leonard Pearson (1908), dean of the University of Pennsylvania veterinary school, in his paper "A note on the occurrence in America of chronic bacterial dysentery of cattle." The distribution of JD is considered to be worldwide and would seem most likely to be present in every country that has livestock industries. Mptb belongs to the family of Mycobacteriaceae, and is a Gram-positive, strictly aerobic, non-motile, acid fast rod-shaped bacterium (Griffiths, 2006).

This thesis follows the style and format of Preventive Veterinary Medicine.

It is resistant in the environment, even under harsh conditions, and remains viable for extended periods of time. Environmental resistance of Mptb is due to the complex tripartite lipid-rich mycobacterial cell wall (Manning, 2001). Mptb is resistant to low pH, high temperature, and chemical agents (Manning, 2001). This means that pasteurization and water treatment procedures are insufficient for killing the bacteria. Studies have shown that current milk pasteurization methods in the US do not completely eliminate Mptb (Grant et al., 1996; Grant et al., 2002) and viable Mptb has been detected in US retail pasteurized milk (Ellingson et al., 2005). Another study found Mptb in the municipal water supply of a major US city (Mishina et al., 1996). In contrast to the bacteria's resistance, the *in vitro* growth requirements of Mptb are quite specific and include mycobactin, an iron binding agent. Soils that have a low pH (acidic) are much more suitable for Mptb survival than alkaline soils (Johnson-Ifearegulu and Kaneene, 1997a).

Transmission & Susceptibility

Infection of cattle with Mptb can occur via the fecal-oral and in-utero routes. Calves can become infected via consumption of colostrum or milk. Cross-species transmission has also been documented (Muskens et al., 2001). Fecal-oral is considered the most common route of transmission. Calves can become infected by nursing on udders that are contaminated with feces, or via a contaminated calving environment (Taylor et al., 1981). It has also been shown that calves can become infected by other calves, usually shortly after infection and most probably through the fecal-oral route

(Roermund et al., 2007). In-utero transmission occurs most commonly when an infected dam has clinical Johne's disease (Manning, 2001). Mptb can persist in the mammary tissues of infected cows and detection of infection generally requires bacterial culture of the colostrum or milk (Taylor et al., 1981). Wild animals, for example rabbits, bighorn sheep and mountain goats, can become infected by Mptb. In one study, no evidence that wild animals were a source of infection for domestic animals was found (Beard et al., 2001). However, the longer that Mptb remains endemic in a wildlife population, the greater the possibility that it can be introduced into domestic animal populations.

Age appears to be one of the most important factors affecting transmission, with an age dependent increase in resistance to Mptb infection (Collins, 2003b). Although cattle of all ages are susceptible to infection with Mptb, infection most commonly occurs in calves <6 months of age (Larsen et al., 1975). The infective dose of Mptb is unknown (Collins, 2003a), but it appears a relatively small dose is needed to infect a young calf (up to 6 months of age) (Whittington and Sergeant, 2001) while a much larger dose is required to infect cattle over 2 years old (Collins, 2003b). Once infected, the individual likely remains permanently infected and can shed Mptb in their feces for extended periods of time. Clinical illness generally occurs later in life, several years after infection.

Pathogenesis

Shortly after ingestion of Mptb, the bacteria pass through the intestinal tract. The site of infection is thought to be in the region of the distal small intestine and proximal large intestine (most likely in the ileum). Mptb is taken up by M cells that line the

intestine (Rebhun, 1995; Collins, 2003a) and the distribution of M cells in the intestine explains the predilection of the ileum as the primary, although not unique, site for lesion development (Whitlock and Buergelt, 1996). The M cells (specialized absorptive mucosal cells) overlying small bulges of lymphoid cells near Peyer's patches engulf Mptb from the lumen, then discharge the organisms into the sub-epithelial dome without metabolically changing the bacilli (Momotani et al., 1988; Whitlock and Buergelt, 1996). Calves appear to be more susceptible to Mptb infection because mucosal lymphoid tissue occupies more of the intestines (8.6% of the upper small intestine and two-thirds of the ileum) at this age compared to older cattle (Lepper, 1992; Whitlock and Buergelt, 1996). After uptake of the organism, Mptb bacilli are transported to the underlying lymphoid tissue. Experts believe bacilli are then carried via lymphatics to the blood stream and localize in secondary sites, such as the liver, spleen and peripheral lymph nodes (NRC 2003, p. 37). The time from infection to clinical disease (incubation period) can range from 4 months to 14 years (Manning, 2001; Whittington and Sergeant, 2001).

Prevention

Voluntary JD control programs exist in most states. The major components for JD control programs are based on dairy herd recommendations, but they also can be implemented in herds of other animals, such as beef cattle, sheep, goats and exotics. Recommendations include taking precautions against introducing disease via purchased animals, isolating/slaughtering clinically affected animals, culling recent offspring of clinical cases as soon as possible, removing calves from dams immediately upon birth

(before nursing) and isolating in separate calf-rearing areas, harvesting colostrum from cows with clean and sanitized udders, feeding colostrum to calves by bucket and only feeding milk replacer or pasteurized milk thereafter, preventing contamination of calf feedstuffs, water or bedding from the adult herd and applying manure from the adult herd only to cropland or pastures grazed by adult stock (Collins, 1994; Rossiter and Burhans, 1996; NRC, 2003). Also, it is important to prevent feces from contaminating water sources, such as runoff water from the milking area that can come in contact with streams. Keeping feedstuffs from coming in contact with contaminated fecal material should reduce the risk of spreading Mptb.

Economic Impact

Johne's disease has the greatest negative economic impact on the dairy industry (Collins, 1994). Approximately 22% of US dairy herds are classified as JD positive (NAHMS, 1997); however, within-herd seroprevalence is $\leq 10\%$ in 85.9% of operations (NAHMS, 2002). Approximately 7.8% of US beef herds are classified as having one JD positive animal and a within-herd seroprevalence of 0.4% (Dargatz et al., 2001). It was estimated that over 90% of beef cow-calf operators knew very little about the disease in 1997 (NAHMS, 1998). Direct quantifiable losses associated with clinical JD have been documented (Benedictus, 1987; Johnson-Ifeorulundu et al., 1999). These losses can be associated with reduced productivity and salvage value, and purchase of replacement animals (Johnson-Ifeorulundu et al., 1999). The losses in milk production, meat and genetically important animals (culled due to infection with Mptb) has greatly decreased

profits for the industry (Johnson-Iferulundu and Kaneene, 1997b). Beef herds with infected cattle are also affected by production losses, but little information has been published (Hutchinson, 1996). It has been estimated that the economic losses to the cattle industry in the US are about \$1.5 billion annually (Stabel, 1998). In the national survey for Johne's disease in the US during the 1980s, results showed that infection was present in 1.6% of all cattle and 2.9% of cull cows (Merkal et al., 1987; Griffiths, 2006). The United States Department of Agriculture reports that 20 – 40% of US dairy herds are infected with Mptb (Broxmeyer, 2005; Griffiths, 2006) with herd prevalence being strongly associated with herd size (Manning, 2001; Griffiths, 2006). Endemic transmission of this disease will continue when proper control and preventive measures are not employed.

Clinical Disease

Many cattle infected with Mptb do not develop clinical signs of disease prior to slaughter or death (Rebhun, 1995). Clinically affected cattle are infectious and can transmit Mptb to other cattle in the herd. Also, females can produce infected offspring. Cattle that develop clinical signs shed large numbers of organisms in their feces and therefore represent the biggest threat to control efforts. However, clinical cases only represent a small portion of infected cattle that may be present in a herd.

Clinical signs in cattle include diarrhea, progressive wasting and loss of condition, and in extreme cases, ventral edema secondary to hypoproteinemia. Temperature and vital signs typically remain within normal limits (Rebhun, 1995).

Diarrhea associated with Mptb infection is defined as a pea-soup consistency. A JD affected animal can best be described as one that has looser than normal feces compared to the other individuals in the herd, based on the type of diet that is being fed (Rebhun, 1995). Cattle in early stages of disease maintain a normal appetite and attitude, but their hair coat quality and body condition deteriorate due to protein loss (Rebhun, 1995). High-producing animals may experience episodes of indigestion and inappetance, due to calcium deficiency, a side-effect of the hypoproteinemia (Rebhun, 1995). Moderate to advanced cases exhibit muscle wasting, poor dry hair coat, significant production loss, dehydration and inappetance (Rebhun, 1995). Abomasal displacement has also been observed in cattle with moderate to severe JD. Ventral, intermandibular, udder and lower limb edema is present in advanced cases. Constant diarrhea causes discoloration of the tail, perineum, hindlimbs and hindquarters (Rebhun, 1995). Cows with JD typically do not appear seriously ill until the later stages of disease (Rebhun, 1995).

According to Whitlock (1996), clinical cases are only the “tip of the iceberg”. There are four stages generally associated with JD; Stage I – silent infection, Stage II – subclinical disease, Stage III – clinical disease and Stage IV – advanced clinical disease. Stage I generally includes young cattle (up to 2 years of age) that are in the early stages of infection, with no overt evidence or clinical signs (Lepper, 1992; Whitlock and Buergelt, 1996). These animals seem identical to non-infected herdmates with regards to growth, weight gain and appearance. Detection of disease by routine or special clinicopathologic tests is not possible and the only reliable means of detecting individuals in this stage is from finding the organism in the tissues either by culture or

histiologic confirmation of Mptb in intestines (Whitlock and Buergelt, 1996). These animals can shed the organism into the environment at levels below the threshold of detection (Whitlock and Buergelt, 1996).

Stage II animals do not have diarrhea or visible signs of JD, but they may have detectable antibodies to Mptb and altered cellular immune response and are prone to other diseases, including mastitis and infertility (Whitlock and Buergelt, 1996). Similar to stage I animals, stage II animals that test negative on fecal culture might still be shedding low numbers of bacteria into the environment, posing a threat to other animals. Of the shedders, only a small percentage of infected cattle (15%-25%) may be detected by fecal culture and removed from the herd (Whitlock and Buergelt, 1996). Most animals in this stage are still undetectable by available diagnostic techniques, and animals will slowly progress to stage III (Whitlock and Buergelt, 1996). Often cattle in this stage (II) are culled from the herd for other conditions, with JD unrecognized by the owner or veterinarian (Whitlock and Buergelt, 1996).

Stage III animals begin to show initial clinical signs after a long incubation period of many years, and begin to show gradual weight loss despite normal or increased appetite (Whitlock and Buergelt, 1996). Over a period of weeks, concurrent with weight loss, some other signs of JD can be observed. These include manure consistency becoming looser, intermittent at first, with periods of normal manure consistency and increased thirst (Whitlock and Buergelt, 1996). Vital signs, (heart/respiratory rate and temperature) remain within normal limits (Whitlock and Buergelt, 1996). Animals rarely remain in this stage for more than 3 to 4 months before progressing to stage IV, with a

few unusual cases reverting back to stage II, where they remain for an indeterminable period of time (Whitlock and Buergelt, 1996). In stage III, most animals are fecal culture positive and also have increased antibody detectable by commercial ELISA (Whitlock and Buergelt, 1996).

As the disease progresses into stage IV, affected animals become increasingly lethargic, weak and emaciated. Characteristics of terminal stage disease include intermandibular edema (bottle jaw) due to hypoproteinemia, as well as cachexia and “waterhose” or “pipestream” diarrhea (Whitlock and Buergelt, 1996). Generally animals are culled before this stage due to decreased milk production or weight loss. However, some cattle can progress from stage II to stage IV in only a few weeks. Once profuse diarrhea and hypoproteinemia occur, the animal’s condition deteriorates rapidly, often in a matter of days, and animals are sent to slaughter, or otherwise die from dehydration and cachexia (Whitlock and Buergelt, 1996).

