STATISTICAL ANALYSIS OF THE IDEXX C6 ELISA FOR THE DETECTION OF LYME DISEASE AND EHRLICHIOSIS IN A NON-ENDEMIC AREA

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ABSTRACT

The overdiagnosis of Lyme disease and Ehrlichia has caused concern and debate in the veterinary community. This is of particular importance in areas considered non-endemic for tick-borne diseases, where the common vector, *Borrelia burgdorferi*, is known to be less prevalent. This study was performed to help determine the financial viability of performing the popular IDEXX C6 ELISA for the detection of Lyme disease and Ehrlichia in a non-endemic area. It was hypothesized that there would be a number of false positives due to the low prevalence of the target diseases in East Tennessee.

The study was performed in Maryville, Tennessee at Village Veterinary Hospital. The sample size consisted of 465 canines tested as part of routine yearly analysis. Each owner was asked to fill out a survey regarding travel behaviors, where the canine had lived, and pertinent symptoms. The IDEXX C6 ELISA was performed for each sample. Samples that tested positive were then sent Antech Laboratories for the definitive tests (PCR for Ehrlichia, Western blot for Lyme).

Of the 465 total samples, the IDEXX ELISA found seven samples positive for Lyme disease and one sample positive for Ehrlichia. These samples were all confirmed as true positives by the definitive tests. Due to financial and logistical constraints, the definitive tests were not performed to determine false negatives.

Six of the seven samples positive for Lyme disease and the sample positive for Ehrlichia were obtained from canines living in New York or Pennsylvania. The remaining sample was found from a local dog.

Prevalence of disease was the variable of particular concern in this study, and the positive predictive value was expected to correlate with the values extrapolated from low prevalence. This was not the case due to either the exceptional nature of the test, misjudgment regarding the endemic nature of Tennessee, or unusual data due to a small number of positives. There was, however, a clear indication that presence of Lyme disease or Ehrlichia is linked to geographic location. Based on the results obtained, there is support for performing the IDEXX ELISA on a regular basis in non-endemic areas.

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CHAPTER I

INTRODUCTION

A vector-borne disease is any disease that is transferred to the primary host by one or more intermediate hosts (vectors). The majority of vector-related diseases are related to mosquitoes and ticks. These vectors account for diseases that include malaria, West Nile, dengue, lyme disease, heartworms, Rocky Mountain spotted fever, and ehrlichia. Primary hosts for vector-borne diseases vary, depending on the specific vector and type of disease. For this study, the vector of particular importance is the *Ixodes ricinus* tick. Ticks are ectoparisites that feed on the blood of their hosts allowing viruses, bacteria, and protozoa to be easily transmitted to the organism (Raven & Johnson, 2002).

Lyme borreliosis

Lyme disease (Lyme borreliosis) is the most common vector-borne disease in humans and is prevalent among wild and domesticated animals as well. The disease is caused by the spirochete *Borrelia*, which cannot be seen without advanced microscopy. These spirochetes (*Borrelia burgdorferi sensu lato*) are divided into four genomic species groups. The group seen most often in the United States is Group 1, *B. burgdorferi sensu stricto* (Greene, Appel, & Straubinger, 1998). Because the spirochetes are associated with a primary tick

vector, *Ixodes ricinus*, the disease is thought to be relatively limited to the areas inhabited by this vector (Leib & Monroe et al., 1997).

The *Ixodes ricinus* group includes three host ticks each having a 2 to 3 year cycle (Leib & Monroe et al., 1997). These three host ticks are *I. dammini, I. scapularis, and I. pacificus*. *I. dammini* is the most commonly cited vector with the disease and is typically found on the east coast, which corresponds with the assumed endemic area. *B. burgdorferi* can be transmitted to dogs in the nymph and adult stages of the vector, but most often larger mammals are targeted by the adult ticks. The vector can become infected with Lyme disease by feeding off of a dog harboring the spirochete; dogs are therefore considered reservoir organisms for the disease. Figure 1 shows the life cycle of *I. scapularis*, although all *Ixodes* species are similar in life cycle.

