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AN EPIDEMIOLOGICAL STUDY OF LEPTOSPIRA-INDUCED ABORTION IN MARES IN CENTRAL KENTUCKY (1990-2004)

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture
at the University of Kentucky

By

David C. Hall

Lexington, Kentucky

Director: Dr. Roberta M. Dwyer, Associate Professor of Veterinary Science

Lexington, Kentucky

2005

THESIS

David Christopher Hall

The Graduate School

University of Kentucky

2005

This thesis is dedicated to my wife and best friend, [redacted] Her love, support, and encouragement are always present. Being married to me is difficult, but being married to me and the military is almost unfair.

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The following thesis, while an individual work, benefited from the insights and direction of several people. First, my Thesis Chair, Dr. Roberta Dwyer, provided exceptional guidance and support throughout this endeavor. Her willingness to be available and her epidemiological insight kept this investigation moving smoothly in the right direction. In addition, Dr. Mike Donahue, provided valuable inputs at each stage of the investigation, allowing me to quickly solve problems and find a common ground for the taxonomy of this organism. Also, Dr. David Bolin provided instrumental instruction on the necropsy floor at the University of Kentucky Livestock Disease Diagnostic Center. This instruction allowed me to visualize the disease in its final effects. Furthermore, Dr. Daniel Howe provided timely and instructive comments throughout the investigative process. Each committee member, previously mentioned, worked hard to make the finished product accurate in its conclusions.

Other than my committee members, I received vast amounts of technical support from other sources. The laboratory staff, especially Barbara Smith and Judy Donahoe, and faculty at the University of Kentucky Livestock Disease Diagnostic Center provided the positive identifications of a leptospira abortion. Without this identification, the research would not have been possible. Furthermore, the cooperation of the various farms that supplied feedback in the form of questionnaire return or allowing me to visit strengthened the results of this investigation. It would be remiss not to mention the Gluck Equine Research Center's librarian, Gracie Hale, because her hard work and instruction made finding previous leptospirosis literature quick and effective. Also, Gail Watkins completed the paperwork necessary to purchase the mare breeding data reports from the Jockey Club helping to contribute to this study. Additionally, two staff members from the University of Kentucky College of Agriculture's weather department provided unparalleled support. Dr. Tom Priddy gave exceptional guidance in the analysis of temperature and precipitation patterns for this study. Dr. Dennis Hancock, an agricultural extension associate, gave much of his own time to educate me on the use of geographical systems technology and to help generate the maps seen in this study. Finally, Diane Furry, possessed the computer knowledge necessary to bring this document together.

In addition to the technical assistance previously mentioned, I received equally important assistance from family and friends. My wife [redacted] provided the support and understanding needed for the timely completion of this investigation. My entire family, too numerous to mention by name, have always shown unconditional love and support throughout my life. Without their support, this thesis would not have been possible.

ABSTRACT OF THESIS

AN EPIDEMIOLOGICAL STUDY OF LEPTOSPIRA-INDUCED ABORTION IN MARES IN CENTRAL KENTUCKY (1990 - 2004)

Leptospirosis can cause fetal abortion in pregnant mares. The number of abortions attributed to leptospiral infection in central Kentucky can differ greatly from year to year. This study makes comparisons of years having a higher than average prevalence of leptospiral abortions to those years having an average or below average prevalency of such abortions in the horse population. Environmental factors such as temperature and precipitation, as well as, geographical location using graphical information system (GIS) technology were examined. Other factors including time of year, gestational age of the foal, age of the mare, parity of the mare, future reproductive success of the mare, and contact with wildlife were also examined.

KEYWORDS: Leptospirosis, Equine Placentitis, Equine Abortions, GPS/GIS Technology, *Leptospira interrogans*

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Leptospirosis can cause fetal abortion in pregnant mares. The number of abortions attributed to leptospiral infection in central Kentucky can differ greatly from year to year. This study makes comparisons of years having a higher than average prevalence of leptospiral abortions to those years having an average or below average prevalency of such abortions in the horse population. Environmental factors such as temperature and precipitation, as well as, geographical location using graphical information system (GIS) technology were examined. Other factors including time of year, gestational age of the foal, age of the mare, parity of the mare, future reproductive success of the mare, and contact with wildlife were also examined.

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20 JAN 2005

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Chapter One: Literature Review

Leptospirosis is the most widespread zoonosis throughout the world, though the diagnosis is infrequent in humans in the United States (Meites et al., 2004). The human disease is characterized by fever, headache, chills, muscle aches, vomiting, jaundice and an occasional rash. A non-treated patient could develop more serious conditions such as kidney damage, meningitis, liver failure, and even respiratory distress. In very rare occasions leptospirosis can cause death in humans (Chin, 2000; Center for Disease Control and Prevention, 2004). The national levels of leptospirosis diagnosed in the human population in the United States decreased rapidly throughout the later part of the 20^{th} century and the disease was removed from the nationally reportable disease list in 1995 (Meites et al., 2004; Center for Disease Control and Prevention, 2004).

In the late 1980s it became apparent, however, that although leptospirosis was not a significant threat to the human population in the United States, it was a greater threat than previously recognized as a form of placentitis in the horse population (Hong et al., 1993a, 1993b). The signs of a horse experiencing an acute leptospirosis infection include mild depression, loss of appetite, and a fever of 103 to 105 degrees Fahrenheit that may last up to three days. Several weeks after the fever a pregnant mare may experience an abortion and all horses can experience uveitis (moonblindness) several months after the fever. It appears, in the case of uveitis, that leptospires infect the aqueous humor of the horse's eye, and that the resulting sight problems may be the result of the infection itself or because of an autoimmune response from the horse (Bernard, 1993c). This study, however, will concentrate on the disease as it relates to the cause of abortions in the horse population.

Prior to 1990, most of the research concerning leptospirosis as a cause of abortion in horses was done in Northern Ireland (Ellis et al., 1983; Ellis and O'Brien, 1988; Ellis, 1999). However, early in the 1990s studies were done in central Kentucky because of the concentration of Thoroughbred breeding, the incidence of leptospiral abortions in that area, and the location of the University of Kentucky Livestock Disease Diagnostic Center within the region (Donahue et al., 1991; Donahue et al., 1992; Donahue et al., 1995). A primary reason for determining the epidemiology of leptospirosis as it relates to equine abortion is because sometimes mares may not show any of the clinical signs mentioned previously before they abort or produce a stillborn foal (Wilkie et al., 1988; Donahue et al., 1991; Donahue at al., 1992; Donahue et al., 1995). Thus, in many cases, treatment can not be provided in a timely manner to prevent the abortion from occurring.

Previous research endeavors have determined the abortions to occur from 6 months to almost full term in the gestation period (Roberts et al., 1952; Jackson et al., 1957; Hanson, 1977; Ellis and O'Brien, 1988; Hodgin et al., 1989; Donahue et al., 1991; Donahue et al., 1992; Donahue et al., 1995). The outcome of the fetus (aborted, stillborn, or live foal) can depend on the age of the fetus at the time of infection and the virulence of the *Leptospira* serovar (Hanson, 1977). Previous studies have further indicated that age of the mare might have some epidemiological factor increasing the mare's exposure or susceptibility to *Leptospira*, particularly if the mare is between the age of 7 and 11 years old (Carpio et al., 1979; Park et al., 1992; Williams et al., 1994; Rocha et al., 2003). As Dr. William Ellis (1999) indicates, given the results of a leptospiral infection in a mare, it is most unfortunate that so little is known about the infection in the horse. In fact, Abhineet Sheoran and coworkers (2000) noted that it is unknown what percentage of abortions are the results of a *Leptospira* infection only, and not in combination with other causes of placentitis.

Because most research in the United States concerning leptospiral infections in the horse population did not begin until the 1990s, many of the methods of transmission and epidemiology of leptospirosis have not been identified. Current research does indicate that transmission can occur through direct or indirect transmission from a host mammal (Hartskeeri and Terpstra, 1996). Indirect transmission through contact with *Leptospira* contaminated water or soil, is thought to be responsible for most human cases (CDC, 2004). In the case of horse infections, the same transmission logic applies. Leptospira bacteria can be spread through the urine of infected animals, through placental fluids, and through contaminated feed and water. The bacterium is thought to be transmitted to the horse population from other species seen on many farms, such as cattle, deer, and elk. It is also possible that wet environmental conditions seem to increase the risk of exposure (American Public Health Association, 2000; Ward et al., 2004). Other factors might also raise the risk of exposure to the bacteria, such as a mild climate, a high population density, and an increased degree of contact between maintenance and accidental hosts (Levett, 2001).

Current epidemiological beliefs concerning leptospirosis are that the disease is seasonal, being diagnosed in the late summer or fall, with the highest seasonal prevalence in hospital cases (in canines) occurring during the fall, where temperature is the limiting factor in the survival of leptospires. Rainy seasons prevent the rapid dessication of the leptospires in warm-climate regions (Bolin, 1996; Brown et al., 1996; Harkin and Gartrell, 1996; Levett, 2001; Ward et al., 2004). These epidemiological patterns are summarized by Solomon Faine (1994a) in three categories. First, infections can occur in temperate climates and involve few *Leptospira* serovars. Secondly, infections can occur in tropical wet climates, involve a large number of reservoir species, and therefore involve many serovars. Lastly, a rodent-borne infection can exist in the urban environment. Dr. Michael Ward (2004) accomplished several studies on dogs and the encroachment of urbanized areas onto rural land which supported Faine's last epidemiological pattern. The following study will use the first epidemiological pattern, a temperate climate involving few serovars, as its model.

Leptospirosis is a disease caused by leptospires, which are long, thin, and motile spirochetes (0.1 μm by 6 μm to 0.1 μm by 20 μm). These spirochetes may be free in the environment or associated with animal hosts. The individual organisms are highly complex and over 200 known pathogenic serologic variants have been determined (Faine et al., 1999; CDC, 2004). *Leptospira* are divided into 12 species and 5 genomospecies (Brenner et al., 1999). The 12 species of leptospires are further divided into approximately 23 serogroups containing more than 200 pathogenic serovars (Chin, 2000).