Diagnostic Testing

Two classes of diagnostic tests exist for JD detection: direct and indirect methods. Direct methods, such as acid-fast staining of fecal smears, microscopic sections of tissue, culture of the organism and polymerase chain reaction (PCR) of feces, detect the organism. The most widely used diagnostic test for Mptb is culture of the organism from fecal or tissue samples. This requires several steps in order to process the specimen, and isolation takes anywhere from 8 to 16 weeks (Nielson et al., 2001). Processing includes concentrating the organism and reducing the amount of bacterial and fungal

contamination, without reducing the viability of Mptb. Such processing is done for the majority of culture-based analytic methods, including conventional bacteriological methods, radiometric culture media and non-radiometric automated culture systems. Conventional methods generally use media such as Herrold's Egg Yolk Medium and Lowenstein-Jensen medium, both with the addition of mycobactin J (Nielson et al., 2001). Radiometric media is used within the BACTEC system, and has been adapted from use in human medicine by Collins (1990). This system is advantageous because of increased analytical sensitivity, speed and automation of detection. Disadvantages include the cost of instrumentation, safety, and regulatory concerns with radioisotopes (Nielson et al., 2001). Non-radiometric automated culture systems are theoretically capable of detecting Mptb, but published evaluations of these systems are not available. Radiometric systems use special medium formulations in sealed vials inserted into instruments that simultaneously incubate and read the culture (vials with evidence of growth are reported by indicator lights). Supplemental tests are generally required to determine what organism growing in the vial triggered the positive signal (Nielson et al., 2001). Genetic systems used are based on Polymerase Chain Reaction (PCR) technology and are designed to identify a specific repetitive DNA sequence such as IS900 for Mptb (Collins et al., 1989; Green et al., 1989; Nielson et al., 2001). These should provide a rapid and highly sensitive diagnostic tool, but are often complicated by other factors (Nielson et al., 2001). Immunologic labeling has been reported, but due to background staining, this technique has yet to be adopted for routine testing. High-performance

liquid chromatography (HPLC) also has the potential for detection of Mptb cell wall components (Nielson et al., 2001).

Indirect methods of diagnostic testing assess hosts' response to infection. These include clinical signs, gross and microscopic pathology, and immunologic markers of infection. Immune response can be evaluated by cell-mediated immune responses (CMI), including skin testing after intradermal injection of antigens used to diagnose bovine tuberculosis, and also by interferon-gamma (Nielson et al., 2001). Various serological methods have been developed for the detection of humoral immune responses elicited by infection with Mptb. These are generally not recommended for use on cattle before the age of 15 months since humoral immunity does not typically occur prior to 10-17 months after infection (Nielson et al., 2001). The 3 main serological methods are agarose gel immunodiffusion (AGID) test, complement-fixation (CF) test and absorbed indirect enzyme-linked immunosorbent assay (ELISA). These methods all share the common factor of dependency on specific antigens and antigen selection is critical for serologic tests. A short description of the first 2 methods will be followed by a more in-depth explanation of ELISA. The AGID test was one of the first serologic methods developed for Mptb diagnosis, but is now considered less sensitive than both ELISA and CF tests, because of its high antibody detection limit (Nielson et al., 2001). The CF detects complement-fixing antibodies specific for Mptb in serum and is reported to detect antibodies 1-5 months later during the course of infection than the ELISA and also has a lower specificity than both ELISA and AGID tests (Nielson et al., 2001). ELISA is currently the most widely used screening method and thought to be the best of the 3

(Nielson et al., 2001). ELISA measures exposure to Mptb by detecting if antigen or antibodies specific for Mptb are present. The type of ELISA most commonly used is the indirect method, which employs an Mptb antigen bound to a matrix, such as a micro titer plate. Test serum is added, allowing Mptb-specific antibodies in the serum (if present) to bind with antigen. Enzyme-labeled antibody specific to the target species antibody (antiglobulin) is added after washing. In the final step, addition of an enzyme substrate creates a reaction to the enzyme-labeled antiglobulin, producing a color change in a positive sample (Figure 1.1).

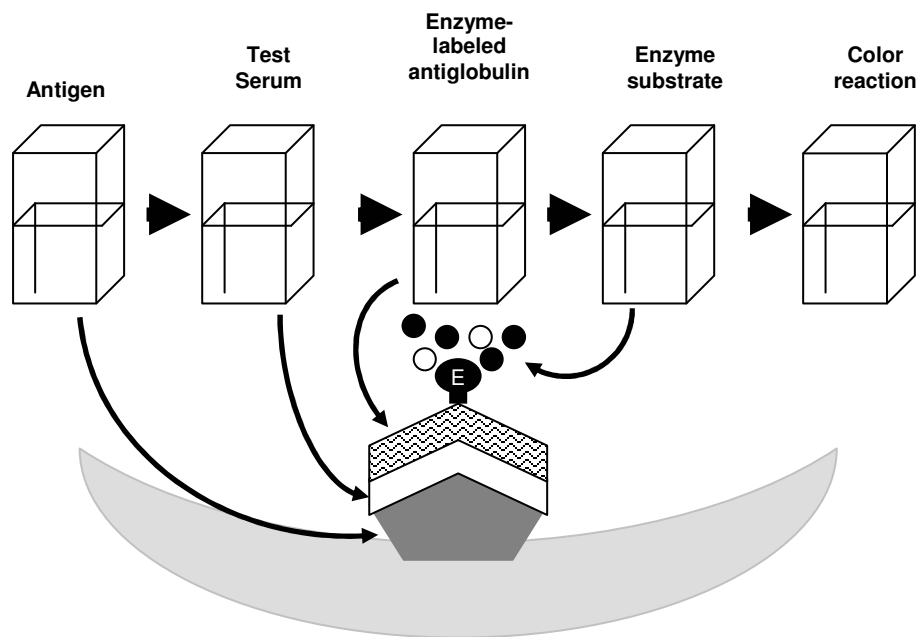


Figure 1.1. Indirect ELISA technique. Antigen is bound to test tube wall. Bound antibody presence detected by enzyme-labeled antiglobulin. Enzyme substrate addition gives color change proportional to bound antibody amount. Based on diagram by Tizard (2000).

Optical density (OD) measurements of the color change quantify reaction intensity and is proportional to the amount of antibody present in the test sera (Tizard, 2000). The ODs can then be transformed to sample-to-positive (S/P) ratios based on the sample OD and the positive and negative control ODs included on the test plate. The S/P ratios are calculated by the equation: $S/P \text{ ratio} = (\text{sample OD} - \text{negative control OD}) / (\text{positive control OD} - \text{negative control OD})$ (Collins, 2002). Samples are then classified as positive or negative based on the manufacturer's recommended S/P cutoff value.

Kriging

Kriging is a geostatistical tool for modeling spatial patterns (Rossi et al., 1994). Kriging was developed in 1971 by G. Matheron based on an idea of D.G. Krige (Carrat and Valleron, 1992). The key feature of kriging is the ability to describe processes in space while taking into account spatial correlations in observations (Buckland and Elston, 1993). Kriging can be performed on nominal as well as continuous variables, allowing estimation of a binary variable (Rossi et al., 1994).

Kriging allows for an optimal spatially continuous description of the latent risk surface from observed regional risk estimates (Berke, 2005). Interpolation is based on distance weighting techniques. This relates semivariance to spatial separation and provides a concise and unbiased description of the scale and pattern of spatial variability (Curran, 1988). As O'Sullivan and Unwin (2003) stated, "all kriging does is to use control point data as a sample to find optimum values of the weights for the data values

included in the interpolation at each unknown location”. Three steps are involved when interpolating this way; 1) producing a description of the spatial variation in the sample control point data, 2) summarizing the spatial variation by a regular mathematical function and 3) using the model to determine the interpolation weights (O'Sullivan and Unwin, 2003).

The first step is one of the most important, because it describes the spatial structure of the values of the variable-of-interest at sample locations: similarity or dissimilarity of nearby values is quantified. It is during this process (variography) that the correlation structure is estimated. Correlations among the values of data points throughout the study area of interest are evaluated, providing a covariance function. The kriging predictor is a weighted average, but it is calculated from the whole sample with the weight depending on the spatial dependence structure.

The second step is to summarize the spatial variation, using a mathematical function. The parameters of this function are then used to approximate a very general form of the model. The variogram is based on the known sample control points of a continuous function that describes the way in which the intensity of the field changes with distance (O'Sullivan and Unwin, 2003). The parameters of the function are the nugget, range and the sill. The nugget is the variance at zero distance, which would ideally be zero. A zero nugget value (absence of variability) however is rare for a best-fit function to experimental data (O'Sullivan and Unwin, 2003). This value is indicative of uncertainty or error in the attribute values (O'Sullivan and Unwin, 2003). The range is the distance up to which the spatial covariance varies and beyond which the

semivariance is constant (no increased effect with added distance). No change in the spatial structure of the data exists beyond the range (O'Sullivan and Unwin, 2003). The last feature is the sill, the constant semivariance value beyond the range. The sill may be approximated by measuring its total variance (O'Sullivan and Unwin, 2003). The spherical model (Figure 1.2) starts at a nonzero variance for the nugget and rises in an elliptical arc to maximum value (sill), at some distance (range). The value of the sill should be equal to the variance of the function (O'Sullivan and Unwin, 2003). Although this model is only an approximation of the spatial variation in real data, it is a powerful summary of the overall properties of a spatial data set (O'Sullivan and Unwin, 2003).

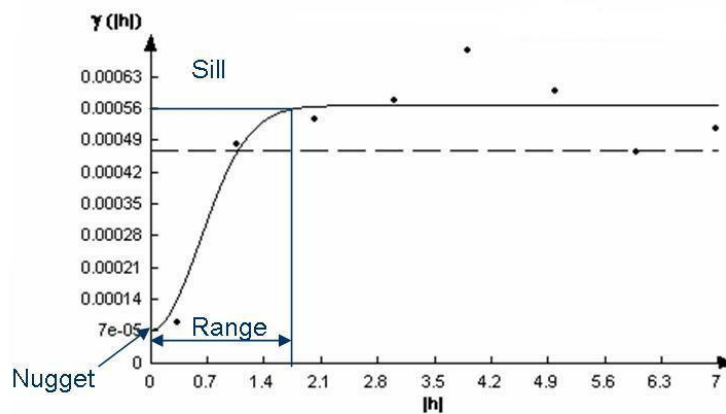


Figure 1.2. Example semivariogram produced using Variowin (Variowin 2.21, Yvan Pannatier). The range, sill and nugget are displayed on the semivariogram curve.

The final step is to use the model for kriging to determine the interpolation weights. The aim is to find the best combination of weights for an un-sampled location,

based on its spatial relationship to the control points and on the relationships between the control points summarized in the semivariogram. In ordinary kriging this is done based on several assumptions, such as the surface having a constant mean and being isotropic. The semivariogram is a simple model with clearly defined properties and the same semivariogram applies over the entire area (O'Sullivan and Unwin, 2003). Kriging is very intensive computationally; therefore most of the actual kriging computations are concealed in the programs that run the procedure.

Kriging can be used to describe geographic variations in the incidence or prevalence of a disease over a certain area, as well as variations in risk factor frequencies (Carrat and Valleron, 1992). If the correct model is used, kriging has the advantage over other interpolation procedures, since estimated values have minimum, quantifiable error (O'Sullivan and Unwin, 2003). Kriging is consistent with the “first law of geography”, that samples closer together in space are more similar than those farther apart (Rossi et al., 1994).

Objective

The objective of this study is to identify if differences exist in Johne's disease seropositivity estimated using 3 different surveillance systems. The study objective will be achieved using serological data collected and analyzed from 3 surveillance systems implemented throughout the state of Texas between 2005 and 2007.