Clinical signs of Lyme disease have been a source of debate. Recently, however, experimental infection has been successful allowing better observation. Symptoms occur 2-5 months after inoculation. Initial clinical signs among canines include fever, lameness, articular swelling, lymphadenomegaly, and anorexia (Greene, Appel, & Straubinger, 1998). This makes diagnosis difficult due to the fact that fever, articular swelling, and anorexia occur equally in seropositive (testing positive for antibodies) and seronegative (testing negative for antibodies) dogs. The most common experimentally documented symptom has been polyarthritis (Shin et al., 1992). This often continues even with antimicrobial treatment. In long-term infections, nonerosive polyarthritis is

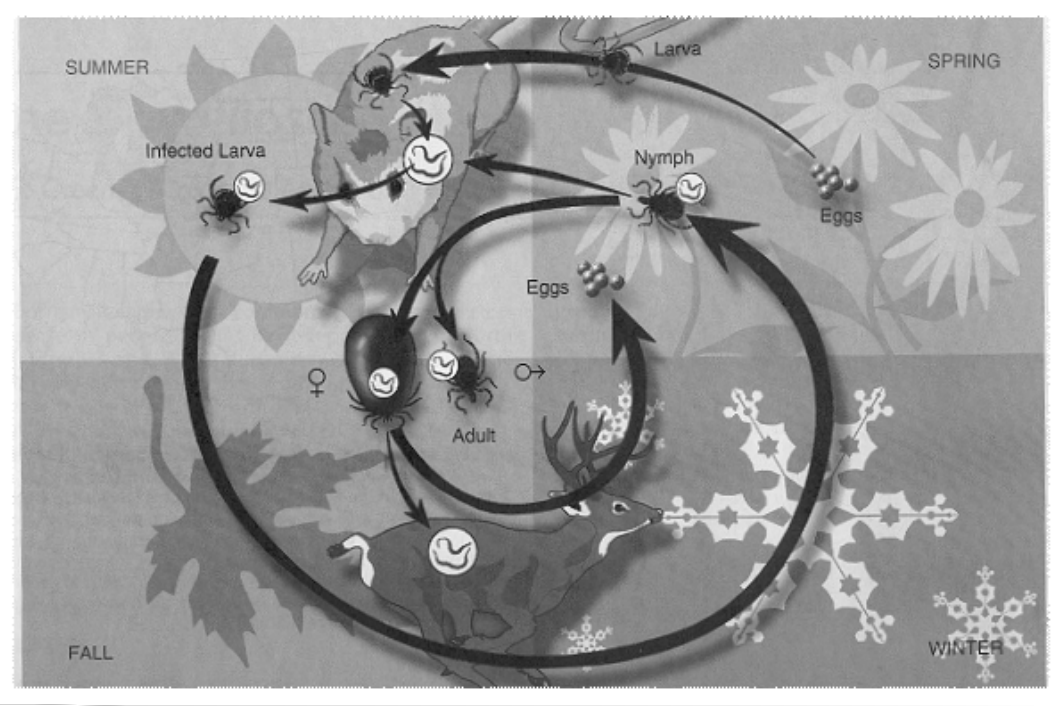

Figure 1. Eggs are oviposited in the spring and hatch approximately one month later. They then feed once in the summer followed by an overwinter period. The spring of the second year, they molt into nymphs and feed during spring or summer. During the fall, they molt into adults and feed on large mammals (Greene, Appel, & Straubinger, Appel, & Straubinger, 1998, 284).

often commonplace. In rare cases renal failure as well as azotemia, uremia, proteinuria, peripheral edema, and body cavity effusions have been seen. This occurred most often in labradors and golden retrievers. Occasionally, neurologic signs are found in the form of Lyme neuroborreliosis, which is caused by a central

nervous system infection by the same spirochete (Cuoto & Nelson et al., 1999). The symptoms of Lyme neuroborreliosis closely resemble central nervous system disease. Lyme carditis is also found and causes a number of cardiac related symptoms.