Research indicates that specific serovars are maintained by a specific host species that acts as a reservoir for the leptospire. The maintenance host is usually an asymptomatic chronic carrier. For example *Leptospira interrogans* serovar icterohaemorrahagiae is most likely a serovar maintained within the rat population (Hanson, 1982; Birnbaum et al.,1998). This serovar and maintenance host relationship is of great epidemiological importance. An identification of the maintenance host would provide further understanding of where the disease was originating in an accidental host population (Hartskeeri and Terpstra, 1996; Bernard, 2003). An accidental host is a periodic carrier that usually develops acute disease.

Because of the complex taxonomic system for *Leptospira*, it is important to discuss how this paper will address the naming of the organisms. Serovars have no taxonomic standing (Levett, 2001), but they help to provide better epidemiological understanding. In literature leading up to the publication of Emergent Causes of Placentitis and Abortion (Donahue and Williams, 2000) serogroup and serovar were often used interchangeably (Dr. Mike Donahue, University of Kentucky Livestock Disease Diagnostic Center, personal communication, 2004). In most cases, unless the study specified serovar determination, the indicated strain was most likely a serogroup. For instance in older North American literature, any of the strains involved in leptospiral infections of the horse were reported as *Leptospira interrogans* serovar pomona. As the studies progressed, the same strain would be listed as *Leptospira interrogans* serovar pomona genotype kennewicki, or *Leptospira interrogans* serovar kennewicki (Donahue and Williams, 2000). This study will adopt the same taxonomic system used by Dr. Donahue and Dr. Williams in their 2000 study, and therefore, a serovar would be written as *Leptospira interrogans* serogroup Pomona serovar kennewicki. It can be assumed that all strains in the serogroup Pomona that occur in horses in North America are most likely serovar kennewicki, because all Pomona serogroup leptospiral strains that have been identified by restriction endonuclease analysis (REA) have proven to be serovar kennewicki (Donahue and Williams, 2000).

It is very important to determine the serovar of leptospira in horses in North America because many previous studies have been done outside the United States and have indicated other serovars as the infectious agent, such as serovar bratislava in Northern Ireland (Ellis et al., 1983). This is not unusual, however, because it has been proven that there are variations in maintenance hosts and the serogroups/serovars that they carry throughout the world (Hartskeeri and Terpstra, 1996).

This study will be concentrating on the effects of temperature, precipitation, and naturally occurring water location on equine leptospiral abortions. It is important, therefore, to look at some previous studies and determine the average length of survival of leptospires in each condition. First, mild to warm temperatures promote a more lengthy period of survival for leptospires, especially if these temperatures are accompanied by more humid conditions (Everard and Everard, 1993; Ratnam, 1994; Levett, 2001). It is generally accepted that leptospires survive in soil of a high moisture content and low acidity (Twigg et al., 1969). In fact, even slightly acidic soil (pH 6.2) sampled from an Australian cane field sustained serovar australis for seven weeks (Smith and Self, 1955).

Secondly, high periods of precipitation are thought to increase the exposure to leptospires by releasing the leptospires from the soil and bringing them to the surface in standing water or even floods (Hellstrom and Marshall, 1978; Carroll and Campbell, 1987; Miller et al., 1991; Rentko et al., 1992; Barwick et al., 1997 and 1998; Adin and Cowgill, 2000; Ward, 2002; Meites et al., 2004). It is thought, however, that survival of leptospires in river water is shorter than in flooding areas or in standing water most likely because of cooler water temperature in the former (Chang et al., 1948; Crawford et al., 1971). Because much research has been done to support the relationship between soil and water in a leptospiral infection it is assumed that transmission usually occurs through contact with contaminated soil and water (Ward, 2002; Twigg et al., 1969). Furthermore, it is important to note that horses do not need to come into direct contact with infected animals to be exposed to leptospires (Barwick et al., 1998). This is especially true since *Leptospira interrogans* is able to survive up to one month in optimum conditions of moisture, pH, and temperature when in soil (Babudieri, 1958).

Lastly, location of the abortions is of great importance during an epidemiological investigation. Variability in environmental factors and inconsistency in geographic data can skew the investigation (Ward, 2004). In this study the region was limited to central Kentucky, referred to as the bluegrass region, which is comprised of Fayette county and surrounding counties. This region experiences the same weather patterns and climate (Dr. Tom Priddy, University of Kentucky Agricultural Weather, personal communication, 2004) and therefore helps to eliminate any climate variability.

Climate is not the only concern when it comes to location of the incidents. In this study an attempt is made to show the location of the incidents of leptospira-induced abortion in relation to major waterways and minor waterways in Kentucky. For this reason, a Global Positioning System (GPS) receiver and Geographic Information Systems (GIS) software were used to pinpoint locations and overlay maps. This is a new and upcoming technology for epidemiological investigations. Through the use of the GIS software, landscape features attributing to the risk of exposure of horses to leptospires can be identified (Ward, 2004). Dr. Michael Ward (2004) used overlay analysis, a GIS tool, that uses specific longitude and latitude points and overlays maps of terrain, waterways, or satellite photos to estimate the exposure of dogs to specific types of vegetation, land use, geology, hydrography, topography, and health services. These comparisons had been identified in previous studies by Nicholson and Mather in 1996 and Mott et al., in 1995. As will be shown in this study, the combination of GIS with the traditional epidemiological methods provide a powerful tool to rapidly identify and assess the multiple risk factors that may be associated with the spread of zoonotic diseases over large areas (Glass et al., 1995).

Chapter Two: Descriptive Information

Introduction

A confirmed case of leptospiral abortion was determined in this study by having pathologists at the University of Kentucky Livestock Disease Diagnostic Center (LDDC) examine the aborted equine fetus and diagnosing leptospirosis as the cause of abortion. A diagnosis of leptospira-induced abortion was made when leptospires were detected in fetal tissues by a fluorescent antibody test (FAT) and/or by detecting antibodies against leptospires in the fetal pericardial fluid and/or the mare's serum using the microscopic agglutination test (MAT).

The serovar of *Leptospira* involved was determined by culture of the bacterium at the LDDC and identification of submitted isolates by personnel at the National Veterinary Services Laboratories or Agricultural Research Service, Ames, Iowa using the restriction endonuclease analysis (REA) technique. Results of the MAT, especially since the 1999 foaling years, were also used to determine the serovar involved (Dr. Mike Donahue, LDDC, per personal communication, 2004).

This study used foaling years (FY) as the increments in time that were analyzed. A foaling year begins July 1st and ends on June 30th. Breeding generally takes place in February through April prior to the foaling year. The breeding is done so that a foal born after full gestation will be as close to a January 1st birth date as possible. Because the Thoroughbred industry in Kentucky is primarily comprised of commercial operations, most Thoroughbreds will follow this breeding and foaling calendar.

Another reason Thoroughbred data is very good for an epidemiological study of this type is because most Thoroughbreds are registered at the Jockey Club. The Jockey

Club is responsible for maintaining the American Stud Book. This book lists "all Thoroughbreds foaled in the United States, Canada and Puerto Rico as well as Thoroughbreds imported into those countries from nations around the world that maintain similar Thoroughbred registries" (www.jockeyclub.com, 2004). If a Thoroughbred is not registered in this book, that Thoroughbred will not be allowed to be sold at most Thoroughbred auctions, nor likely race in any Thoroughbred stakes races. The Jockey Club has been maintaining the bloodlines of Thoroughbreds since 1896.

Because the Jockey Club maintains excellent records, analyzing specific characteristics of the mare such as age and parity was possible in this study. Parity is the number of live foals born to a mare. In this study, parity was further defined as the number of live foals born to a mare prior to experiencing a leptospiral abortion.

This chapter accumulates the descriptive data necessary to begin an epidemiological investigation. The years of high prevalence, the serovars involved, the epidemiological curves, and the characteristics of a mare most likely to experience a leptospiral abortion will be identified by the end of this Chapter.

Materials and Methods

Accession Sheets

The LDDC provided accession sheets for five foaling years (July 1 – June 30) covered in this epidemiological study (2000-2004). An accession sheet is the document completed by the farm manager, veterinarian, or other farm employee when an aborted fetus is submitted to the LDDC for evaluation. The information on these documents provides the date of the animal submission, which was used as the incident (abortion) date. A blank accession sheet is provided in Appendix A.

Necropsy Reports

Necropsy reports were also provided for the 2000 through 2004 foaling years. The necropsy reports contain the pathologist's gross and histological findings, the bacteriological findings, the serological findings, the virological findings, and the final diagnosis.

Microbiology Spreadsheets

Prior to the 2000 foaling year, accession sheet information and necropsy reports were kept at the Livestock Disease Diagnostic Center in a different format. To protect confidentiality of cases not related to this study, data sheets for equine leptospiral abortions were provided by the LDDC, which contained the submission date, farm identification, mare identification, gestational age of foal, histological findings, and serological data (FY 1999 – FY 2004). Dr. Mike Donahue collected this information since 1990.

Mare Data

All Thoroughbred mares having had a leptospiral abortion diagnosed at the LDDC from July 1, 1989 to June 30, 2004 in this study should have been registered by the Jockey Club. To obtain a breeding history report on all registered mares, the Jockey Club database was accessed at www.equineline.com through a user ID and a password, then the Thoroughbred database was selected. The breeding history reports analyzed for the mares in this study provided the mare's sire and dam, the mare's date of birth, the stallion bred to each year, and the results of the breeding (live birth, abortion, or barren).

This data was analyzed to determine the significance in mare age, parity, and future breeding performance after a leptospiral abortion.