CHAPTER II
THREE SURVEILLANCE SYSTEMS FOR DESCRIBING THE SPATIAL
DISTRIBUTION OF JOHNE'S DISEASE SEROPOSITIVITY IN TEXAS
CATTLE

Introduction

Johne's disease (JD) is a chronic, enteric infection of ruminants and other species caused by *Mycobacterium avium* subsp. *paratuberculosis* (Mptb) (Beard et al., 2001; Collins, 2003a, b). JD was first recognized in the 1800s, but it wasn't until 1895 that Johne and Frothingham demonstrated the presence of mycobacteria in the intestines of affected cattle in Germany (Twort and Ingram, 1912). Many cattle infected with Mptb never develop clinical signs of disease prior to slaughter (Rebhun, 1995). Clinically affected cattle are infectious and can transmit the organism to other cattle in the herd. Cattle that develop clinical signs are responsible for shedding large numbers of organisms in their feces and therefore represent the biggest threat to control efforts. Clinical signs in cattle consist of diarrhea, progressive wasting and loss of condition, and in extreme cases, ventral edema secondary to hypoproteinemia. Temperature and vital signs typically remain within normal limits (Rebhun, 1995). However, clinical cases only represent a small portion of infected cattle that may be present in a herd.

Mptb belongs to the family of Mycobacteriaceae, and is a Gram-positive, strictly aerobic, non-motile, acid fast rod-shaped bacterium (Griffiths, 2006). It is extremely resistant in the environment, even under harsh conditions, and remains viable for

extended periods of time. Environmental resistance of *Mptb* is due to the complex tripartite lipid-rich mycobacterial cell wall (Manning, 2001). *Mptb* is resistant to low pH, high temperature, and chemical agents (Manning, 2001). Fecal-oral is considered the most common route of transmission (Sweeney, 1996). Age appears to be the most important factor affecting transmission, with an age dependent increase in resistance to *Mptb* infection (Collins, 2003b). Although cattle of all ages are susceptible to infection with *Mptb*, infection most commonly occurs in calves <6 months of age (Larsen et al., 1975). Once infected, the individual likely remains permanently infected and can shed *Mptb* in their feces for extended periods of time. Clinical illness generally occurs later in life, many months or years after infection. Direct quantifiable losses associated with clinical JD have been documented (Benedictus, 1987; Johnson-Ifearulundu et al., 1999). It has been estimated that the economic losses to the cattle industry in the US are about \$1.5 billion annually (Stabel, 1998). These losses are due to reduced productivity and salvage value, and purchase of replacement animals (Johnson-Ifearulundu et al., 1999).

Quantification of production losses requires accurate information concerning the level of morbidity and mortality in the population attributed to the disease. Surveillance and monitoring are performed to obtain information concerning the occurrence of disease and control measures for their prevention. Disease surveillance is the ongoing observation using methods that are practical, uniform and rapid, with the purpose being trend detection and implementation of control measures. Disease monitoring is often episodic and generally focused on routine measurements to detect changes in health status or without the implied necessity of control measure implementation. Targeted

sampling is a means to achieve these methods by selecting sites according to particular conditions such as when particular individuals are considered to be at higher risk for the disease in question.

Kriging is a geostatistical tool for modeling spatial patterns (Rossi et al., 1994) that can be used to create risk maps of diseases. Kriging is an appropriate method for creating JD maps because of the recognized environmental predictors of Mptb survival. Kriging was developed in 1971 by G. Matheron based on an idea of D.G. Krige (Carrat and Valleron, 1992). The key feature of kriging is the ability to describe processes (such as disease occurrence) in space while taking account of spatial correlations in observations (Buckland and Elston, 1993). Based on distance weighting techniques, kriging can be used for interpolation, providing a concise and unbiased description of the scale and pattern of spatial variability (Curran, 1988). Kriging allows for an optimal spatially continuous description of the latent risk surface from observed risk estimates (Berke, 2005). The objective of the study reported here was to identify if differences exist in spatial distributions of Johne's disease ELISA sample-to-positive values estimated by different surveillance systems. This objective was achieved using cattle serological data collected and analyzed from 3 surveillance systems implemented throughout the state of Texas between 2005 and 2007.

Materials and Methods

Surveillance Systems

Serum samples were collected from cattle using 3 surveillance systems. In the first system, individual cattle within selected herds were sampled. Whole blood was collected from cattle in 13 herds, located in 10 of the 15 ecological zones of Texas, based on the Texas Agriculture Statistical Districts (USDA, 2003). Within-herd sample sizes for each zone were selected proportional to the total number of cattle in the zone, based on the number of cattle reported in the 2002 Agricultural Census (USDA, 2002). Adjacent ecological zones were combined when the number of cattle was small, resulting in 10 zones for cattle sampling. In each sampled zone, herds maintained at Texas Agricultural Experiment Research and Extension Centers (TAEREC) were sampled. Private herds were recruited for zones that did not have a TAEREC facility. Whole blood samples were collected during 2004. Samples were transported on ice to the laboratory where serum was separated and stored at -80°C until testing.

In system 2, samples were collected from cattle markets throughout the state of Texas. Twenty-five head of cattle were sampled at 4 different cattle markets on a monthly basis, by the Texas Animal Health Commission (TAHC). Blood samples were collected, and the sera sent, each month during the period May 2006 to April 2007, inclusive. Sera were refrigerated when received from the TAHC, prior to submission for JD testing.

In system 3, samples were received from bovine veterinarians throughout Texas. Veterinarians were recruited based on membership in a national organization with a

reported practice that included cattle work in Texas. Whole blood was collected by veterinarians that agreed to participate from December 2005 to April 2007, and sera were separated from all samples for testing. Samples were submitted from cattle examined by their practice, on a per case basis. These samples were collected from sick cattle in which a diagnosis by the examining veterinarian could not be made based on clinical signs alone. Cattle were not necessarily selected because they were suspected of having JD. Data collected for these cattle included age, breed, sex and presenting signs observed by the veterinarian. Herd-of-origin location was recorded as street address or road/intersection, county and zip code. Sera were submitted for testing immediately upon receipt from the submitting veterinarian.

Diagnostic Testing

All samples were submitted to the Texas Veterinary Medical Diagnostic Laboratory (TVMDL) to test for antibodies specific for Mptb using a commercially available ELISA (HerdCheck ELISA, IDEXX Laboratories Inc, Westbrook, ME). The sample-to-positive (S/P) ratio was recorded for each sample. The manufacturer's recommended cutoff value of 0.25 was used for estimating prevalence of Johne's seropositivity within each surveillance system.

System 1 serum samples were removed from storage, thawed and submitted to TVMDL in 100 sample batches from May 25th to June 26th, 2006. System 2 serum samples were submitted to TVMDL as they were received, generally at the beginning of

each month, in a batch of 100 samples. System 3 serum samples were submitted to TVMDL as received.

Spatial Modeling and Statistical Analysis

The proportions of positive JD results were calculated for each system and compared using chi-square tests in available software (EpiInfo version 6.04d for Windows, CDC, Atlanta, GA). Descriptive statistics (including the mean, median, minimum, maximum and standard deviation) were calculated for the S/P values for each system.

The sample locations were georeferenced and converted to the North American Datum (NAD) 1983 coordinate system and projected using Universal Transverse Mercator (UTM) zone 14. Moran's I was performed in available software (ArcGIS 9.0 ESRI Corp. Redlands, CA) to assess spatial autocorrelation within all data using the S/P ratio for each point. In addition, Cuzick and Edwards test (Cuzick and Edwards, 1990) was used to analyze this data by categorizing the JD test result as a 0 or 1 category, based on positive or negative ELISA results, and assessing the spatial relationship up to the 10 nearest neighbors (ClusterSeer version 2.0. TerraSeer™, Ann Arbor MI, 2002).

Spatial risk maps were created using kriging. First a semivariogram was constructed from the data: semivariance versus lag distance (using up to 10 bins) between all pairs of locations. This allowed for estimation of the nugget (variance at zero distance), range (distance at which the semivariogram levels off) and sill (constant semivariance beyond range) using a parametric model (exponential, spherical, power or

Gaussian) (O'Sullivan and Unwin, 2003). Calculations were performed using an available freeware program (Variowin 2.21, Yvan Pannatier), for each of the 3 surveillance systems. Using the estimated semivariogram parameters, kriging was performed with commercially available software (ArcGIS 9.0, ESRI Corp. Redlands, CA) to produce isopleths maps of sample-to-positive ratios. Maps were created, independently, for each surveillance system. The variable-of-interest for kriging was the S/P ratio for each spatially unique point, with an average of the S/P ratio used at points that were not spatially unique. Cut-points, for presentation purposes, were chosen based on the Johne's ELISA interpretation previously reported by Collins (2002), with 3 extra lower range categories. These categories were added to account for the large proportion of results within the negative range.

Kriged ELISA S/P ratio values between systems were compared with non-parametric tests. The kriged values were recorded for each pixel in the maps for the 3 systems independently and system by system correlations were assessed using Spearman's rho. Kriged values at the sample locations (subset of entire map) were compared (pixel by pixel) using the Friedman test overall and Wilcoxon signed ranks tests for pairwise comparisons of systems. Calculations were performed within a commercially available software package (SPSS version 13.2 for Windows, Chicago, IL) and all statistical tests were interpreted at the 5% level of significance.

Results

Sera were evaluated for Mptb antibodies from 2358 cattle with 1084 animals in system 1, 1200 from system 2 and 74 from system 3. System 1 had 13 herds (15 different sampling locations) with several sample sizes (Table 2.1). System 3 had the highest prevalence of positive animals, as well as the highest individual S/P (Table 2.2). System 3 also had the highest number of S/P values in the strong positive category (Table 2.2 and 2.3 and Figure 2.1). System 3 animals consisted of 57 (77%) beef cattle and 17 (23%) dairy cattle, with 9 (16%) males and 48 (84%) females and 4 (24%) males and 13 (76%) females in each production type, respectively. There were 3 positive dairy cattle (all female) and 4 positive beef cattle, 2 female and 2 male, respectively (Table 2.2). In system 3, the prevalence of positive animals was 17% (3/17) for dairy and 7% (4/57) for beef. Within beef cattle, the prevalence was 22% (2/9) for males and 4% (2/48) for females. Within dairy cattle, the prevalence was 0% (0/4) in males and 23% (3/13) in females.

Table 2.1. Herd sample sizes and number of ELISA positive cattle.

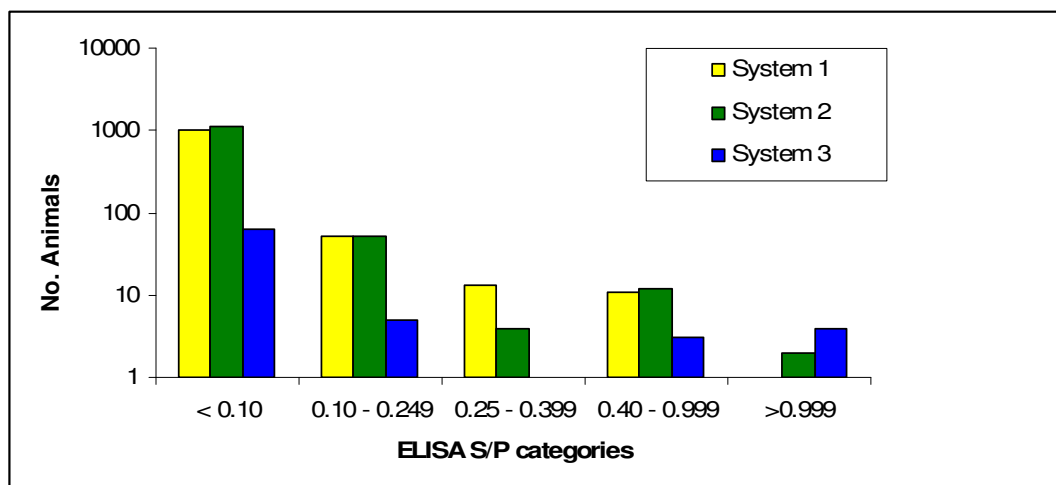
Herd number (herd at second location)	Number sampled (second location)	Number ELISA positive
1	115	21
2	87	0
3	173	0
4	137	1
5	131	1
6	94	2
7	18	0
8	47	0
9 (9b)	63 (60)	0
10	27	0
11	40	0
12	40	0
13 (13b)	19 (33)	0
Total	1084	25

Table 2.2. Descriptive statistics, χ^2 analysis and prevalence of positive results for each surveillance system and overall. Values without superscripts in common are significantly different.