The difficult diagnosis associated with Lyme borreliosis and the presence of several different species of *Borrelia* spirochetes makes determining the exact geographical prevalence difficult, especially in animals. Inferences are typically made regarding endemic areas by evaluating the reported cases in humans, although this is not supported by bacteriological findings from those supposedly acquired from non-endemic areas (Jacobsen, Chang, & Shin, 1996). This leads to a much more generalized idea of what constitutes "endemic" vs. "non-endemic". The Center for Disease Control has reported that in the United States, 85% of cases reported were from the eastern coastal states (Massachusetts to Virginia), and 10% were reported from the upper Midwest (Greene, Appel, & Straubinger, 1998). This broadly corresponds with the distribution of the *Ixodes ricinus* tick, the most common vector, and the percentages have not changed significantly in recent years. These ticks are also a common intermediate host for granulocytic *Ehrlichia*, which will be discussed later.

Treatment of Lyme disease typically involves antibiotics including amoxicillin, tetracycline, doxycycline, ceftriaxone, erythromycin, cefuroxime, and penicillin G for approximately 30 days (Greene, Appel, & Straubinger, 1998). The spirochete is typically nonresponsive to aminoglycosides and quinolones. Doxycycline is used for suspected central nervous system infection because it

more easily penetrates the blood-brain area (Leib & Monroe et al., 1997). Antibiotics may cause a number of side effects, such as gastrointestinal aggravation, which is commonly associated with tetracycline. Restriction of movement and exercise is also recommended. Aspirin can be administered for pain. Those dogs with acute phase Lyme disease respond better than those with the chronic form of the disease.

Prevention of Lyme disease in domestic animals involves either avoiding exposure to the spirochete or being vaccinated against the infection. Avoiding exposure in endemic areas is difficult and is reliant somewhat on vector control. Controlling the tick population would help reduce the prevalence of the disease (Greene, Appel, & Straubinger, 1998). However, the most effective pesticides for controlling tick populations are environmentally destructive chlorinated hydrocarbons, and any pesticide would have to be distributed over extremely large areas. Also, targeting rodents and vaccinating reservoir species is an option, but this is an enormous undertaking and could probably never work completely. Vaccinations against the disease are helpful, but are not wholly effective because of the always changing nature of *Borrelia*. There is also the possibility that vaccinations could cause hypersensitivity in dogs already harboring the spirochete or occasionally cause allergic reactions. Yet another disadvantage of vaccination is the false-positive serological result caused for months to years later on serologic testing. Despite these disadvantages, vaccination is, at the moment, the most helpful course of action in high risk dogs.

Ehrlichiosis

Another tick-borne disease that affects the canine population is Ehrlichiosis, an obligate intracellular parasite of the genus *Ehrlichea* (Neer, 1998). While there are species that affect people, the symptoms are generally mild and do not compare to the severity of the disease in dogs. The species that is most common and affects the family Canidae (i.e. coyote, fox, jackal, and domestic dog) is *E. canis*. The most common vector for *E. canis* is the brown dog tick (*Rhipicephalus sanguineus*). The disease is spread similarly to Lyme disease, and in similar life stages of the tick. Dogs infected with the disease are divided into acute, subclinical, and chronic phases (Harrus et al., 2002). The acute phase starts after incubation and lasts 2 to 4 weeks accompanied by general symptoms such as fever, anorexia, weight loss, and general malaise. The subclinical phase typically lasts 40 to 120 days, but can last years in naturally infected dogs. During this phase, the subject will show little or no clinical signs, and often a strong immune system will eliminate the disease. If the disease remains, chronic phase begins. Chronic phase is sometimes mild but may include impaired bone marrow production.

Symptoms of *E. canis*, in general, include fever, anorexia, weight loss, hemorrhagic diathesis, central nervous system problems, and lymphadenomegaly (Neer, 1998). Dogs may also exhibit ocular problems resulting in blindness, neuromuscular signs, and lameness due to polyarthritis. Laboratory signs of the disease include anemia, leukopenia, marked thrombocytopenia, hyperglobulinemia, pancytopenia, and proteinuria.