Information Processing

Microsoft Excel from Microsoft Office 2000 SR-1 Professional program was used to create spreadsheets of data collected and to create the graphs and charts. Statistical comparisons were accomplished on the averages calculated for parity, mare age at the time of the abortion, and the gestational age of the fetus at time of abortion using a t-test with a pooled variance to calculate the estimated standard deviation. The t-test makes the assumption that the averages are simple random samples from two independent normal populations that have the same variance. The p-value was calculated by looking up the t-value in a t-table (Rosner, 2000) under the correct degrees of freedom. As common in most scientific literature, statistical significance was set at $\alpha=0.05$.

Results

Estimated Prevalence of Leptospirosis in Kentucky per Annum

It is important to note that during the years of the study, the number of live foals per year was not a constant. Therefore a measure, prevalence, is used to correct for this population change. The number of live Thoroughbred foals born in the state of Kentucky for each year studied was obtained from the the Jockey Club Live Foal Reports at the organization's website. The numbers for the state of Kentucky can be seen in Table 2.1.

Table 2.1: Number of Live Thoroughbred (TB) Foals Born in Kentucky per Foaling Year

Foaling Year	Live TB Foals Born in Kentucky	Live TB Foals Born in United States	Percentage of United States Born Live TB Foals Born in Kentucky
1990	7397	40333	18.3
1991	7263	38149	19.0
1992	6871	35049	19.6
1993	7008	33820	20.7
1994	7108	32117	22.1
1995	7683	31882	24.1
1996	8442	32240	26.2
1997	8933	32115	27.8
1998	9495	32941	28.8
1999	9893	33825	29.2
2000	10116	34699	29.2
2001	9843	34636	28.4
2002	8203	32408	25.3
2003	12582	34025	37.0
2004	13797	36274	38.0

The Jockey Club asserts that the figures used in Table 2.1 are 90% accurate and depend heavily on the dependable reporting of breeders throughout the United States. Furthermore, it is important to note that in each of the foaling years represented in this study that Kentucky produced the most live foals per US state.

In order to estimate the prevalence of leptospira-induced abortions in the state of Kentucky, an accurate count of the number of incidents per foaling year in Thoroughbred mares was obtained from the LDDC. If the final diagnosis of the abortion was due to a *Leptospira* infection, that incident was used as a confirmed case in this epidemiological survey. The number of leptospiral cases diagnosed by the LDDC in Thoroughbred mares can be seen in Table 2.2 and in all breeds of horse in Table 2.3. Table 2.2 calculates an estimated prevalence by comparing the number of cases diagnosed by the LDDC in

Thoroughbred mares to the total number of live Thoroughbred foals born in Kentucky (www.jockeyclub.com). The majority of the

Table 2.2: Estimated Prevalence of Leptospira-Induced Abortions in Kentucky by Comparing Cases Diagnosed in Thoroughbreds by the University of Kentucky Livestock Disease Diagnostic Center to the Number of Live Thoroughbred Foals Born in Kentucky During Specific Foaling Years

Foaling Year	Cases Diagnosed by LDDC (Thoroughbreds)	Live Foals Born in Kentucky (Thoroughbreds)	Estimated Prevalence of Leptospira-Induced Abortions per Live Foal in Kentucky*
1990	27	7397	**.0037**
1991	7	7263	.0010
1992	14	6871	**.0020**
1993	34	7008	**.0049**
1994	2	7108	.0003
1995	3	7683	.0004
1996	7	8442	.0008
1997	9	8933	.0010
1998	2	9495	.0002
1999	6	9893	.0006
2000	1	10116	.0001
2001	29	9843	**.0029**
2002	4	8203	.0005
2003	4	12582	.0003
2004	24	13797	**.0017**

*Bold numbering indicates a foaling year of high prevalence

Thoroughbred farms in Kentucky are located in the bluegrass region of Kentucky (Kentucky Thoroughbred Farm Managers' Club Directory, 2003), a geographical region including Fayette, Woodford, Scott, Bourbon, Clark, and Jessamine counties. The LDDC is located in the heart of the bluegrass region where a more representative prevalence might be calculated by comparing the number of cases diagnosed by the LDDC in Thoroughbred horses to the total number of live Thoroughbred foals born in Kentucky.

Table 2.3 calculates an estimated prevalence by comparing the number of cases diagnosed in all breeds of horses to the total number of live Thoroughbred foals born in Kentucky during the same years. The years of higher than average prevalence are the same in both Tables 2.2 and 2.3. The high-prevalence years are marked by bold type.

Table 2.3: Estimated Prevalence of Leptospira-Induced Abortions in Kentucky by Comparing Cases Diagnosed in all Horse Breeds by the University of Kentucky Livestock Disease Diagnostic Center to the Number of Live Thoroughbred Foals Born in Kentucky During Specific Foaling Years

Foaling Year	Cases Diagnosed by LDDC (All Breeds)	Live Foals Born in Kentucky (Thoroughbreds)	Estimated Prevalence of *Leptospira*-Induced Abortions per Live Foal in Kentucky*
1990	39	7397	**.0053**
1991	12	7263	.0017
1992	22	6871	**.0032**
1993	47	7008	**.0067**
1994	5	7108	.0007
1995	6	7683	.0008
1996	7	8442	.0008
1997	12	8933	.0013
1998	3	9495	.0003
1999	9	9893	.0009
2000	5	10116	.0005
2001	34	9843	**.0035**
2002	6	8203	.0007
2003	7	12582	.0006
2004	36	13797	**.0026**

*Bold numbering indicates a foaling year of high prevalence

A closer look at the frequency of leptospira-induced abortions diagnosed by the LDDC over the 15 years of this study indicates the number of abortions in specific breeds as 173 in Thoroughbreds, 12 in Standardbreds, 6 in Quarter Horses, 1 in Belgians, 1 in Miniature Horses, 1 in Tennessee Walking Horses, and 47 in undetermined breeds. The 47 in undetermined breeds includes those horses indicated as Thoroughbreds on the LDDC accession sheets but not found in the Jockey Club registry.

Epidemiological Curves for Time of Year

After determining the years most likely to correspond to high prevalence rates of leptospira-induced abortion per live birth Thoroughbred foals in Kentucky, the data was further expounded to include incidents of leptospira-induced abortion per month per foaling year. The dates used for this data were taken from LDDC accession sheets and the spreadsheets created by Dr. Donahue, and the incidents include all breeds of horses diagnosed at the LDDC. The epidemiological curves that were generated from this data can be seen in Figure 2.1 through Figure 2.15.

Figure 2.1: Overall Epidemiological Curve Depicting the Number of Incidents of Leptospira-Induced Abortions In All Horse Breeds per Year

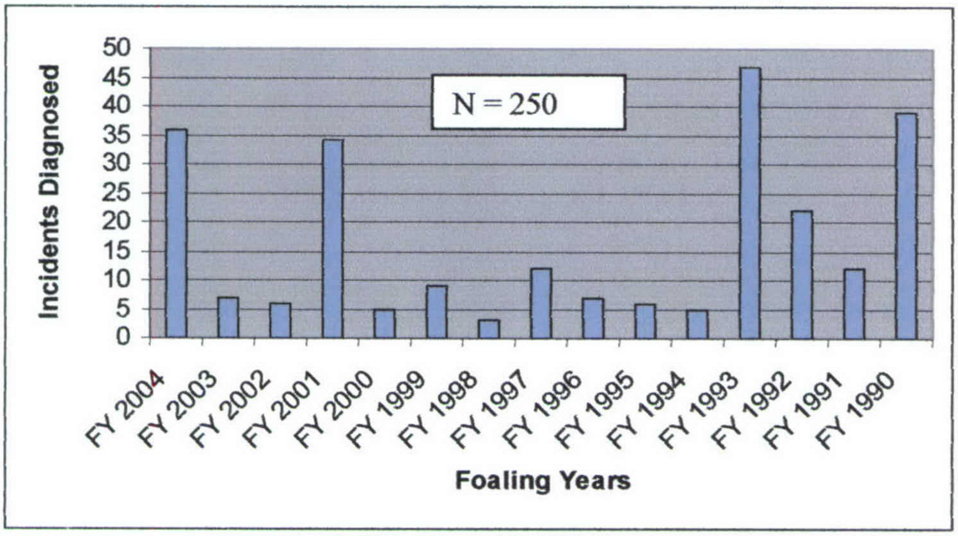


Figure 2.2: Epidemiological Curve for Leptospira-Induced Abortions in All Horse Breeds per Month During the 1990 Foaling Year

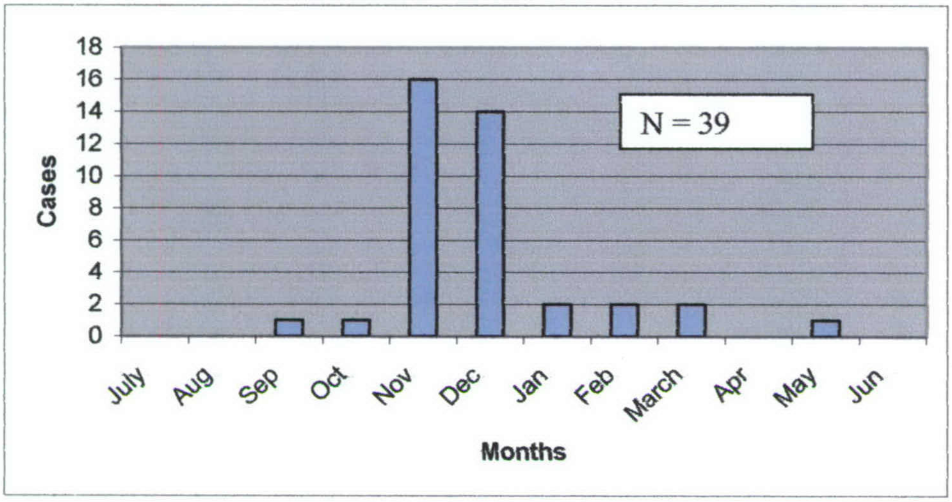


Figure 2.3: Epidemiological Curve for Leptospira-Induced Abortions in All Horse Breeds per Month During the 1991 Foaling Year