Category	Number of Positive S/P values	Number of Cattle	Prevalence	Minimum S/P	Maximum S/P	Median S/P	Mean S/P	Standard Deviation
Overall	51	2358	2.16%	-0.30	5.456	0.003	0.02666	0.1592
System 1	25	1084	2.31% ^a	-0.30	1.639	-0.004	0.01764	0.1028
System 2	19	1200	1.58% ^{a,b}	-0.056	1.753	0.007	0.02523	0.0952
System 3	7	74	9.46% ^c	-0.066	5.456	0.0175	0.18240	0.6974

Table 2.3. Sick animal descriptive information for ELISA positive cattle from system 3.

Sample ID	Breed	Age (Years)	Sex	S/P Value	Presenting Complaints
5	Brahman	10	M	0.559	Chronic weight loss, emaciated, diarrhea
11	Brangus cross	5	F	1.793	Diarrhea, weight loss, decreased rumen motility
17	Jersey	3	F	1.253	Decreased milk production, decreased rumen motility, diarrhea
27	Holstein	5	F	5.456	Increased rumen motility, diarrhea, mastitis
38	Holstein	6	F	0.934	Weight loss, decreased milk production, diarrhea
57	Angus	2.5	M	0.655	Emaciated, increased temperature
73	Longhorn	1	F	1.283	None listed

**Figure 2.1.** Distribution of S/P values for five categories of an indirect ELISA used to diagnose Mptb infection in 2358 cattle from 3 surveillance systems.

Location of cattle from which sera were collected did not encompass all areas of Texas and did not necessarily correlate with cattle density (Figure 2.2). Some of the cattle locations appeared clustered (Figure 2.2) upon visual inspection of their mapped coordinates. Moran's I demonstrated significant spatial autocorrelation of the S/P value (Moran's I = 0.007189; P = 0.011). The data also showed significant (Bonferroni P = 0.01) clustering when JD test result was categorized as binary (positive, negative) data. Based on the kriged maps produced, areas of increased seropositivity exist for cattle throughout Texas (Figures 2.3 -2.5). The areas identified varied with the type of surveillance system used to collect samples. Spearman's rho estimated the correlation of predicted (kriged) S/P values between systems as 0.278, 0.355 and -0.166 for systems 1&2, systems 2&3, and systems 1&3 respectively. Kriged values at the sample locations (Table 2.4) were different overall based on the Friedman test (P < 0.001) and all pairwise comparisons between systems (P < 0.001).

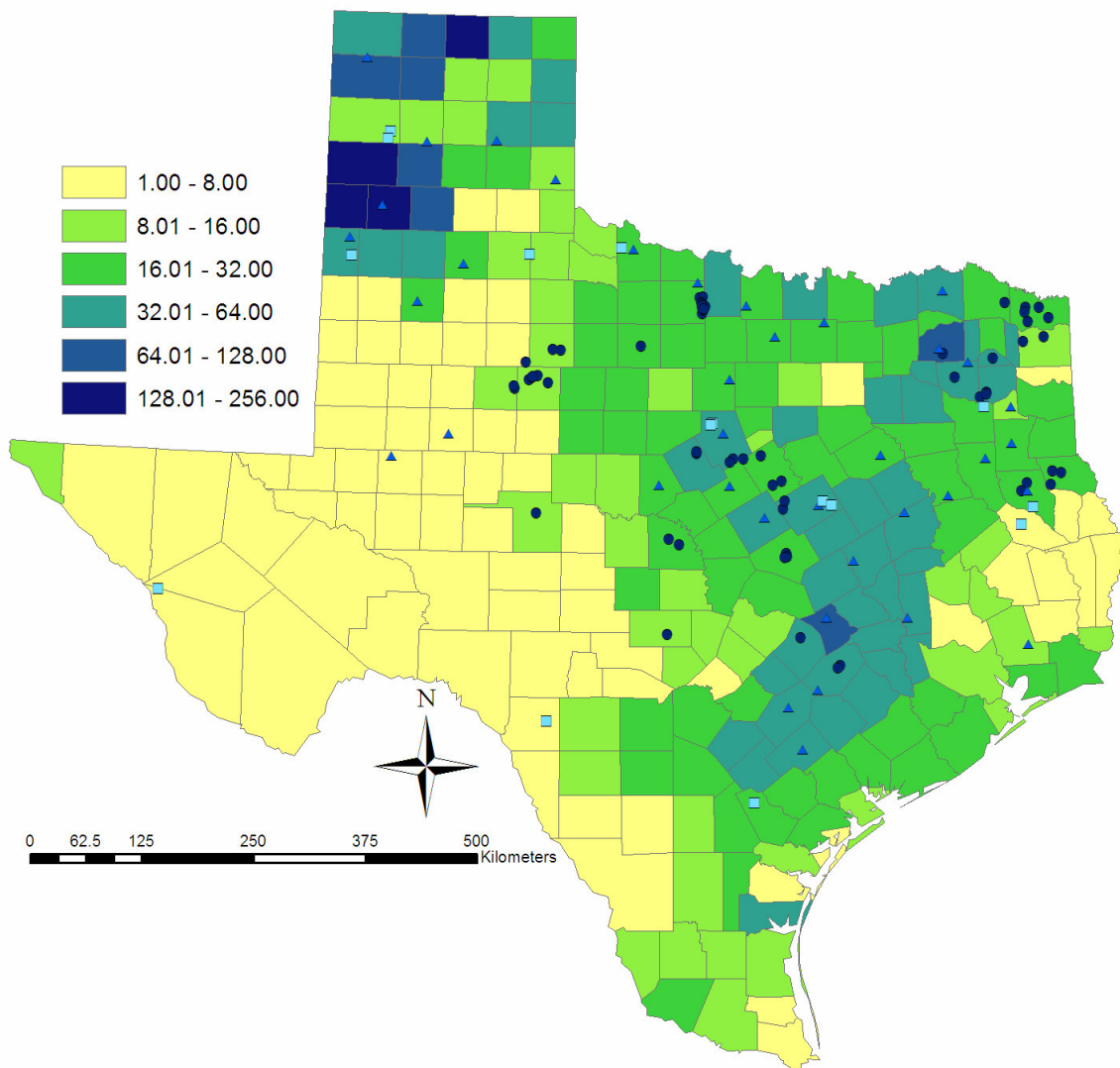


Figure 2.2. Distribution of locations for cattle serum samples received based on each system. Squares, circles and triangles represent system 1, system 2, and system 3, respectively. Cattle density per km² is defined by the color scale.

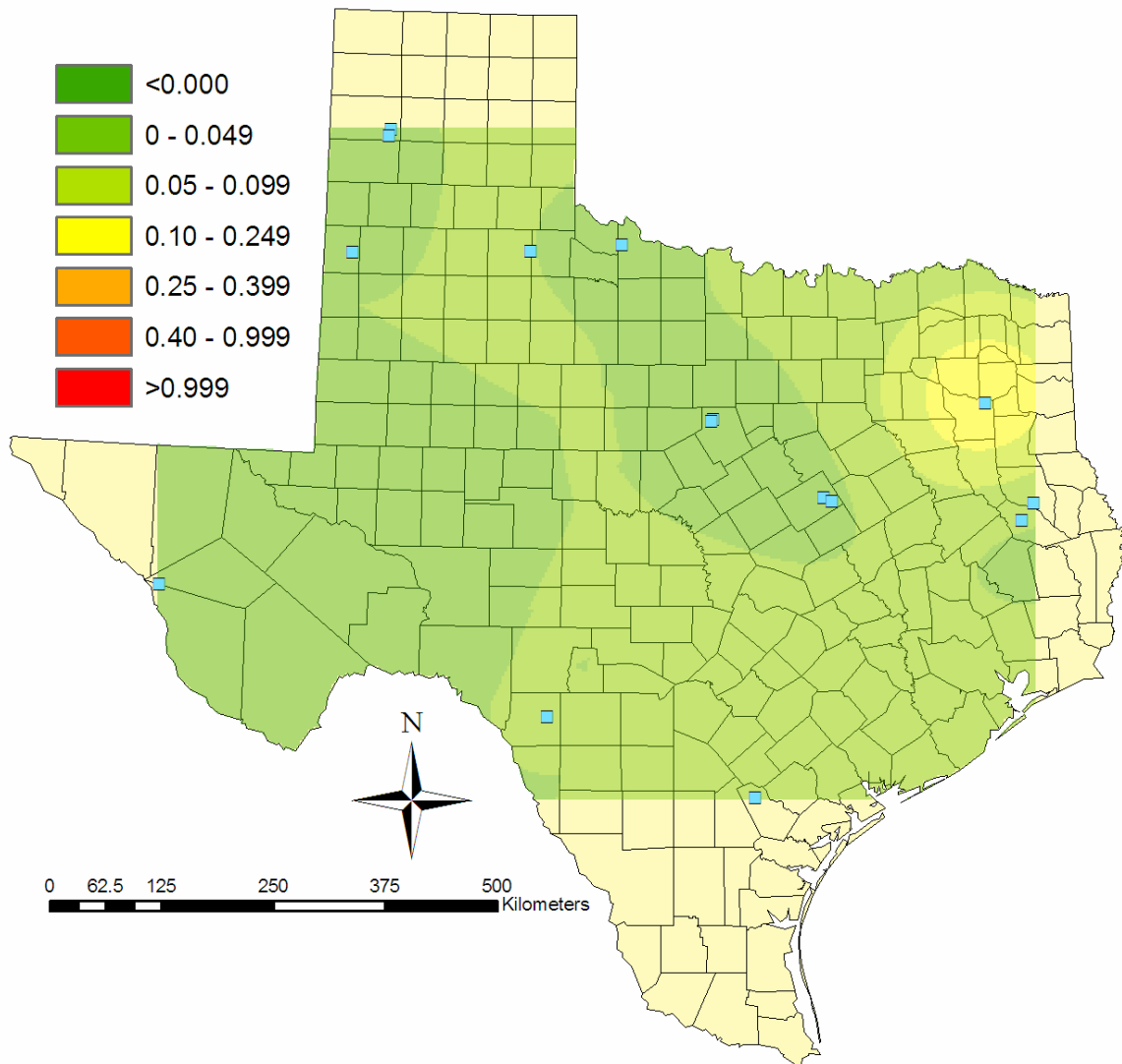


Figure 2.3. Distribution of kriged S/P ratios, by seropositivity category, in relation to system 1 cattle locations.