Diagnosis of *Ehrlichiosis* is based on the combination of the listed clinical signs, hematology, thrombocytopenias, and serology (Neer, 1998). Serological diagnosis for the disease is typically by an indirect fluorescent antibody (FA) test. Now, as in Lyme disease, the use of an enzyme-linked immunosorbent assay (ELISA) test is conducted frequently to detect antibodies to *Ehrlichiosis*. Similarly, Western blotting is the definitive test following an ELISA or indirect FA test. For *E. canis*, the immunodominant proteins are typically between 22 and 29 kDa (broadly 27 kDa). With the Western blot test, the disease can be seen as early as 2 days after infection. Serology is often positive for dogs with no history of illness or clinical signs because of the high prevalence of the subclinical form (Cuoto & Nelson et al., 1999). Also, as in serology for Lyme disease, cross-reaction with similar microorganisms often occurs.

Treatment of *Ehrlichiosis* is most effective early in the disease. Effective drugs include tetracycline, oxytetracycline, doxycycline, and minocycline (Neer, 1998). Tetracycline and oxytetracycline are the classically used drugs, while doxycycline and minocycline are used more frequently today. Use of one of these antimicrobial drugs is often accompanied by fluids for dehydration or blood transfusions when anemia is present. Time frame of treatment varies, but dramatic improvement typically occurs within 2 days of starting treatment. The time frames for the antimicrobial drugs vary, but usually are given for 10 or more days.

Evaluation of Tests for Lyme Disease

There are three types of serologic tests used to help diagnose infection of Lyme borreliosis: the Western blot (immunoblot), ELISA, and IFA (indirect fluorescent antibody). Western blot is the definitive test, and is used typically following an ELISA or IFA test, both of which are less expensive. Western blot analyzes humoral immune response in Lyme borreliosis by dectecting the presence of immunodominant proteins found in *Borrelia burgdorferi* (Zoller, Cremer, & Faulde, 1993). For acute-phase infection, this test is based on the proteins 21 kDa band and 41 kDa band. For chronic-phase infection, the presence of 94 kDa, 39 kDa, 31 kDa, 30 kDa, and 21 kDa bands are noted. The assays detect specific antibodies against the specific disease tested for. The ELISA used for this study detects the IgG and IgM antibodies against *B. burgdorferi*.

Recent studies have indicated that not all tests for diagnosing diseases such as Lyme disease are as useful as many individuals believe. For this reason, the evaluation of statistical information provided by the companies manufacturing the tests must be done with great care. This requires having knowledge of the terms and methods used to describe a test performed on a population. The subtle differences between the terms often cause confusion regarding the subject, and many medical professionals fail to grasp the topic fully. A few key terms that are an integral part of beginning to understand the analysis of testing for disease are sensitivity, specificity, prevalence, incidence, and predictive value.

Sensitivity and specificity

Sensitivity can be thought of as positivity in disease (Galen & Gambino, 1975). For the purpose of this study, sensitivity is used to describe the overall probability of a test; the term is often otherwise used to describe ability to detect low concentrations. Sensitivity is the incidence of true-positive results when the test is applied to a group of individuals that are known to have the disease. The percentage denoting sensitivity is the percentage of positive results obtained in a population consisting wholly of diseased individuals. A test with 95% sensitivity would, therefore, detect the disease in 95 out of 100 patients that actually have the disease. Five of the 100 patients would be deemed false-negatives.

Specificity can be described as negativity in health (Galen &Gambino, 1975). It is the incidence of true-negatives when a test is performed on a population known to be without the disease. The percentage denoting specificity is the percentages of negative results obtained in a population free of diseased individuals. A test with 95% specificity would show negative results for 95 out of 100 patients without the disease. Five of the 100 patients would be deemed false-positives.

Prevalence and incidence

Prevalence and incidence are often incorrectly used interchangeably. Prevalence is derived from the word prevail and refers to the commonality of a disease (Galen & Gambino, 1975). The prevalence of a disease is the number of individuals in a population that have the disease out of a specific total number of individuals. The incidence of a disease refers to a specific time frame. For

instance, the incidence of a disease would be the number of patients out of the specific total who developed the disease during an allotted time frame. Therefore, incidence may be low while prevalence is high. This would occur if very few individuals became infected with the disease while many were already infected. If the disease itself does not last for a long period of time, incidence may be high while prevalence is low. Many individuals could contract the disease in a certain time frame while very few already had the disease.