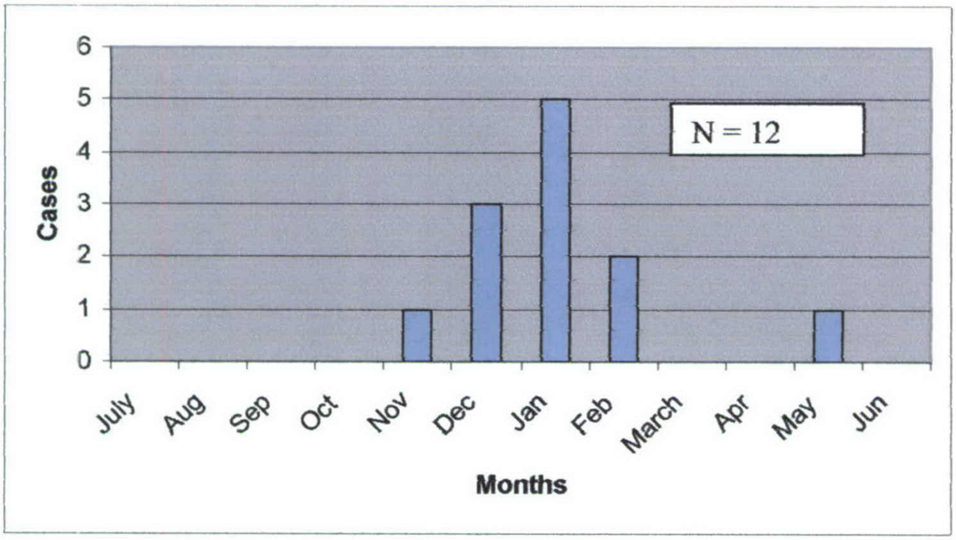


Figure 2.4: Epidemiological Curve for Leptospira-Induced Abortions in All Horse Breeds per Month During the 1992 Foaling Year

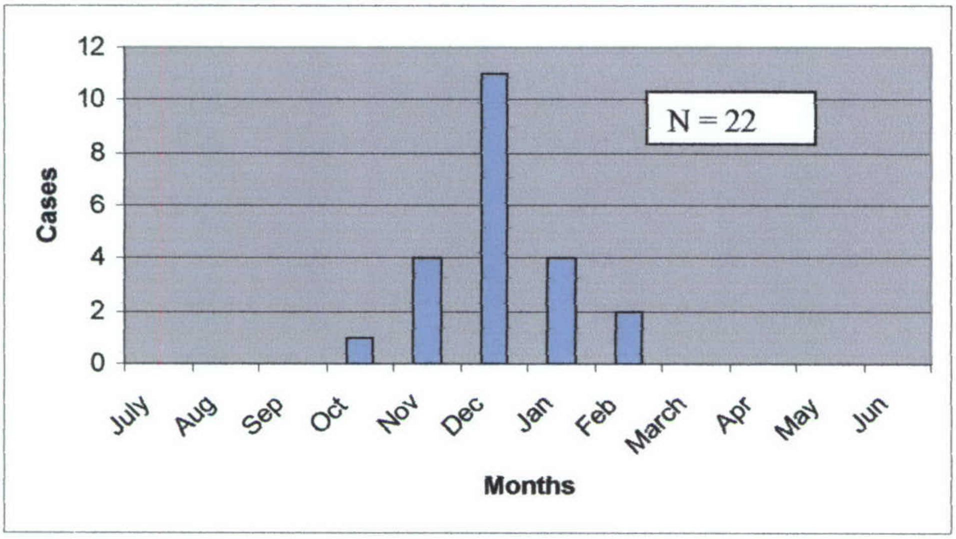


Figure 2.5: Epidemiological Curve for Leptospira-Induced Abortions in All Horse Breeds per Month During the 1993 Foaling Year

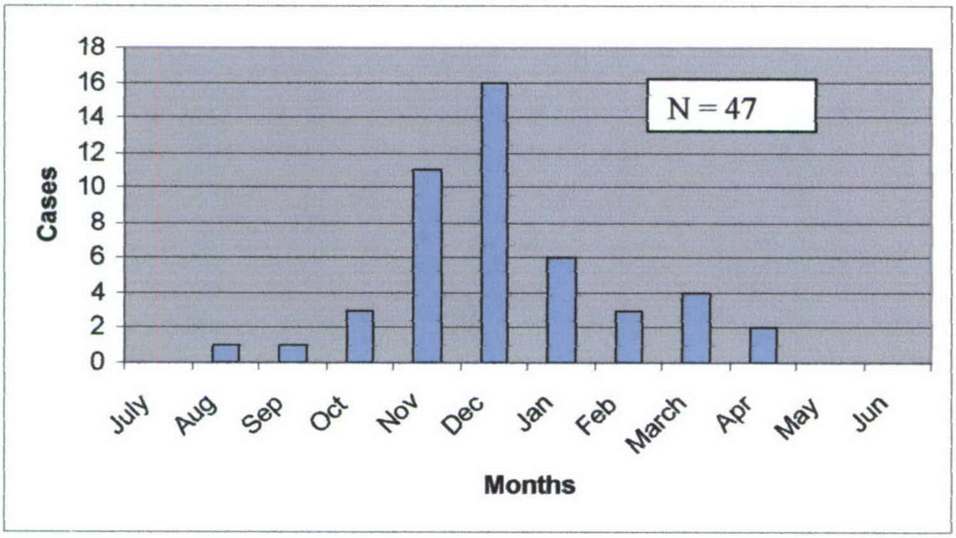


Figure 2.6: Epidemiological Curve for Leptospira-Induced Abortions in All Horse Breeds per Month During the 1994 Foaling Year

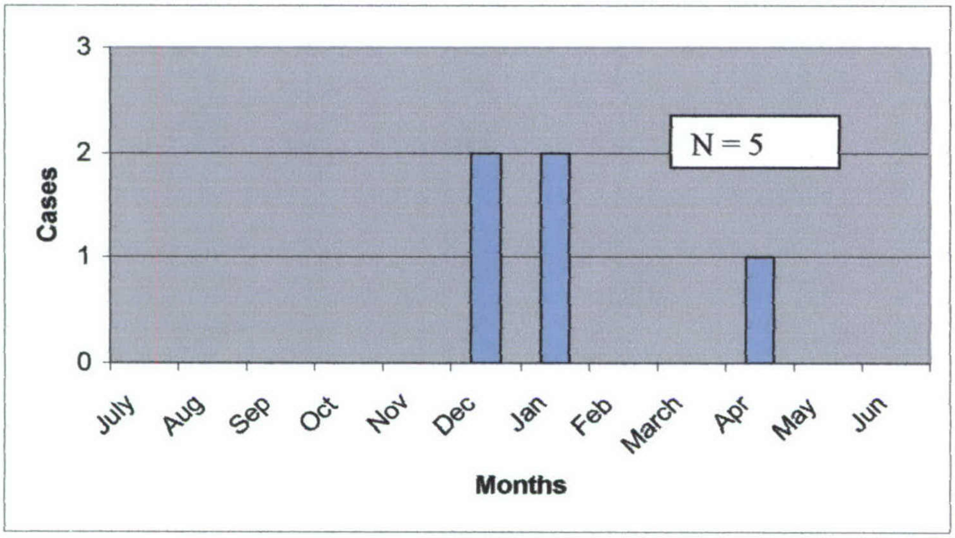


Figure 2.7: Epidemiological Curve for Leptospira-Induced Abortions in All Horse Breeds per Month During the 1995 Foaling Year