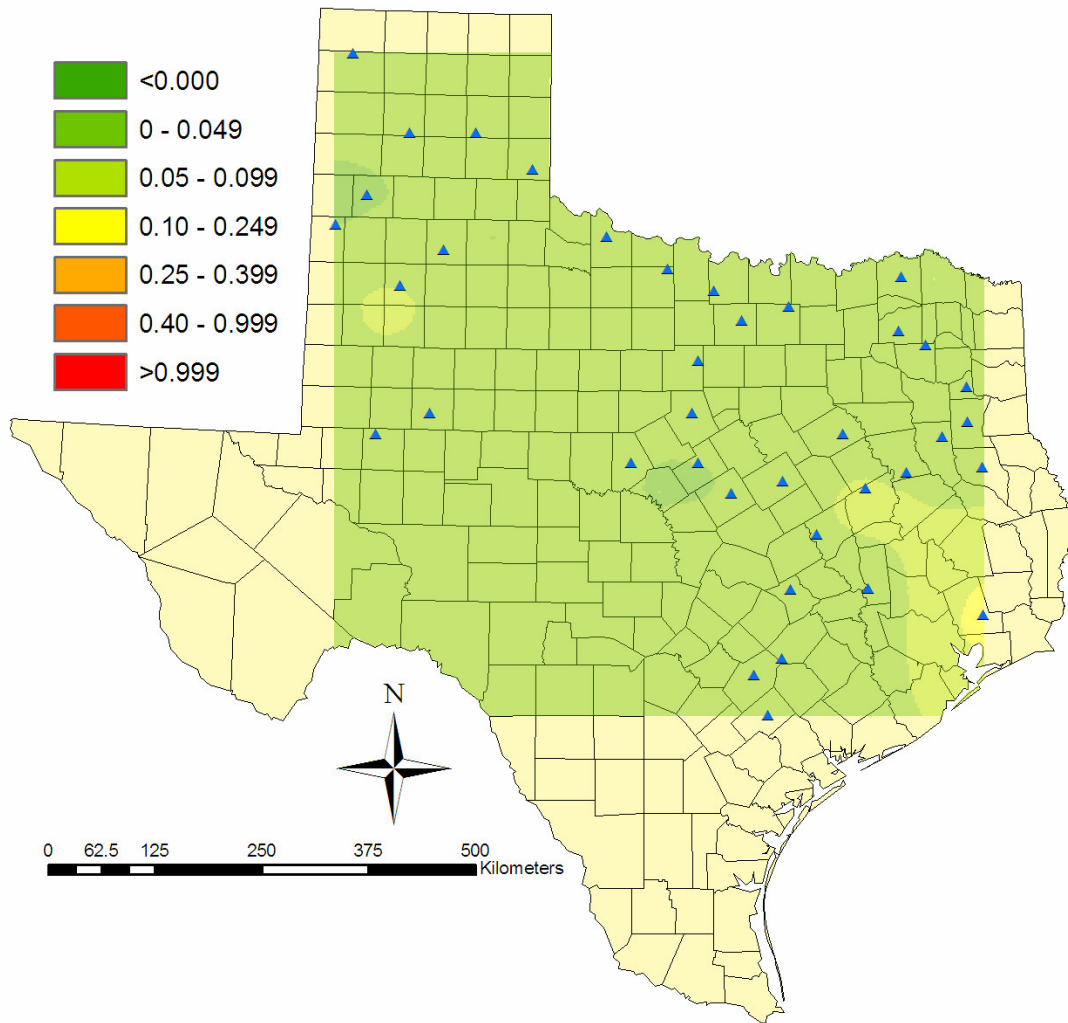


Figure 2.4. Distribution of kriged S/P ratios, by seropositivity category, in relation to system 2 cattle locations.

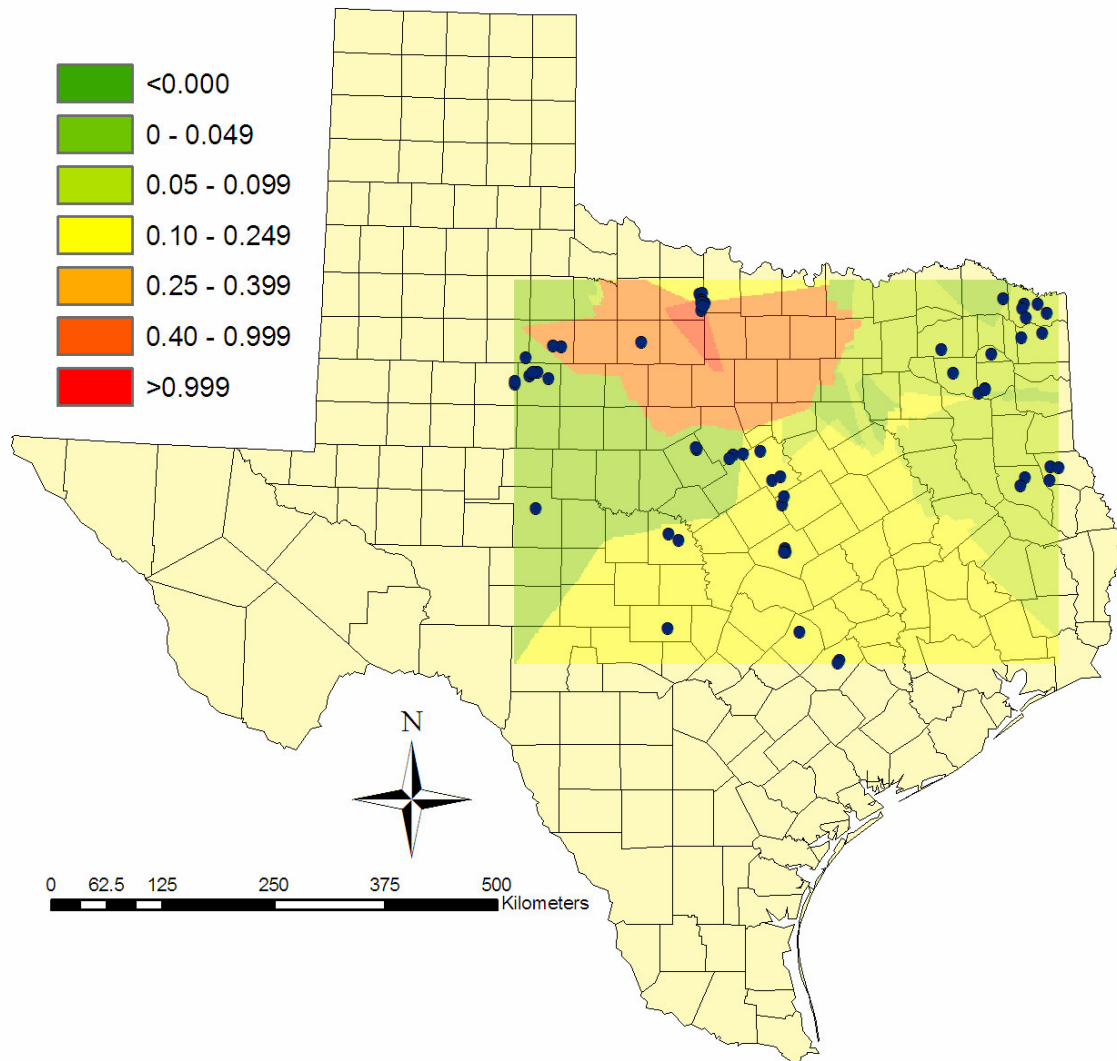


Figure 2.5. Distribution of kriged S/P ratios, by seropositivity category, in relation to system 3 cattle locations.

Table 2.4. Descriptive statistics for kriged S/P values from each system at sample locations.

System	No. Locations	Mean	Std. Dev.	Minimum	Maximum
1	82	0.0178	0.0342	-0.0198	0.1301
2	82	0.0215	0.0120	-0.0177	0.1294
3	82	0.1693	0.2204	0.0067	1.3586

Discussion

Johne's disease detection and control is important for animal health, cattle productivity and possibly human health. The long incubation period and potential for cattle to be silent shedders of the bacteria while contaminating the environment, makes the disease difficult to control. Absence of clinical signs in many animals until the later stages of disease also allows for animals to remain infectious for the entire herd over long periods of time. The identification of areas that have a higher propensity for seropositive animals could be used to help control JD. In our study a very low prevalence of JD seropositivity was observed in Texas overall from the 3 systems. Differences in the areas of high seropositivity were seen from the kriged maps produced for each system.

In a previous study of the prevalence and risk factors for paratuberculosis in purebred beef cattle in Texas (Roussel et al., 2005) ELISA results classified 3% of cattle (137/4579) as positive and 44% of herds (50/115) had at least 1 seropositive animal. It was concluded that seroprevalence of paratuberculosis among purebred beef cattle may, in general, be greater than seroprevalence among beef cattle in the US. The previous

study also estimated a higher spatial odds ratio for ELISA S/P in the central eastern parts of Texas, which is different than the high-risk areas identified in the present study.

The overall prevalence of positive JD ELISA results was 2.2%. This overall estimate is quite low and is also lower than the previous study in Texas cattle (Roussel et al., 2005). For system 1 cattle, the prevalence was 2.3% overall, which would indicate that beef herds in Texas have relatively low numbers of Mptb infected cattle. The prevalence for herds in this study (31%) was also lower than the previous study (Roussel et al., 2005), where herd prevalence was 44%. However, herd 1 had the most positive ELISA results, with a prevalence of 18.3% while the remaining herds (2-13) had a pooled prevalence of only 0.4%. This leaves two possibilities for herd 1; either infection with Mptb is very common in this herd or the high prevalence is due to many false-positive serologic responses. Since these animals are extensively managed beef cattle and prevalence of this magnitude is improbable, the most likely explanation is exposure to environmental mycobacteria causing false-positive ELISA results. System 2 cattle had the lowest prevalence (1.6%), but similar to the findings for system 1. Most market cattle in Texas are beef cattle, with a few areas having dairy-specific markets. The diagnostic specificity of this ELISA has been estimated to be between 95 and 99% (Whitlock et al., 2000; Collins, 2002; Kalis et al., 2002; Collins et al., 2005; McKenna et al., 2005), suggesting that all positive JD results in herd 1 (system 1) and system 2 could be due to false-positive ELISA results.

System 3 had the highest prevalence of JD seropositive animals (9.5%) and the highest overall S/P ratio (5.5). Based on these factors, it appears that this system is the

most sensitive for detecting JD seropositive cattle. This indicates that cattle that are singled out by the owner for health or performance issues, have a greater chance of being a JD seropositive animal. However, in comparison to systems 1 and 2, the number of tested animals was much lower. According to the ELISA S/P categories, most animals from all systems had results that fell within the negative and suspect ranges. However, system 3 had the highest prevalence of positive results and also the highest proportion of strong positive results.

System 3 animals had a higher proportion of beef cattle than dairy cattle and there was also a greater proportion of females tested than males. Seven of the 74 cattle in this system were considered JD positive based on their S/P ratio. Of these 7 animals, 2 were male and 5 were female, with 4 beef cattle and 3 dairy cattle. The prevalences within dairy cattle and beef cattle were 17% (3/17) and 7% (4/57) respectively. These results are consistent with the general opinion that dairy cattle tend to be affected with JD more frequently than beef cattle. The proportion of males in most cattle populations is generally quite low and the relatively high prevalence within male beef cattle in system 3 was unexpected.

Samples from the evaluated systems did not cover all areas of Texas and collection locations visually appeared clustered. This was most apparent in system 3, where many locations were geographically close. This was probably due to the areas that participating veterinarians cover for their clinics, and the fact that some clinics sent more samples than others. Areas of increased seropositivity were dependant upon the surveillance system from which the samples were received. System 1 samples had a

concentration of suspect positive values in the north eastern corner of Texas, along with slightly higher negative S/P ratios in the north central and south west areas of Texas.

System 2 had several higher seropositive areas throughout eastern Texas along with one higher area in western Texas. System 3 had a very strong positive area covering most of the north central area of Texas, along with a higher seropositive area in the south central to south east portion of Texas (where locations were observed), and a slightly higher area in eastern Texas. These findings, especially the higher areas of system 3 and system 1, seem to correlate with the dairy cattle population of Texas. These dairy areas are concentrated in the north eastern areas around Sulphur Springs and Stephenville (areas near Dallas) and in the panhandle areas around Amarillo and Lubbock.

Collection of samples based on the 3 systems in this study might not necessarily be possible in other regions of the country, or in other countries. A more appropriate testing scheme might be possible for systems 1 and 2, which averaged S/P ratios for animals with the same spatial locations. Better participation by practitioners and from a greater area throughout the state could change the overall interpretation of system 3. Inclusion of cattle production type, proportional to the true distribution, might also improve predicted S/P distributions. However, our results show that based on the type of surveillance system employed, varying detection will be achieved. Using only a single type of system might not appropriately predict the distribution of JD seropositivity and the type of system to employ should depend upon the objective of the monitoring or surveillance program.

Conclusions

Spatially predicted JD seropositivity depends upon the system employed for the collection of samples. No method of surveillance is deemed to be better than another, but the purpose of the surveillance system must be carefully considered during its design and interpretation of results.

CHAPTER III

DISCUSSION AND CONCLUSION

As stated in Chapter II, JD detection and control is important for animal health, cattle productivity and possibly human health. The long incubation period and potential for cattle to be silent shedders of the bacteria while contaminating the environment, makes the disease difficult to control. Absence of clinical signs in many animals until the later stages of disease also allows for animals to remain infectious for the entire herd over long periods of time. The identification of areas that have a higher propensity for seropositive animals could be used to help control JD. In our study a very low prevalence of JD seropositivity was observed in Texas overall from the 3 systems. Differences in the areas of high seropositivity were seen from the kriged maps produced for each system.