Predictive value

Predictive value involves the specificity and sensitivity when applied to a population of healthy and diseased individuals (Galen & Gambino, 1975). The positive predictive value is the percentage of positives that are true positives out of all of the positive results obtained. The negative predictive value is the percentage of negatives that are true negatives out of all of the negative results obtained. Table 1 from Galen and Gambino's *Beyond Normality* illustrates this idea. For instance, in Galen and Gambino, the example of a test with 95% sensitivity and 95% specificity is used. Table 2 shows the effect of prevalence on predictive value for this test. This table illustrates how greatly predictive value is affected by prevalence. Even with excellent values for sensitivity and specificity, predictive value suffers enormously in a population with very low prevalence, and many tests lack the specificity and sensitivity of the example test.

Table 1. Mathematical basis for sensitivity, specificity, and predictive value.

	Number with positive test result	Number with negative test result	Totals
Number with disease	TP	FN	TP+FN
Number without disease	FP	TN	FP+TN
Totals	TP+FP	TN+FN	TP+FP+TN+FN

TP = true positives, FP = false positives, TN = true negatives

sensitivity = $\frac{TP}{TP + FN} x100$

specificity = $\frac{TN}{TN + FP} x100$

positive predictive value = $\frac{TP}{TP + FP} x100$

negative predictive value = $\frac{TN}{TN + FN} x100$

Source: (Galen and Gambino, 1975, p.13)

Medical Applications

The aforementioned terms can be readily applied to common medical practices. Sensitivity, specificity, prevalence, and predictive value factor into the type of test that is appropriate for each situation. The values for sensitivity and specificity are almost always inversely related. Increasing the sensitivity of a test

will, most likely, decrease the specificity and visa versa. High sensitivity is desired in conditions where the disease being tested for is extremely serious and can be tested for (Galen & Gambino, 1975). It is also important that false-positives do not lead to serious repercussions for the patient, or in the case of a pet, the patient's owner. High specificity is desired in cases where the disease is not curable and false-positives could lead to devastating effects on the patient. The most important idea to consider for this particular study is when a high positive predictive value is important. An acceptable positive predictive value is important when the false-positives cause treatment that is greatly detrimental to the patient or the patient's owner. This includes a large number of factors.

Table 2. Effect of prevalence on predictive value for a test with 95% sensitivity and specificity.

Prevalence of disease (%)	Positive predictive value (%)
0.1	1.9
1	16.1
2	27.9
5	50.0
50	95.0

Source: (Galen and Gambino 1975, 16)

The consequences can include financial, emotional, and psychological strain. This is in addition to any physical consequences that occur due to reaction to the treatment. This leads to the idea that solely relying on a test, as many professionals do, is not the best method for diagnosing disease. Tests should be used as aids in association with physical symptoms and/or other tests.

Normally, there are several specific tests for a disease, and they should all be considered when determining if a test is appropriate for that specific situation. The functions of tests include providing diagnosis for an individual known to have symptoms of disease, providing a prognosis for an individual already known to have the disease, providing knowledge of a disease in a healthy individual, and providing data for determining the effects of treatment (Galen & Gambino, 1975). Each situation warrants a different type of test. To provide knowledge of a disease in a healthy animal, prevalence of the disease is essential.

For this particular study, Western blot is used as the definitive test. This test is highly sensitive and specific due to its unusual nature. It targets six distinct molecular masses associated with Lyme disease (Jacobson, Chang & Shin, 1996). This allows for human interaction and decision-making when it comes to the number of bands found to interact with the molecules. Also, the prevalence of the population tested is much higher due to the fact that most Western blot tests are performed on samples thought to be infected with disease. ELISA and IFA tests typically have much lower values for specificity and sensitivity.