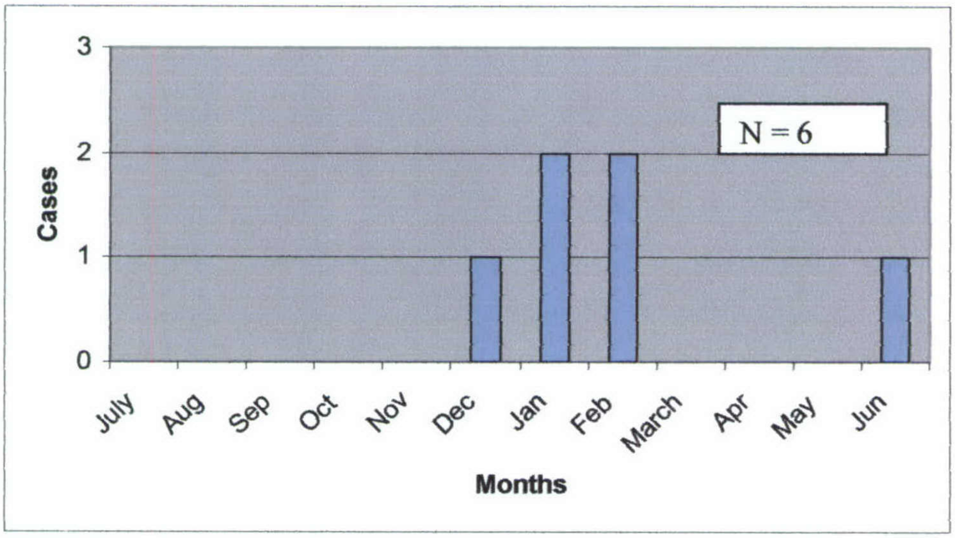
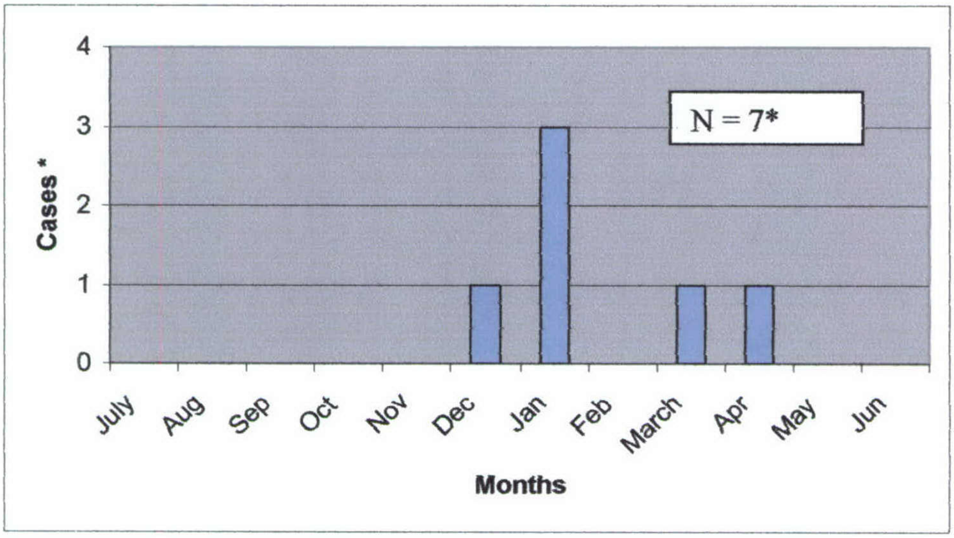
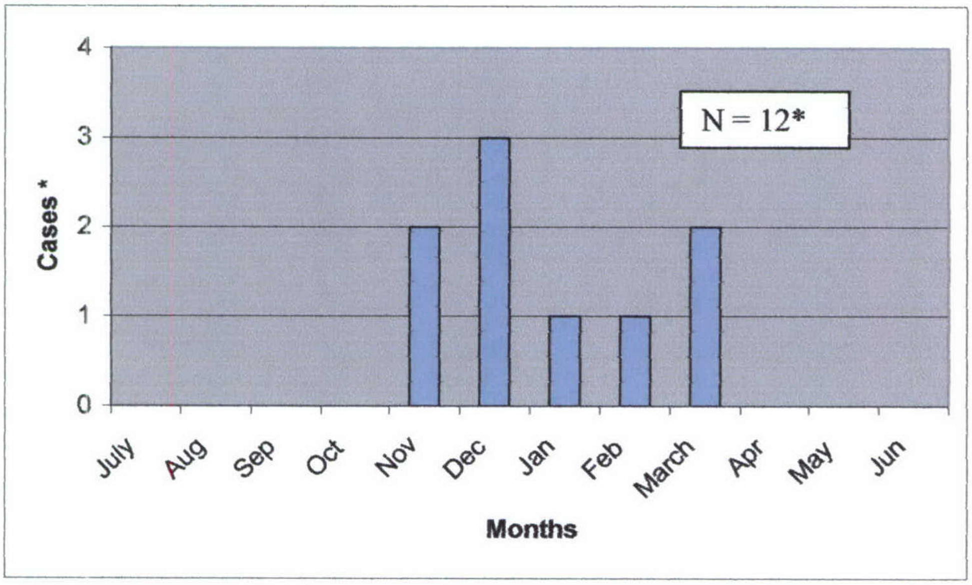


Figure 2.8: Epidemiological Curve for Leptospira-Induced Abortions in All Horse Breeds per Month During the 1996 Foaling Year



*A Case Exists Between January and March but the Exact Date is Unknown

Figure 2.9: Epidemiological Curve for Leptospira-Induced Abortions in All Horse Breeds per Month During the 1997 Foaling Year



*Two Cases Exist Prior to the November Cases and One Case Exists Between December and January but the Exact Dates are Unknown

Figure 2.10: Epidemiological Curve for Leptospira-Induced Abortions in All Horse Breeds per Month During the 1998 Foaling Year

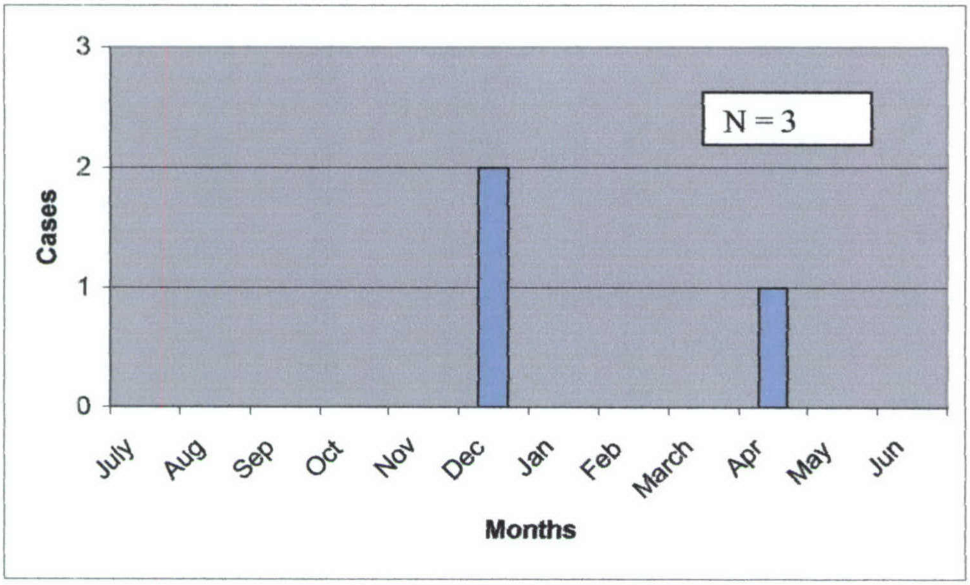


Figure 2.11: Epidemiological Curve for Leptospira-Induced Abortions in All Horse Breeds per Month During the 1999 Foaling Year

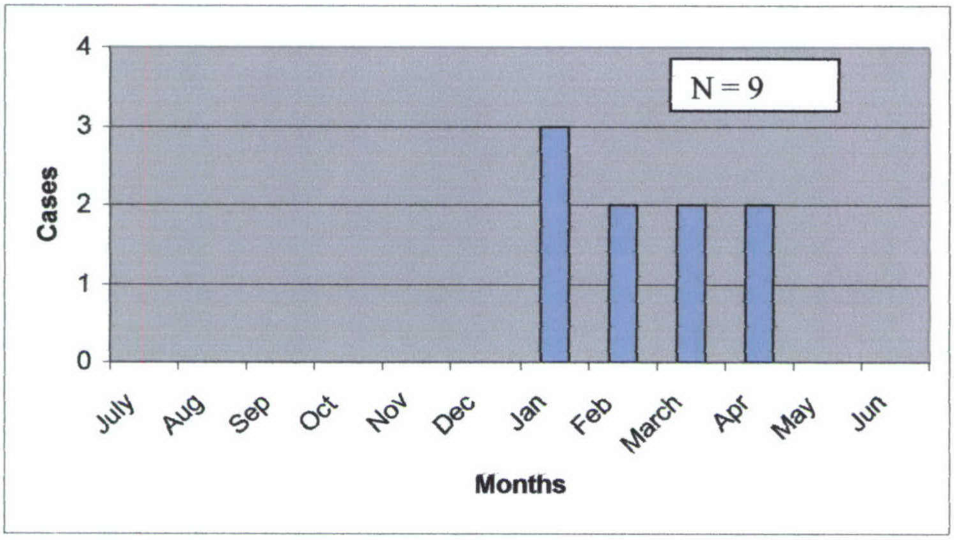


Figure 2.12: Epidemiological Curve for Leptospira-Induced Abortions in All Horse Breeds per Month During the 2000 Foaling Year

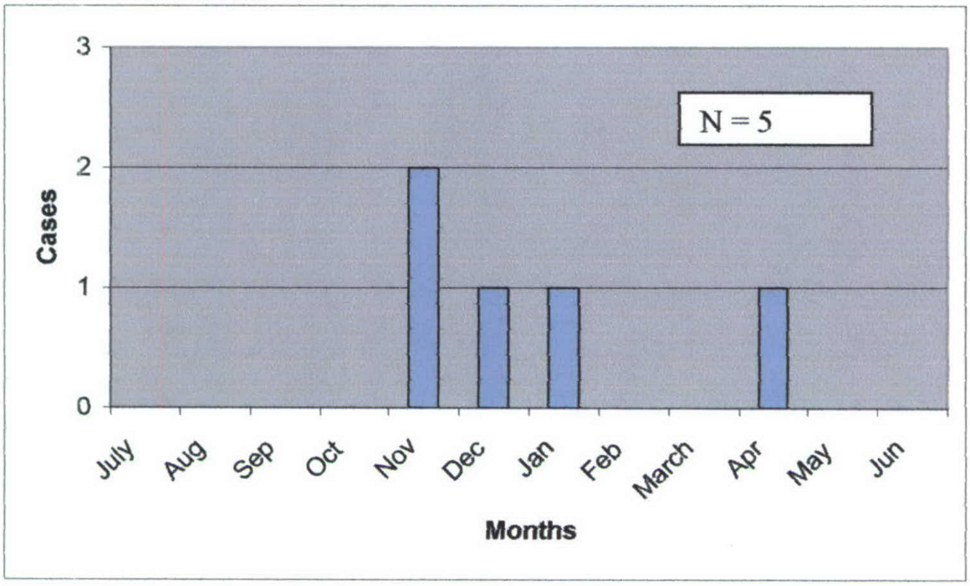


Figure 2.13: Epidemiological Curve for Leptospira-Induced Abortions in All Horse Breeds per Month During the 2001 Foaling Year