Difficulties were encountered creating kriged maps for the data in this study. The actual process for kriging is a complicated and computationally intensive process, but when using programs that do the calculations for you, it becomes a bit easier. This is done by using programs designed to estimate the semivariogram and then using those parameters in the mapping program. The difficulty in the procedure was estimation of the kriging parameters, mainly because the best fit of parameters is left to subjective observation. This process was challenging for all systems, but especially for system 3. The biggest challenge of this geostatistical method was that each spatial location can only have one value. Locations that had more than one value (more than one animal at

the site) had to have values averaged. Also, another problem was properly fitting a semivariogram to the data. System 3 posed an enormous challenge for estimating an appropriate semivariogram. The main reason system 3 was so difficult was probably due to the great variance in S/P values among locations. This created problems because large S/P values (high positive values ≥ 1.25) for locations with a single animal exerted a large influence relative to locations where values were averaged over multiple animals. After much trial and error, semivariograms and their corresponding nugget, sill and range were estimated for each system (Appendix).

Samples from the systems did not cover all areas of Texas and some sample locations appeared clustered. Visual clustering was most apparent for system 3, where many locations were relatively close together. This was probably due to the areas that participating veterinarians cover for their clinics, and the fact that some clinics sent more samples than others. System 1 also had 2 areas where the sampled herds were close. This was due to the fact that 2 TAERECs and a private ranch had multiple locations where animals were raised (and subsequently sampled) and 2 private ranches were sampled within another ecological zone (Figure 3.1). Areas of increased seropositivity were observed throughout Texas and depended upon the surveillance system from which samples were received. System 1 samples had a concentration of suspect positive values in the north eastern corner of Texas, along with slightly higher negative results in the north central and south west areas of Texas. System 2 had several higher seropositive areas throughout eastern Texas along with one higher area in western Texas. System 3 had a strong positive area covering most of the north central area of Texas, along with a

higher seropositive area in the south central to south east portion of Texas (where locations were observed), and a slightly higher area in eastern Texas. Based on these findings, especially within systems 1 and 3, the higher areas seem to correlate with the dairy cattle population in Texas. These dairy areas are concentrated in the north eastern areas around Sulphur Springs and Stephenville (areas near Dallas) and in the panhandle areas around Amarillo and Lubbock (Figure 3.2).

Differences of seropositivity in an area could be due to several factors. The production type of the cattle is expected to be associated with the JD test results because dairy cattle are known to have higher infection rates of Mptb compared to beef cattle.

Following the same thought, systems which included more animals of one type (beef or dairy) than another, would likely demonstrate different spatial distributions. Based on the animals selected in the systems, system 1 did not sample dairy animals and therefore would be expected to have very low prevalence of seropositive animals. Although the prevalence was quite low, it was not the lowest compared to the other systems, which included dairy cattle. One of the possibilities for this is the fact that herd 1 of system 1 had more positive ELISA results than the rest of the herds combined. This could be due to herd 1 having a high Mptb infection prevalence and possibly clinically ill cattle. However, since beef herds are generally extensively managed, a more plausible explanation would be that cattle in this herd have false-positive results due to environmental factors, such as exposure to other mycobacteria that can cause false-positive results. System 2 for instance was based on samples from livestock markets throughout the state. Generally markets have a higher number of beef cattle, but there

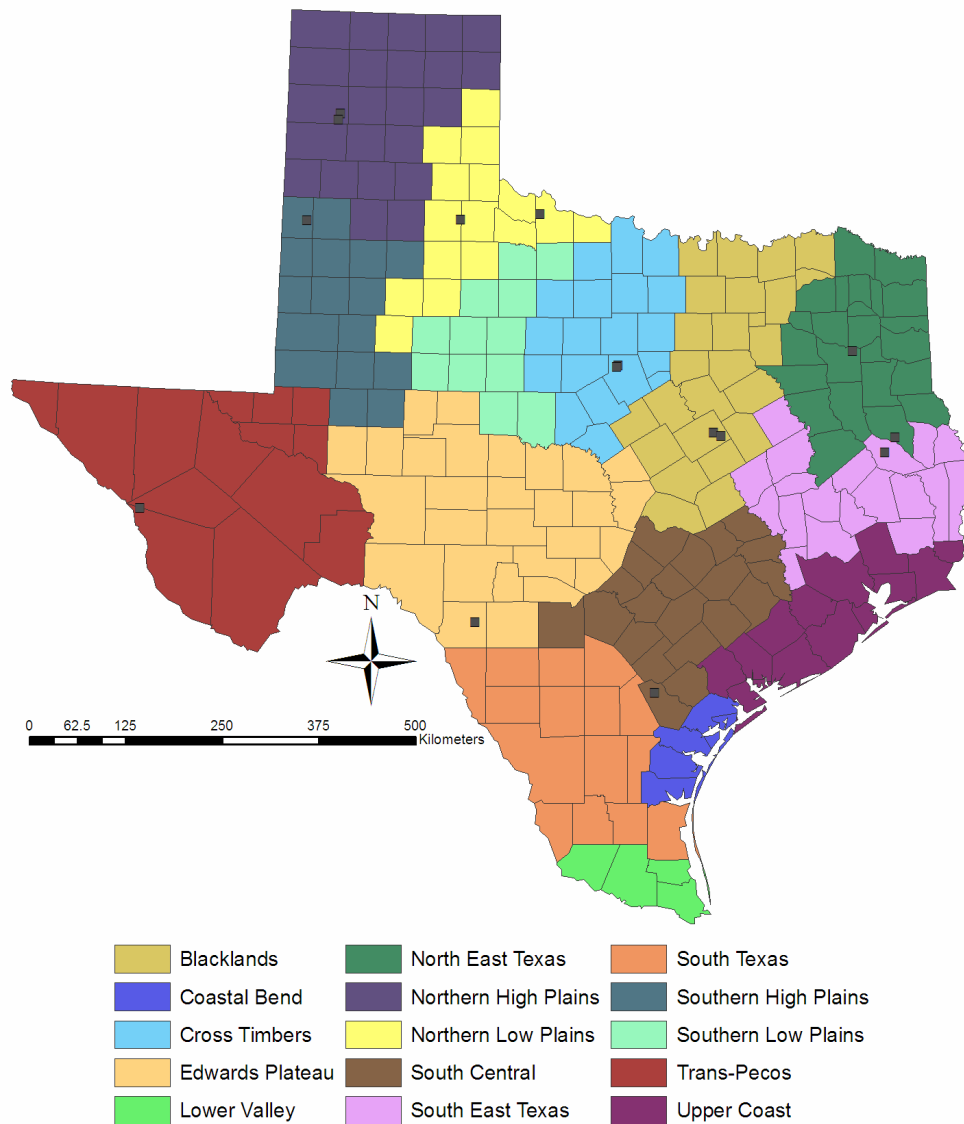


Figure 3.1. The 15 ecological zones of Texas based on the Texas Agriculture Statistical Districts. Gray squares represent sampled herd locations.

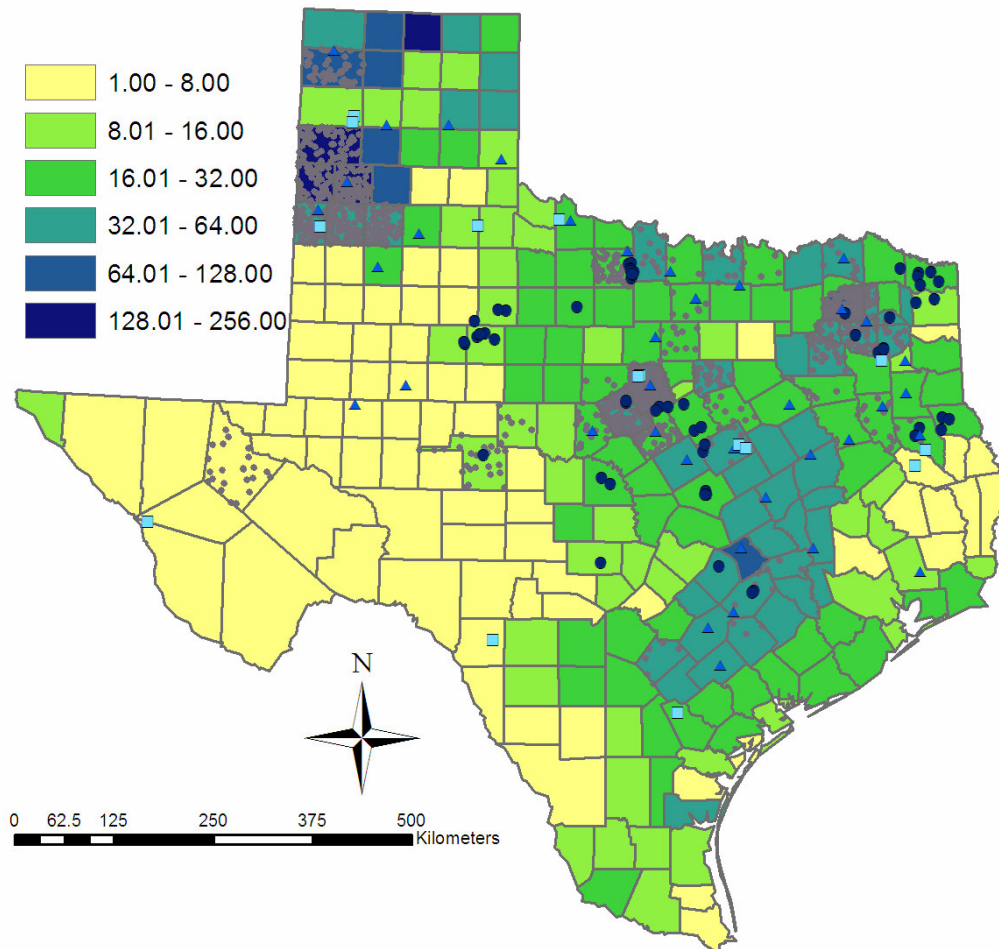


Figure 3.2. Texas cattle density (animal/km²) represented by color scale and dairy cow density (1 dot = 200 cows). System 1, 2 and 3 sample locations are designated by light blue squares, medium blue triangles and dark blue circles, respectively.

are markets that specialize in cull dairy cows. Also if a market is located near the dairy regions of Texas (Figure 3.2), there is an increased likelihood that market animals include dairy cattle. It seems unusual that cattle in the third system had the largest absolute number of positive animals because there were so few cattle sampled. It would seem that a greater chance of receiving samples from infected cattle would exist from the other systems. However, this was not the case and was most likely due to the fact that this system included cattle that were 'sick', thus increasing the likelihood that they could have a chronic disease. JD could also predispose cattle for other disease processes.

Following the same thought, systems which included more animals of one type (beef or dairy) than another, would likely demonstrate different spatial distributions. Based on the animals selected in the systems, system 1 did not sample dairy animals and therefore would be expected to have very low prevalence of seropositive animals. Although the prevalence was quite low, it was not the lowest compared to the other systems, which included dairy cattle. One of the possibilities for this is the fact that herd 1 of system 1 had more positive ELISA results than the rest of the herds combined. This could be due to herd 1 having a high Mptb infection prevalence and possibly clinically ill cattle. However, since beef herds are generally extensively managed, a more plausible explanation would be that cattle in this herd have false-positive results due to environmental factors, such as exposure to other mycobacteria that can cause false-positive results. System 2 for instance was based on samples from livestock markets throughout the state. Generally markets have a higher number of beef cattle, but there are markets that specialize in cull dairy cows. Also if a market is located near the dairy

regions of Texas (Figure 3.2), there is an increased likelihood that market animals include dairy cattle. It seems unusual that cattle in the third system had the largest absolute number of positive animals because there were so few cattle sampled. It would seem that a greater chance of receiving samples from infected cattle would exist from the other systems. However, this was not the case and was most likely due to the fact that this system included cattle that were 'sick', thus increasing the likelihood that they could have a chronic disease. JD could also predispose cattle for other disease processes.