Hypothesis

The purpose of this study is to determine the usefulness of a specific C6 ELISA test manufactured and promoted by IDEXX, a large pharmaceutical company. In the literature published by the company, routine screening of healthy dogs with this test is encouraged. The test is distributed across the nation with the same rationale. This rationale is based on the idea that Lyme disease and *Ehrlichiosis* are spreading rapidly, and thus becoming more endemic in many areas. It is, however, difficult to know the actual prevalence of these diseases in an area. This leads to a fear of an epidemic in areas where these diseases are not seen. This, in turn, leads to unnecessarily using a test for the screening of healthy dogs. However, in the event that the diseases are of low prevalence in that area, the repercussions of using the test as a screen would outweigh the benefits of occasionally finding a true positive result.

This study is also intended to help determine if the false positives and ensuing costs outweigh the true positive results in an area not known for these diseases. This will assist in making an inference about whether or not this specific C6 ELISA should be perfor med in an area similar to East Tennessee. The results will also provide some insight into the actual prevalence of the diseases in the area of testing. As shown in examples previously cited, the prevalence will have to be relatively high to warrant the ELISA being used as a screen test. Also, because the test includes both Lyme disease and *Ehrlichiosis* responses, if either disease is found to be in low prevalence, the use of the test as a screen would not be warranted.

It is hoped that this study will provide increased knowledge about this specific test as well as the idea of diagnostic testing in general. Informing individuals who rely on such testing as a definitive means of diagnosis would decrease the gross overdiagnosis of these diseases. If individuals become better informed about this assay, the financial, emotional, and treatment problems that follow a false positive diagnosis will be reduced.

CHAPTER II

MATERIALS AND METHODS

Survey

Individuals bringing their canines into Village Veterinary Hospital, in Maryville, Tennessee were asked to participate in the study by filling out a survey pertaining to geographic location, habits, history, and current health of their canine. The information needed from the survey included vaccination history, recent diagnoses, tick control, tick findings, areas canine has been exposed to, and hours outside.

Blood Samples

Blood samples (465) were obtained from canines that were otherwise receiving a heartworm test. The sample was immediately transferred from syringe to an anticoagulate test tube. The blood (two drops) was then mixed with the conjugate (five drops). The blood/conjugate mix was immediately placed on the IDEXX Snap test using the provided sample tube. After the test was run for at least two minutes, the results were observed, and positive samples were sent to Antech laboratories and evaluated using western blot analysis (Lyme) and PCR analysis (ehrlichiosis).

IDEXX ELISA

The IDEXX Snap enzyme-linked immunosorbent assay test was used to detect antibody to *Borrelia burgdorferi* and antibody to *Ehrlichia canis* in whole blood. A positive was indicated by presence of color showing that the assay reagents are active and antibodies are present. The blood used was at room temperature and was anticoagulated as indicated in the tests directions.

Western Blot Analysis

For the positive Lyme tests, Western Blotting was done to test for a true positive or true negative. The blood samples were sent to a laboratory for this procedure. The Western Blot serologic assay is far more sensitive and specific than ELISA assays (Greene, Appel, & Straubinger, 1998). Proteins were taken from the blood sample and separated in agar gel with electrophoresis and transferred to nitrocellulose paper. The antigens were then reacted with the test sera and analyzed through staining.

PCR

For positive *Ehrlichiosis* ELISA assays, the samples were sent to the lab for PCR amplification. PCR was particularly useful to detect small amounts of DNA or RNA specific to Ehrlichia (Greene, Appel, & Straubinger, 1998) and to confirm a true positive or true negative as with Western Blotting. A specific nucleic acid primer was then reacted with the genetic material of *Ehrlichiosis*. These results were amplified and electrophoresis was performed. Complementary nucleic acid probes then confirmed the identity of the product.

CHAPTER III

RESULTS

Of the 465 total samples, the IDEXX ELISA found seven samples positive for Lyme disease and 1 sample positive for Ehrlichia. These samples were all confirmed as true positives by Western blotting (Lyme) or PCR (Ehrlichia). Table 3 shows these results.

~~4~~ Four of the seven canines found to be positive for Lyme disease and the only canine found to be positive for *Ehrlichiosis* liv~~ed~~ed in ~~-~~New York. ~~(when??)~~.