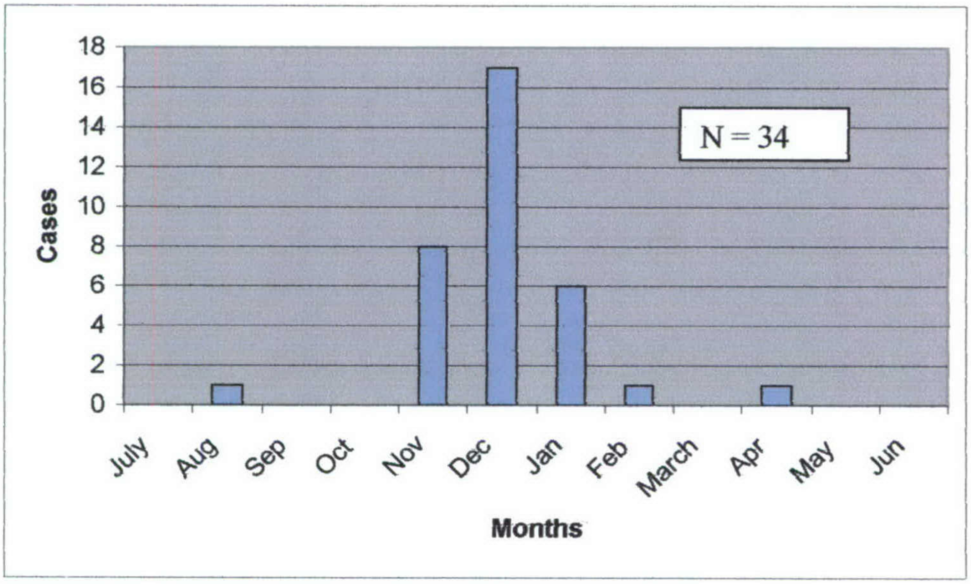


Figure 2.14: Epidemiological Curve for Leptospira-Induced Abortions in All Horse Breeds per Month During the 2002 Foaling Year

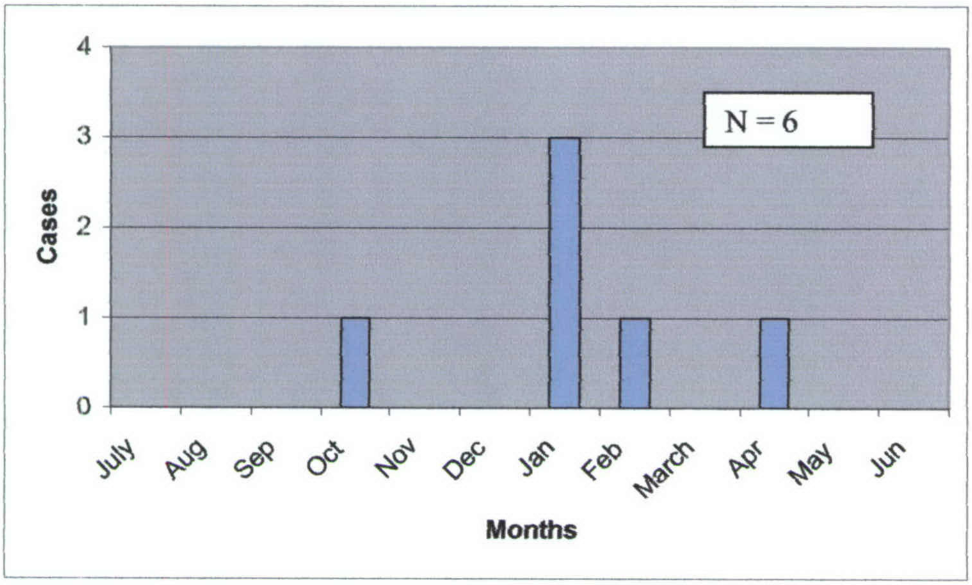


Figure 2.15: Epidemiological Curve for Leptospira-Induced Abortions in All Horse Breeds per Month During the 2003 Foaling Year

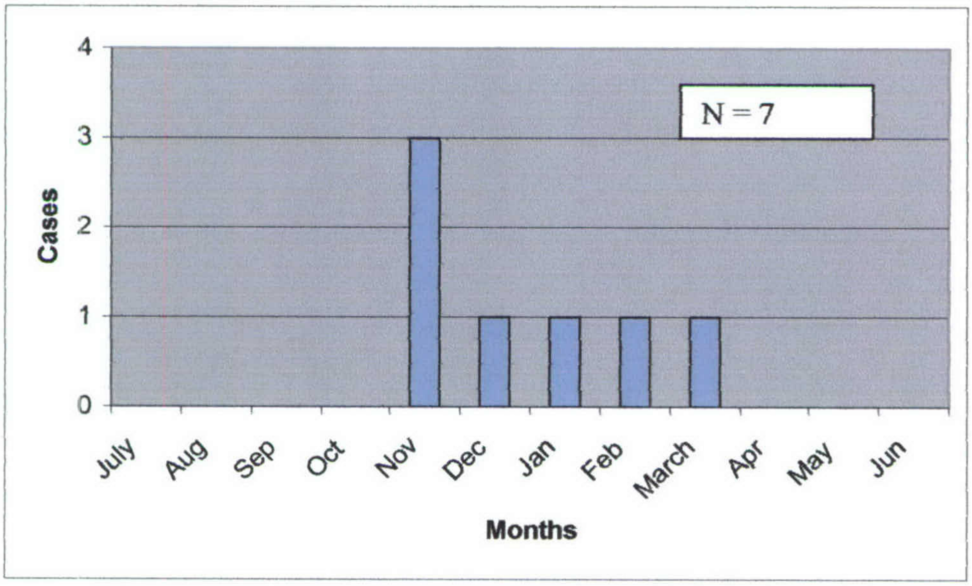
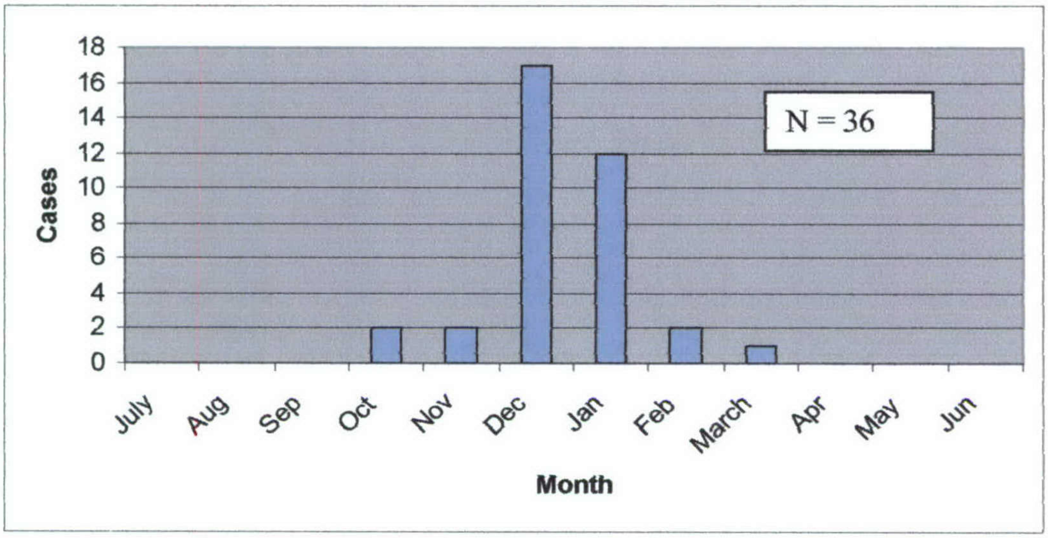
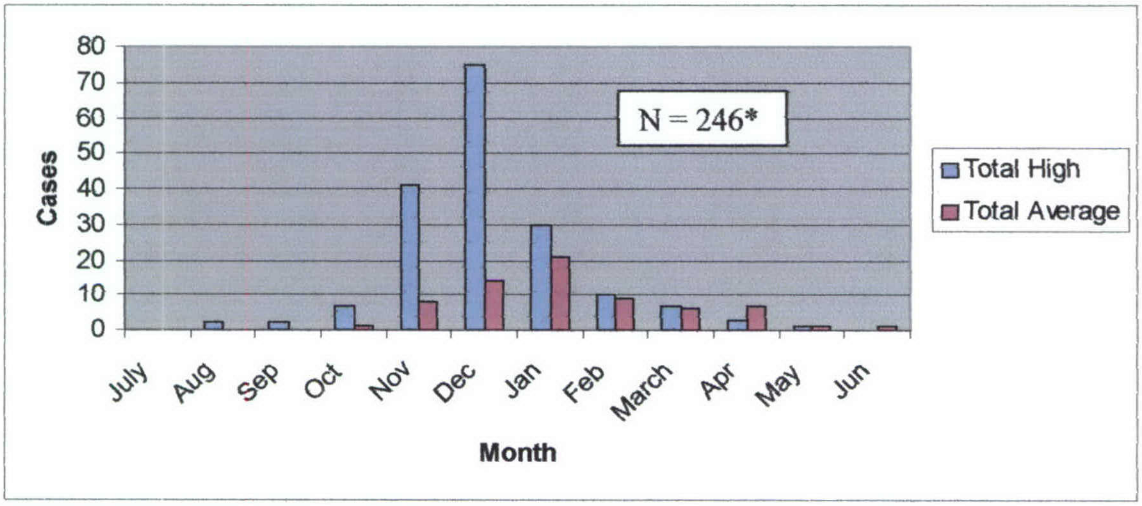


Figure 2.16: Epidemiological Curve for Leptospira-Induced Abortions in All Horse Breeds per Month During the 2004 Foaling Year



The epidemiological curves provide excellent views of the months in each year that the most incidents of abortion occurred. The curves also are able to illustrate trends in the incidents. Therefore, the epidemiological curves were combined for the years of higher than average prevalence (1990, 1992, 1993, 2001, and 2004) to create one epidemiological curve, and the epidemiological curves for the remaining years were also combined to create a separate curve for baseline prevalence. The two separate epidemiological curves were studied for trends within each curve and differences between the two curves. A graph combining the two curves, high-prevalence and baseline-prevalence abortions per month, is depicted in Figure 2.17.