The number of veterinarians contacted that were willing to participate was low. Those who did agree to participate submitted few samples and some did not submit any at all (Figure 3.3). Many of the veterinarians from which samples were received, were the same practitioners from which samples were received on a regular basis. A greater number of samples from all veterinarians that agreed to participate and more widely distributed practitioner locations would have allowed for a better estimation of the distribution of JD seropositivity throughout the state. Better and more detailed information submitted on the information sheets (Appendix) that accompanied each sample, would also have been better for estimation of exact locations and possibly reduce the necessity to average values for non-unique locations.

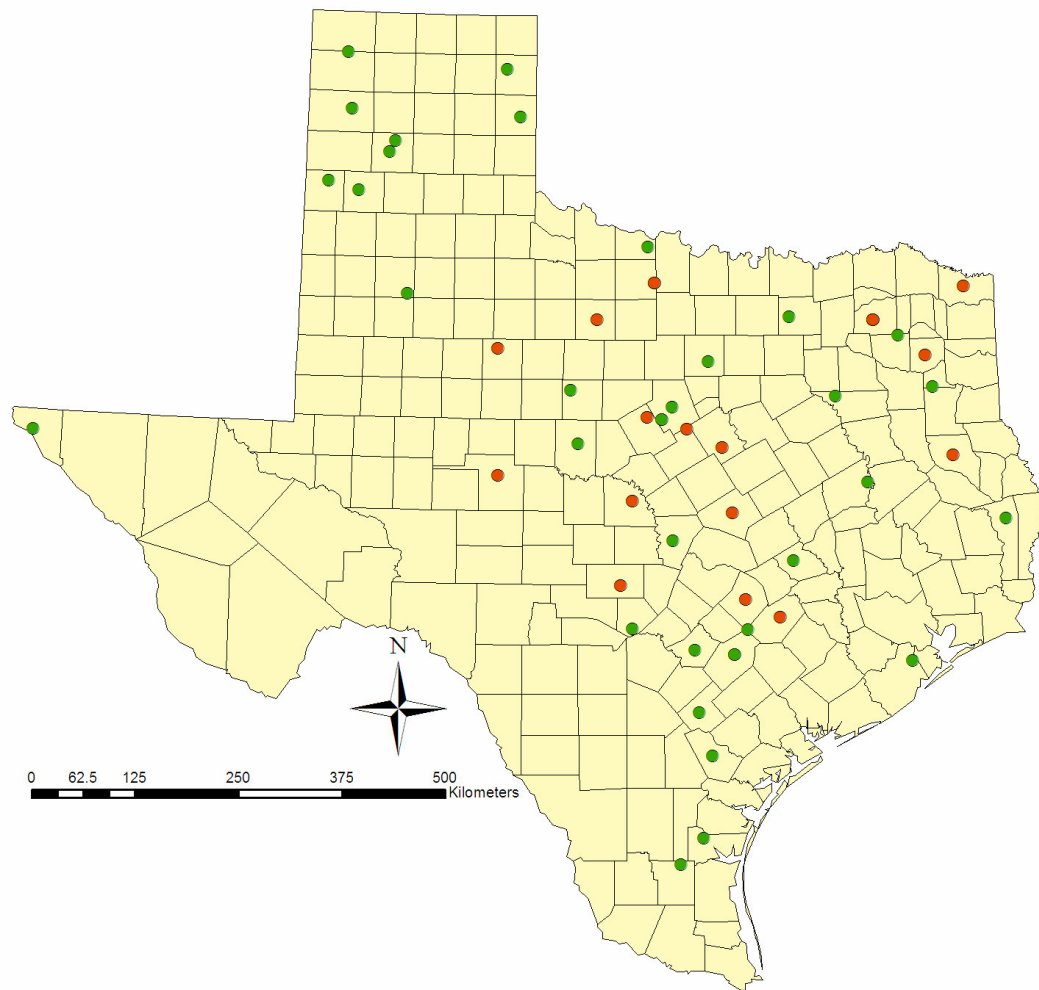


Figure 3.3. Veterinarian locations throughout Texas. Red dots represent participating practitioners who submitted serum samples and green dots represent those who did not submit samples.

Collection of samples based on the 3 systems for this study might not necessarily be possible in other regions of the country, or in other countries. A more appropriate sampling scheme might also be possible for systems 1 and 2, which averaged S/P ratios for animals with the same spatial location. However, our results do show that based on the type of surveillance system employed, varying detection will be achieved.

Based on the 3 systems employed in this study, there are distinct differences in the geographical locations of JD seropositivity, dependant upon the system used. System 1 seems the most plausible system to use, but no system was deemed to be the 'best' or 'most accurate' system for surveillance through this study. System 3 had the greatest sensitivity for the detection of seropositive animals. Validation of this finding or to determine the type of system able to make the most valid predictions concerning seroprevalence, would necessitate further study, possibly in other locations throughout the country.

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APPENDIX

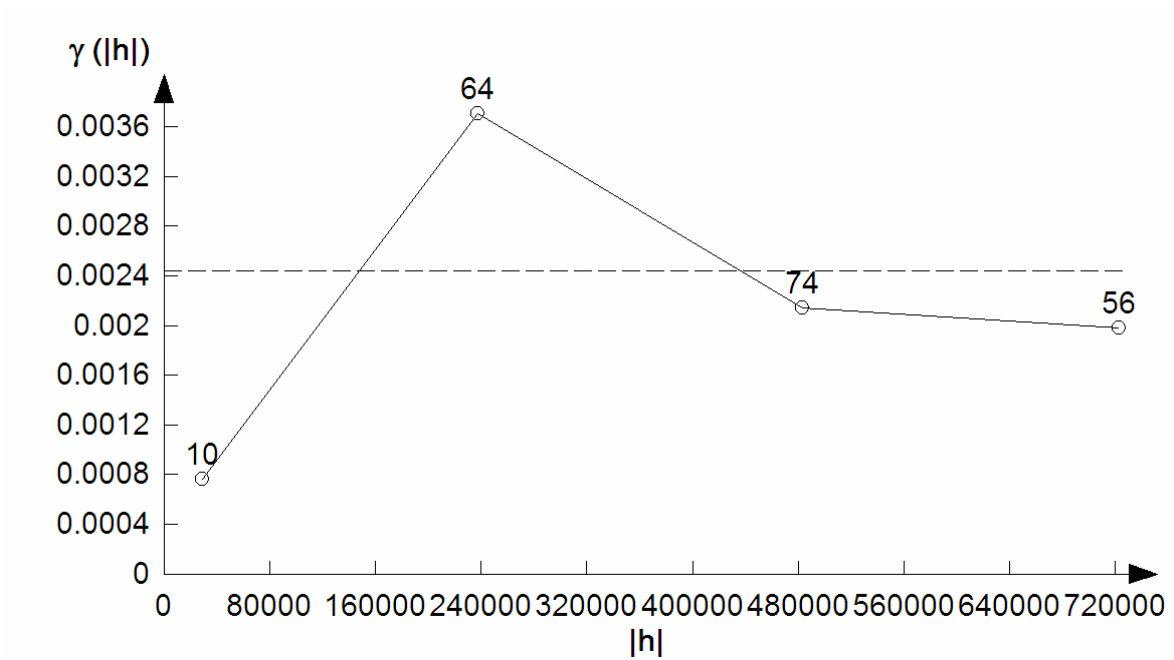


Figure A.1. System 1 variogram.

Indicative goodness of fit

current fit: 2.1953e-01
best fit found: 2.1953e-01

Nugget: 0.00072

1st structure	2nd structure	3rd structure
Dir.: 0	Dir.: 0	Dir.: 0
Model: Gaussian	Model:	Model:
Range: 189800	Range: 0	Range: 0
Sill: 0.00249	Sill: 0	Sill: 0
Anis.: 1	Anis.: 1	Anis.: 1

Store Restore Best fit found

A priori Cov./Corr. >>

Figure A.2. System 1 model parameters.

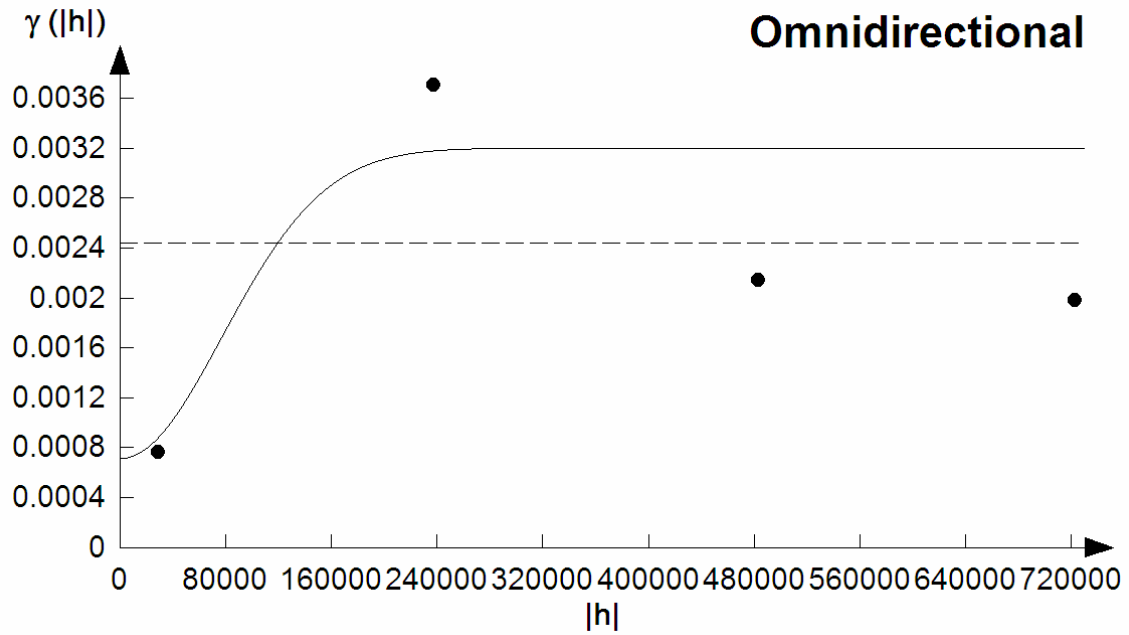


Figure A.3. System 1 model.

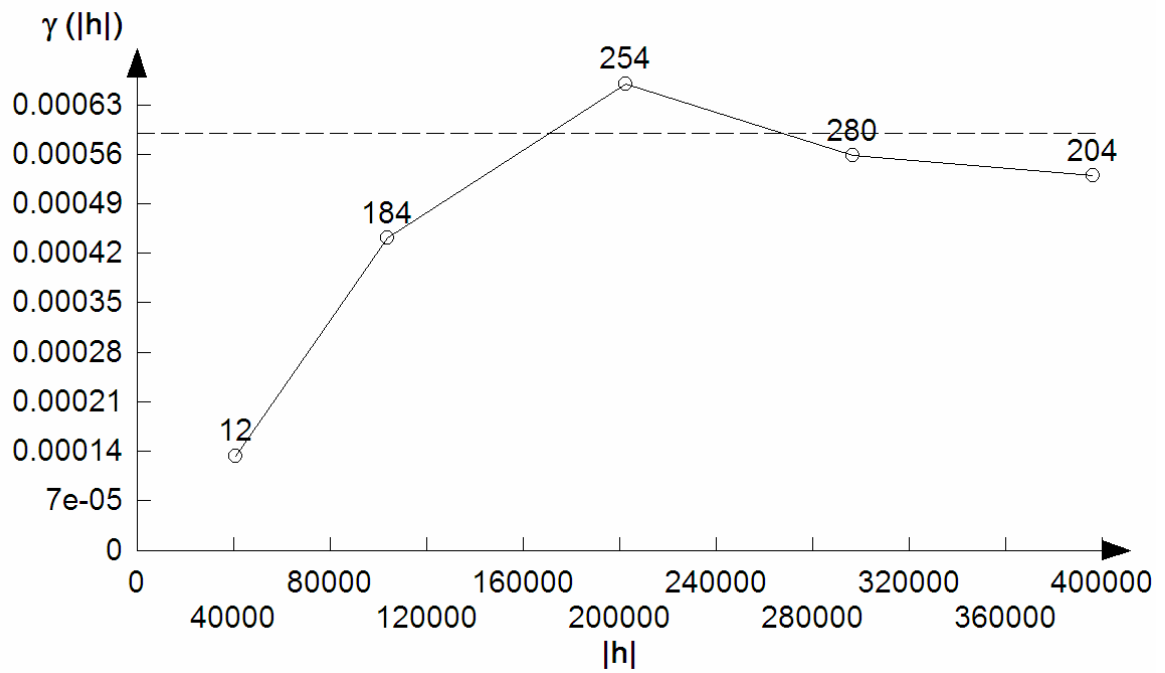


Figure A.4. System 2 variogram.