~~2~~ Two of the canines positive for Lyme disease lived in Pennsylvania. ~~(when??).~~ The other sample positive for Lyme antibodies was found in a dog that had only lived in the East Tennessee area. These results indicate a geographical correlation. This is shown in Figure 2.

Western blot for the one positive sample found from a canine living in Tennessee detected weak bands indicating a minimal reaction. All of the other samples with Lyme disease were found to have immunogenic proteins with a high degree of specificity for *Borrelia burgdorferi*, indicating a strong reaction.

Table 3. Lyme disease and Ehrlichia positives found by the IDEXX C6 ELISA.

Disease	IDEXX Positive	Definitive Test Positive	Positive Predictive Value
Lyme	7	7	100%
Ehrlichia	1	1	100%

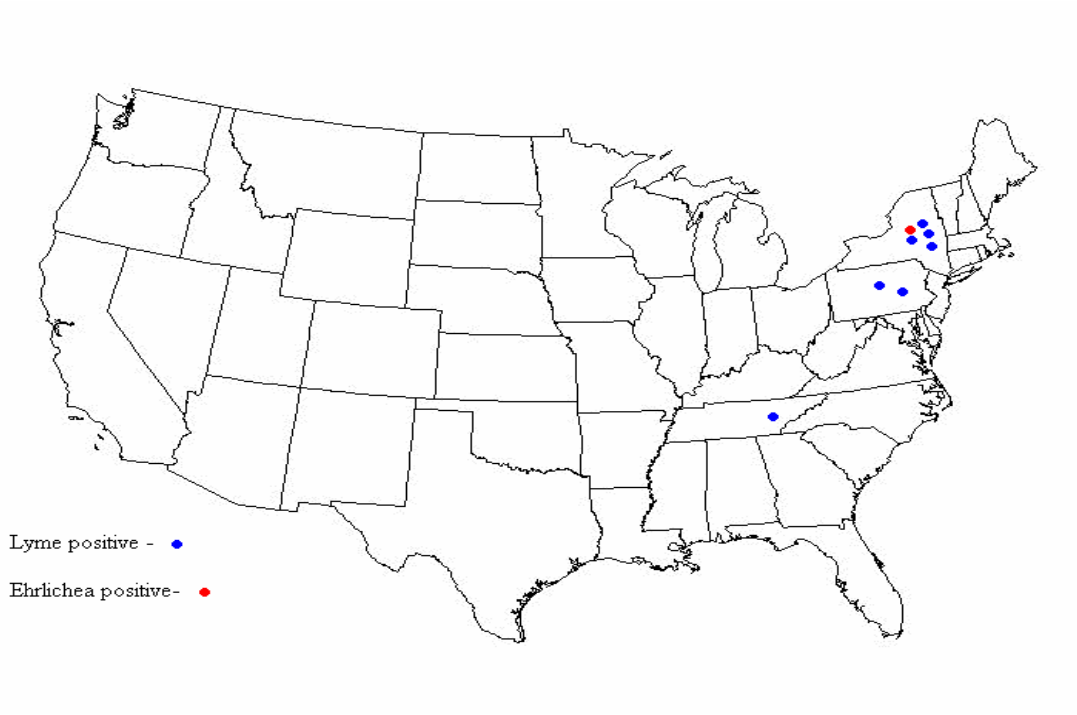

Figure 2. Location of canines with positive samples for Lyme disease and Ehrlichia.

CHAPTER IV

DISCUSSION

This study was designed to determine if it was financially viable for veterinary ~~customers~~ clients to ~~perform~~ have the IDEXX C6 ELISA performed for the detection of Lyme disease and Ehrlichia ~~on a regular basis~~ in a non-endemic area. While there is no definitive answer to this, the study performed would indicate financial justification. This is supported by the fact that there were no false positives~~. The~~, the IDEXX ELISA ~~was~~ being 100% accurate. It was, however, hypothesized that there would be a number of false positives. This hypothesis was based on the generally accepted ideas pertaining to sensitivity, specificity, prevalence, and predictive value. Prevalence was the variable of particular concern in this study, and the positive predictive value was expected to correlate with the values extrapolated from low prevalence. This was not the case. This was due to either the limitations due to a small number of positives, ~~exceptional nature of the test,~~ misjudgment regarding the endemic nature of Tennessee, or the exceptional nature of the test. ~~or aberrant data~~limitations ~~due to a small number of positives.~~