Figure 2.17: Comparison Epidemiological Curves for High Prevalence Leptospira-Induced Abortion Foaling Years Totals per Month and Baseline Prevalence Leptospira-Induced Abortion Years Totals per Month for Foaling Years 1990-2004



*FY 1996 and FY 1997 contain 4 cases with unknown dates

Serovars Most Often Diagnosed by LDDC in Leptospira-Induced Abortions

It has been suggested that certain serovars of *Leptospira* are species-specific and little cross-species infection is seen (Faine, 1994b). Therefore it was important to determine the serovars most commonly involved in equine leptospira-induced abortions. In previous abortion cases in studies completed at the LDDC (Donahue et al., 1991, 1992, 1995) it was demonstrated that fetal fluids with titers against serovar pomona or the mare's serum with high titer's against serovar pomona, and/or copenhageni, and/or bratislava indicated that serovar kennewicki was the causative agent. The necropsy reports obtained from the LDDC for the foaling years 2000 through 2004 contained the laboratory data, which listed the serovars involved from each confirmed incident via a microscopic agglutination test (MAT). Antibodies against the serovars were detected in the pericardial fluid (fetus) and/or serum (mare). In some cases serovar identification was not possible.

Figure 2.18 shows the serovar distribution of both serovar specific cases and serovar combination cases. Additionally, a similar pie chart is shown in Figure 2.19, but the serovar combinations have been treated as individual incidents to help illustrate the involvement of the serovars incriminated by the LDDC in leptospira-induced abortion cases using the MAT. Previous results seen at the LDDC indicated that MAT response to pomona, icterohaemorrahagiae, and bratislava were indicative of serovar kennewicki.

Figure 2.18: Serovar Distribution of *Leptospira* for Foaling Years 2000 through 2004 in Cases of Leptospira-Induced Abortion Diagnosed by the LDDC

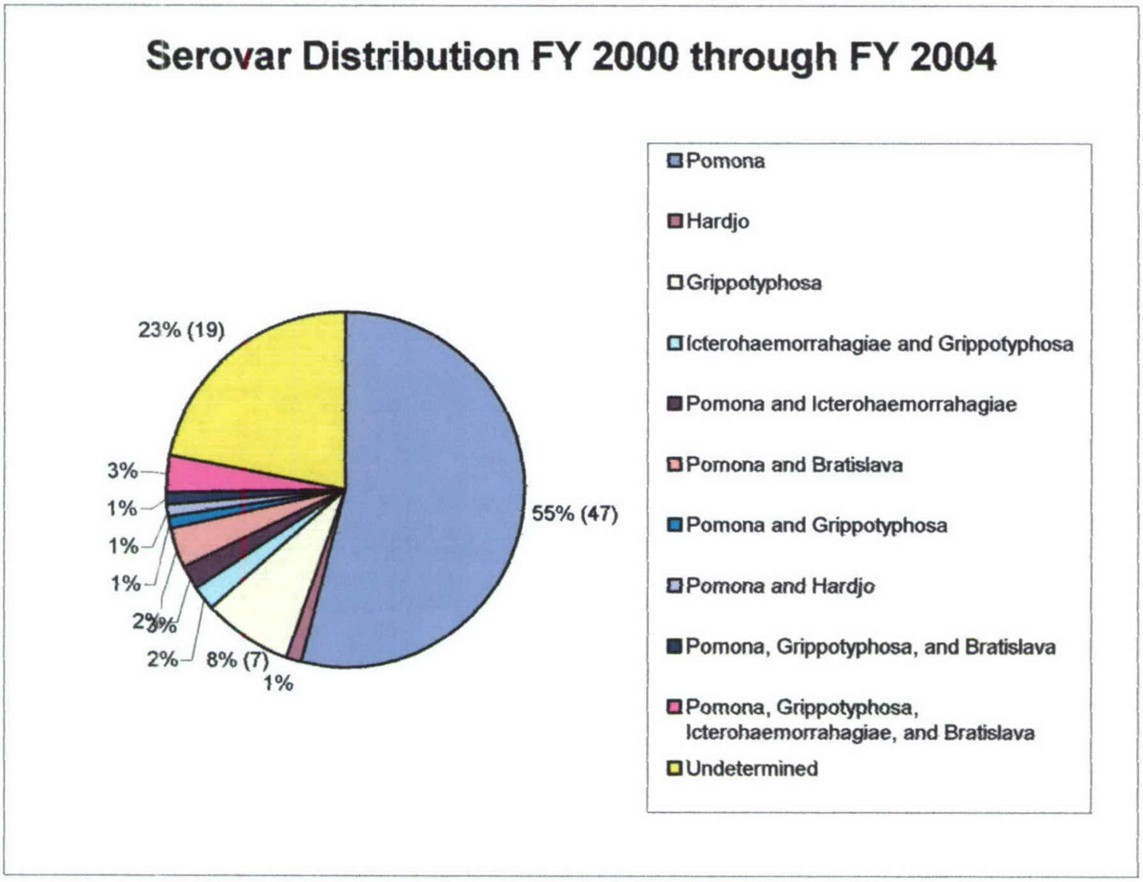
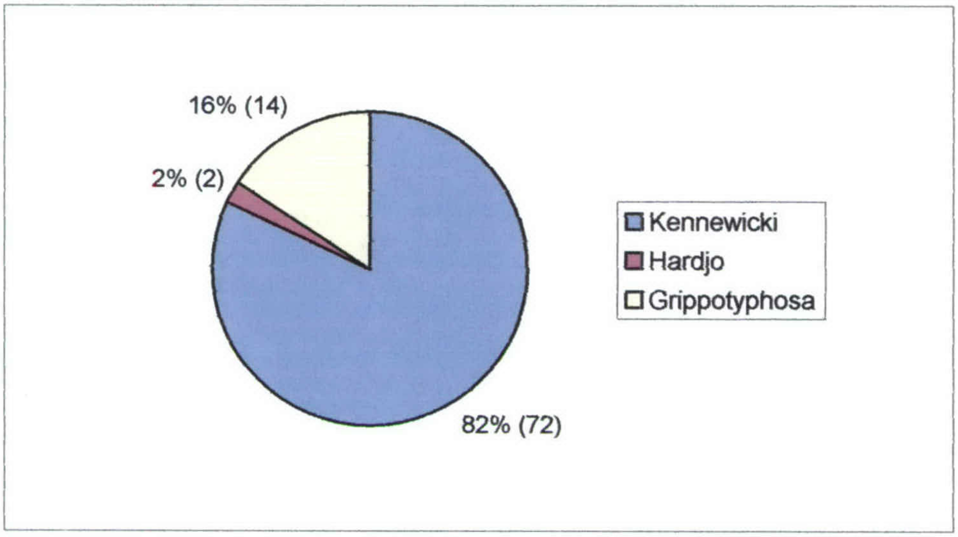


Figure 2.19: Serovars Incriminated by the LDDC to Showing the Distribution of *Leptospira* serovars for Foaling Years 2000 through 2004 in Cases of Leptospira-Induced Abortion Diagnosed by the LDDC



Mare Data

The majority of data that has been used previously in this chapter included all abortion incidents diagnosed by the LDDC in all breeds of horses. As seen with the determination of an estimated prevalence for leptospira-induced abortions, a better understanding of the horse population in which these incidents occur can be gathered by analyzing those incidents that occurred in Thoroughbred mares. Thoroughbred mares are registered with the Jockey Club and a breeding history for each mare is available. The information gathered from a breeding history details the age of the mare, the sire and dam of the mare, the years the mare was bred, the breeding outcome of those years, and the stallion the mare was bred to each year. Similar information on other breeds is not as easily available, if it is available at all.

A mare was confirmed as a Thoroughbred if the owner or farm manager on the LDDC accession sheet classified her as a Thoroughbred and she had been registered with the Jockey Club. Out of 246 confirmed cases over the fifteen years of this study 173 (70.33%) occurred in Thoroughbreds. The information obtained from the Jockey Club registry was analyzed for parity, mare age at time of abortion, and breeding success in the years following the abortion. Additionally, the cases that were diagnosed by the LDDC and appeared in the Jockey Club registry were analyzed to determine trends in the gestational age of the fetus at the time of abortion. The gestational age was found on the accession sheets and the spreadsheets provided by the LDDC and Dr. Mike Donahue.

To determine average parity for a specific foaling year, the numbers of live foals born to each Thoroughbred mare prior to her abortion were determined. The numbers of live foals for each mare were combined and divided by the number of mares. The averages for each foaling year were then combined and divided by 15 to calculate the total average parity for the entire study. These results can be seen in Figure 2.20. The range for parity in all 15 years of the study was 1 to 8 live births. The median was 2.88 live births. The range for parity in high-prevalence years was 3 to 5 live births and the median was 3.58 live births. The range for parity in baseline-prevalence years was 1 to 9 and the median was 3 live births.

As evidenced by Figure 2.20, the average parity of a Thoroughbred mare prior to the incident of a leptospira-induced abortion is approximately 3 live births. There is a slight difference in the averages of the years of high prevalence and those years of baseline prevalence. The difference, however, was proven to be non-significant ($p > 0.05$) following a statistical comparison.

The average mare age was determined in a similar fashion as parity. The results for mare age can be seen in Figure 2.21. For all 15 years of the study the range for the average mare age was 5 years to 15 years old and the median was 8.73 years old. For the high-prevalence years the range for the average mare age was 6.83 to 11.71 years old and the median was 8.74 years old. For the baseline-prevalence years the range for the average mare age was 5 to 15 years old and the median was 8.84 years old.