Indicative goodness of fit

current fit: 1.7309e-02
best fit found: 1.7309e-02

Nugget: 0.00012

1st structure	2nd structure	3rd structure
Dir.: 0	Dir.: 0	Dir.: 0
Model: Gaussian	Model:	Model:
Range: 180000	Range: 0	Range: 0
Sill: 0.000516	Sill: 0	Sill: 0
Anis.: 1	Anis.: 1	Anis.: 1

Store Restore Best fit found

A priori Cov./Corr. >>

Figure A.5. System 2 model parameters.

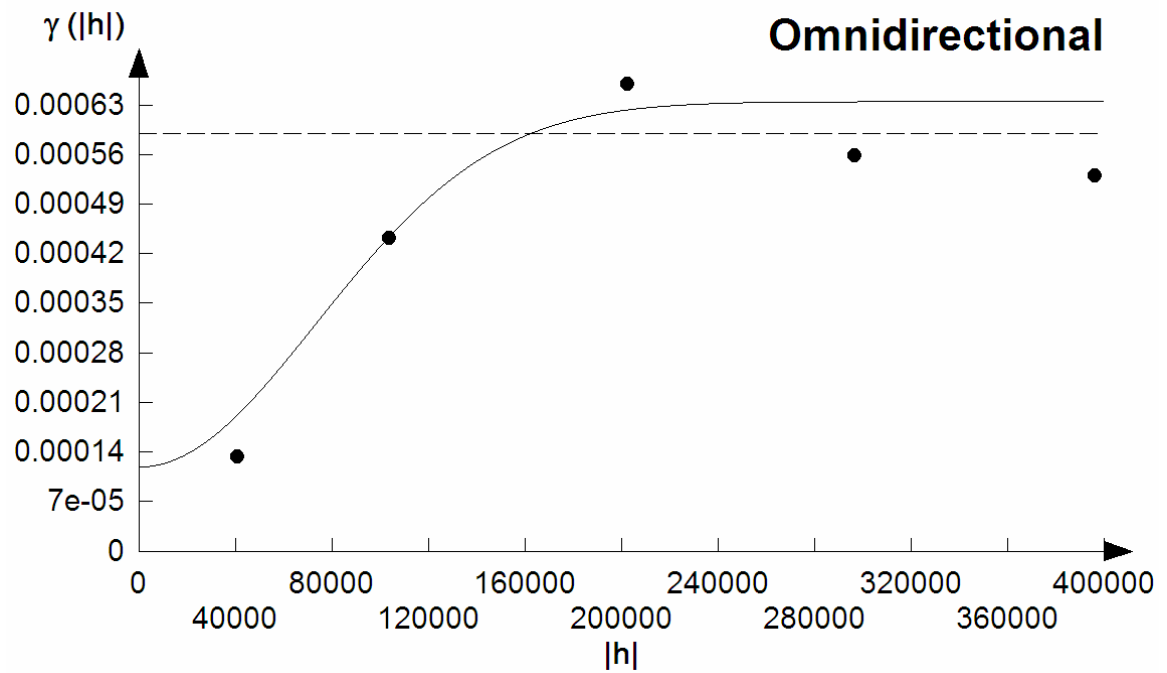


Figure A.6. System 2 model.

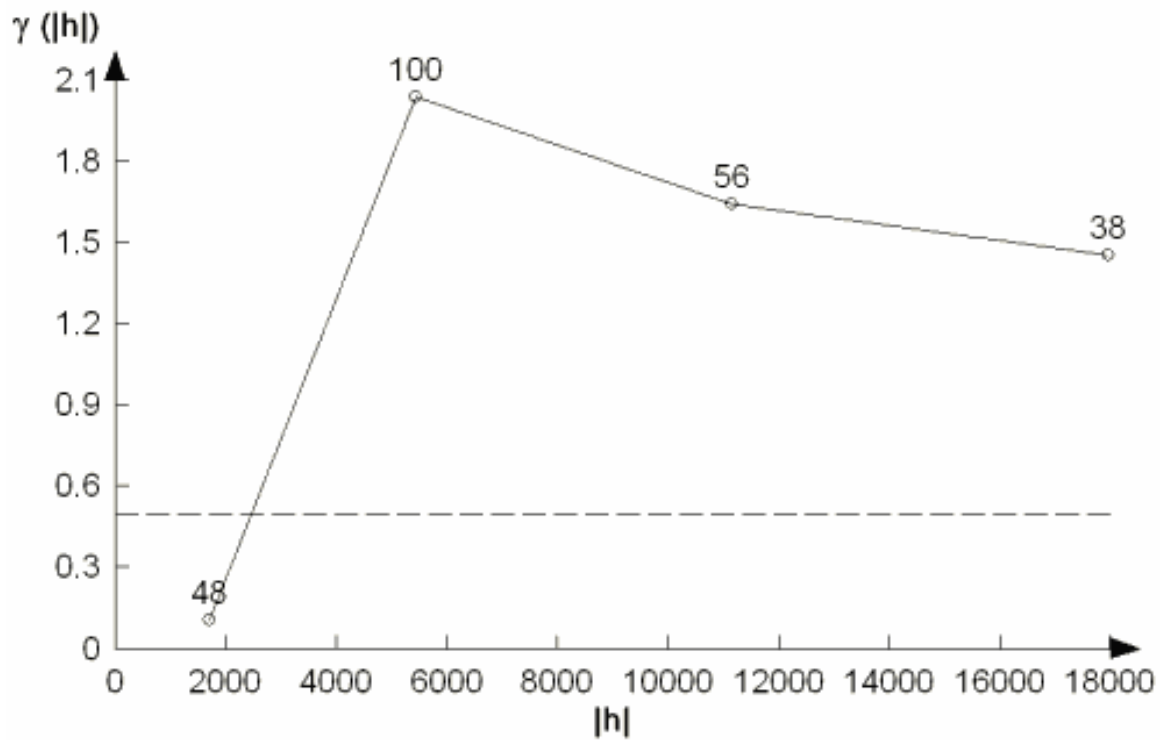


Figure A.7. System 3 variogram.

Indicative goodness of fit

current fit: 1.3662e+01
best fit found: 1.3662e+01

Nugget: 0.115

1st structure	2nd structure	3rd structure
Dir.: 0	Dir.: 0	Dir.: 0
Model: Gaussian	Model:	Model:
Range: 4140	Range: 0	Range: 0
Sill: 0.5	Sill: 0	Sill: 0
Anis.: 1	Anis.: 1	Anis.: 1

Store Restore Best fit found

A priori Cov./Corr. >>

Figure A.8. System 3 model parameters.

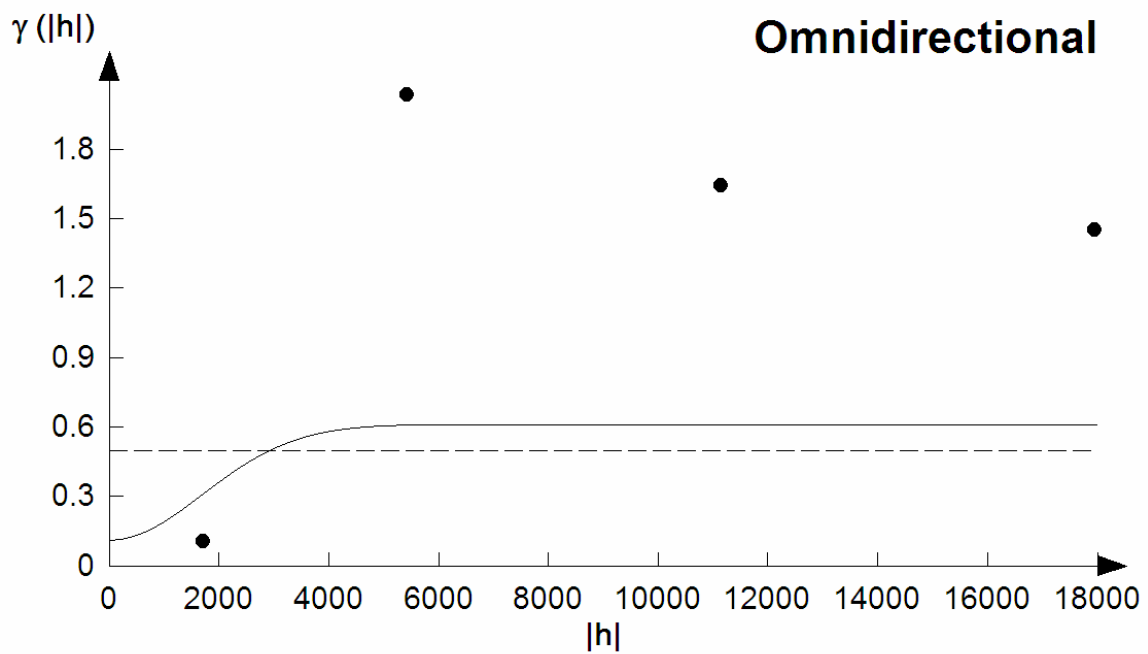


Figure A.9. System 3 model.

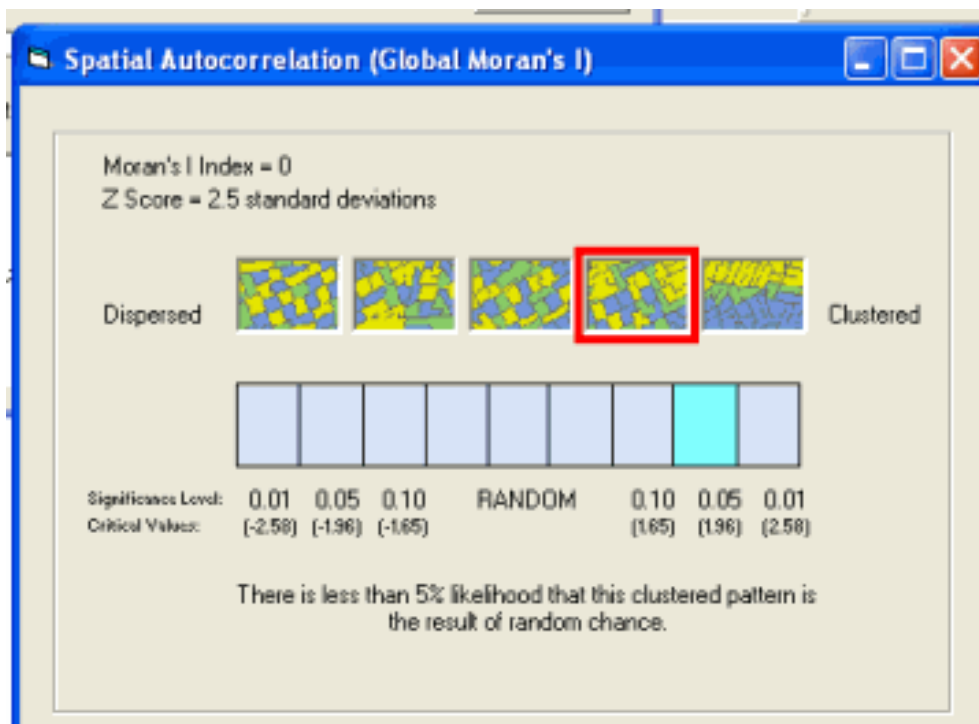


Figure A.10. Spatial autocorrelation for points in all systems using S/P ratios.

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