The ~~aberrant data is very much a possibility~~limited sample size is the most plausible explanation. According to Galen and Gambino, acquiring a positive predictive value of 100% is improbable, even with an extremely sensitive and

specific test (1975). Mathematically, a positive predictive value of 100% would not be obtained unless the sensitivity and specificity of the test were both 100%, which is not the case. However, with a relatively limited group of positives, error in positive predictive value is likely. In any statistical analysis, a small sample size increases the risk of error and decreases the likelihood of an actual correlation between the results and a deduction (McClave & Sincich, 2003). This makes it difficult to deduce the actual positive predictive value of the test. If there had been more ELISA positives, it would be possible to make a more definitive statement regarding the accuracy of the IDEXX test. The fact that there were few positives can be contrasted with a similar study done involving 440 military working dogs, many from endemic areas (Sheets, Rossi, Kearney, & Moore, 2000 ~~199?~~). Out of the 440 canine serum samples, 89 positives were found using a commercial ELISA. This indicates the problematic nature of performing this study in a non-endemic area.

The endemic nature of the area was another variable. Of the 8 total positives, 7 were from states known to have a high tick population. The results show that the actual prevalence of the disease in Tennessee is most likely low. The only canine found to have Lyme disease from the area was shown (by Western blotting) to have weak bands indicative of a minimal reaction to antigens consistent with natural exposure to *Borrelia burgdorferi*. All of the other samples with Lyme disease were found to be strongly positive. While this fact would aid in making a geographical comparison, in terms of the endemic nature of east Tennessee, it simply further confounds the results. The fact that the out of state

canines were in the area supports IDEXX's suggestion to run the test in non-endemic areas. The area of testing is not known for Lyme disease prevalence, but the canines visiting the veterinary hospital are not necessarily from the area. The simple fact that the test accurately detected the 7 out--of--state cases supports the notion of performing this test on a regular basis. Testing is not performed only on canines indigenous to the non-endemic area, therefore the endemic nature of a disease from a -population of dogs visiting a specific clinic is impossible to determine. The fact remains, however, that even with canines from endemic regions, the prevalence should not be high enough to mathematically warrant a 100% positive predictive value (Galen & Gambino 1975).

~~The obvious connection would be using a canine's location to further aid in diagnosis.~~For veterinary ~~customers~~clients with reservations about conducting the IDEXX test, the most important variable influencing the decision would be using the canine's potential exposure to Lyme disease and Ehrlichia.~~ This would be especially important in hospitals reluctant to use an ELISA test all the time.~~ There are, however, problems associated with this as well, because there is a lack of information regarding how endemic an area is. To be able to screen dogs by location would require extensive knowledge of where the endemic areas are. Also, pet owners are not always aware of the geographical areas their canine has lived or visited. There is also a question of the time frame that would constitute a risk for the pet, or how long, if at all, the canine was outside. The variables are far too extensive to use geographic location as a diagnostic tool. This is yet another factor that supports regular testing with the IDEXX C6 ELISA.

The fact that this study found a positive predictive value of 100% would indicate a high specificity. ~~Due to fiscal restraints, a definitive~~However, a Western-blot definitive test ~~could~~ was not ~~be done~~conducted for every sample due to fiscal constraints. Because of this ~~restraint~~, there was no indication as to the sensitivity, and false negatives could have been missed. However, sensitivity and specificity are inversely related (Galen & Gambino 1975). Another possible explanation for the ~~aberrant data~~ unexpected data would be a lower sensitivity. If the specificity of the test is 100% as indicated, then the sensitivity would be significantly lower. If the entire sample population was tested definitively, this explanation could be validated.

Regardless of the explanation, the results support the use of the IDEXX C6 ELISA in non-endemic areas. The true nature of the test could be better determined by testing for false negatives. Further research including more locations and definitive tests for all negative results would help to address any remaining questions.

~~Concluding paragraph. Future research???~~

~~In addition, the~~

APPENDIX

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