Figure 2.20: Average Parity for Thoroughbred Mares Prior to Experiencing a Leptospira-Induced Abortion (All Years, High Prevalence Years, and Baseline Prevalence Years)

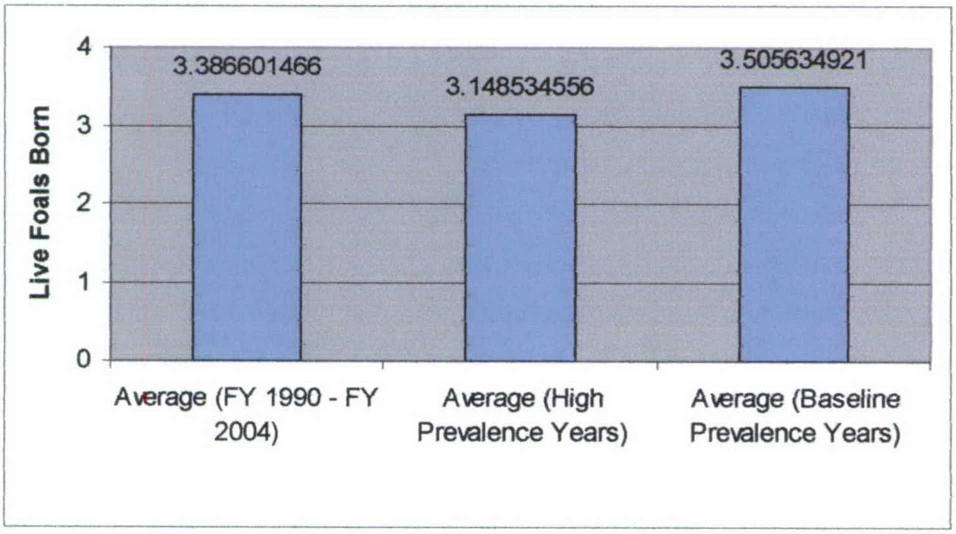
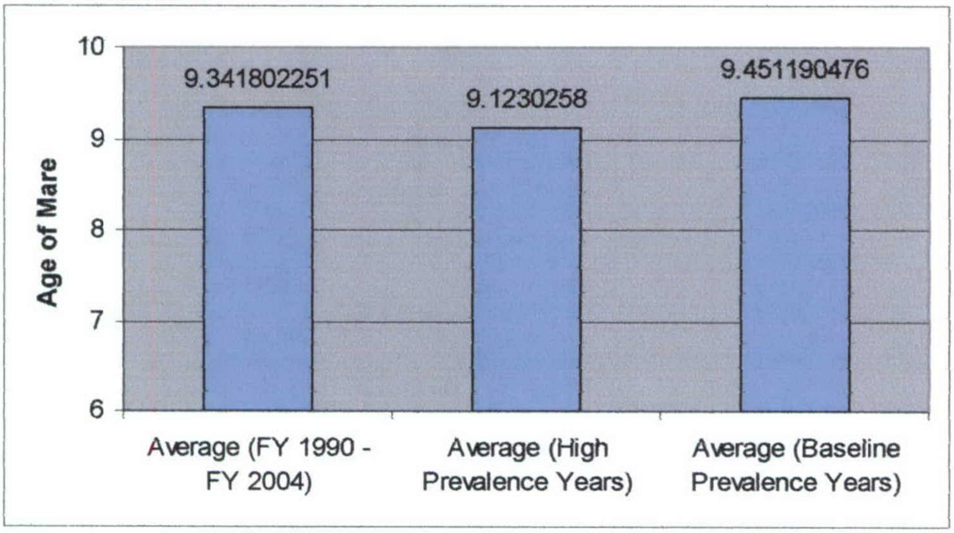


Figure 2.21 indicates the average mare age at the time of abortion for the total years, the high-prevalence years, and the baseline-prevalence years. The figure shows that the average is approximately 9 years old in each case. A statistical comparison was still performed on the difference between the high-prevalence and baseline-prevalence years. This difference was shown to be nonsignificant ($p > 0.05$).

Average gestational age of the fetus at the time of the abortion was the next characteristic analyzed. Again, the averages were calculated in the similar manner to parity and average mare age. The results for gestational age of the fetus can be seen in Figure 2.22. The range for the average gestational age of the fetus was 8 to 10.25 months

Figure 2.21: Average Age in Years for Thoroughbred Mares at the Time of the Leptospira-Induced Abortion (All Years, High Prevalence Years, and Baseline Prevalence Years)



during all 15 years of the study, and the median for the average gestational age was 9.32 months. During the high-prevalence years the range of average gestational age was 8 to 9.16 months old and the median was 8.35 months old. During the baseline-prevalence years the range of the average gestational age was 8.5 to 10.25 months old and the median was 9.625 months old.

Figure 2.22: Average Gestational Age in Months for Thoroughbred Fetuses at the Time of the *Leptospira*-Induced Abortion (All Years, High Prevalence Years, and Baseline Prevalence Years)

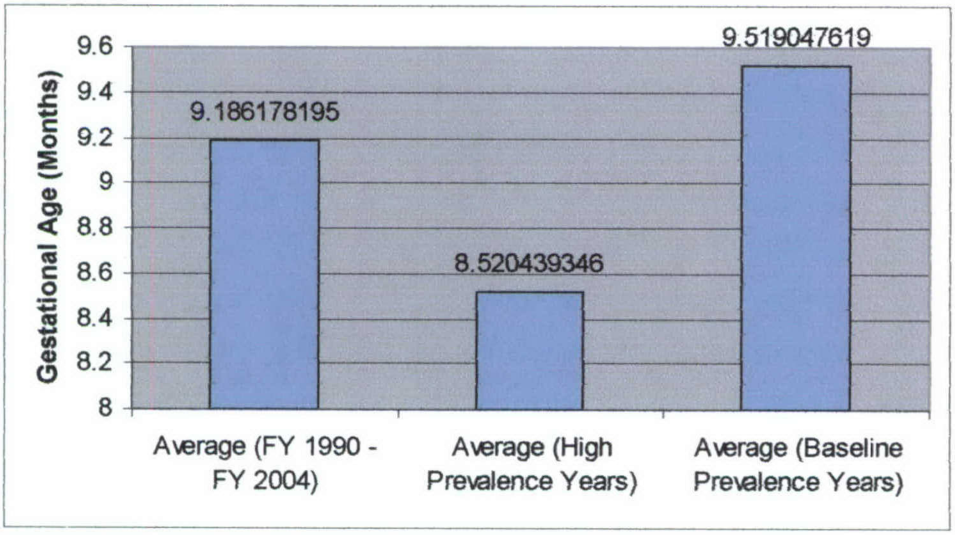


Figure 2.22 indicates the average gestational age of the fetus at the time of abortion for the total years, the high-prevalence years, and the baseline-prevalence years. The average for the total years is approximately 9 months. The average for the high-prevalence years is approximately 8.5 months. The average for the baseline-prevalence years is almost a full month greater at approximately 9.5 months. The average gestational age for the high-prevalence years and the average for the baseline-prevalence years were statistically compared to show a significant difference ($p < 0.005$).

Breeding success was also determined using the mare data from the Jockey Club. The confirmed cases of abortions or the mare being barren the year following a leptospiral abortion are summarized in Table 2.4. In a study of Thoroughbred mares in Kentucky during 1988 and 1989 foaling years, the average live foal percentage for 9-year old mares was approximately 78% (Baker, 1995). This serves as a baseline comparison only, since all Kentucky Thorougbred mares were included in Baker's investigation, whereas only 149 mares, most likely from central Kentucky, are included in the analysis of reproductive success following a leptospira-induced abortion. Additionally, improved veterinary methods for enhancing reproductive performance of mares have progressed during the past 14 years, also likely accounting for the difference in reproductive success between the studies.

Table 2.4: Thoroughbred Mare Breeding Success in the Year Following a Leptospira Abortion

Foaling Year*	Total TB Mares Aborting or Barren in the Year Following a Leptospiral Abortion	Total TB Mares Giving Birth to a Live Foal in the Year Following a Leptospiral Abortion	Percentage of TB Mares Having a Leptospiral Abortion the Year Following a Leptospiral Abortion
1990	3	24	88.89%
1991	1	6	85.71%
1992	2	12	85.71%
1993	4	30	88.24%
1994	0	2	100%
1995	0	3	100%
1996	0	7	100%
1997	1	8	88.89%
1998	0	2	100%
1999	2	4	66.67%
2000	0	1	100%
2001	4	25	86.21%
2002	1	3	75.00%
2003	0	4	100%
Total	18	131	87.92%

*Foaling year 2004 is not included because results are not available

Discussion

Prevalence

The LDDC is one of two state diagnostic laboratories in Kentucky. The other diagnostic laboratory, the Breathitt Veterinary Laboratory, is located in western Kentucky. Historically, the farms in closer proximity to the LDDC will utilize the facility's services. This is especially true since, prior to 2004, all equine necropsies were performed free of charge. This could mean that several cases of leptospira-induced abortion go undiagnosed by the LDDC each year. For this study the assumption was made that if a farm did not utilize the LDDC because of geographic constraints in prior years, it did not use the LDDC for the same reasons in later years. Therefore, when the number of incidents diagnosed at the LDDC is compared to the total number of live Thoroughbred foals born in Kentucky for each Foaling Year, the resulting prevalence may be low, but is equally low for each year.

A prevalence amount higher than or equal to the average prevalence of .0014 was used to determine the foaling years having a higher than normal incidence of abortion. In other words, if there were 14 or more leptospira-induced abortions in Thoroughbreds for every 10,000 foals born alive during a particular foaling year, that foaling year was determined to be a high-prevalence year. The foaling years determined to be those of high prevalency were 1990, 1992, 1993, 2001, and 2004. The corresponding prevalence is marked in bold numbering in Table 2.2.

In order to confirm the years of high prevalence, Table 2.3, comparing all breeds diagnosed with leptospira-induced abortions to Thoroughbred foals born alive in Kentucky, was analyzed in the same manner. In this case, the average prevalence was

.0020 and the years of high prevalence were the same as those previously determined (1990, 1992, 1993, 2001, and 2004). Bold numbering in Table 2.3 marks the corresponding prevalence to these years.

Epidemiological Curves

Figure 2.17 combines the High-Prevalence (HP) years and the Baseline-Prevalence (BP) years and indicates the most noticeable differences in the two curves. First, the two curves begin during different months. The HP curve begins in August and the BP curve does not begin until October. Secondly, the two curves end in different months. It is once again a two-month difference, as the HP curve ends in April and the BP curve does not end until June. Thirdly, the peak number of incidents occurs during different months in each curve. The HP curve illustrates an incident peak in December, while the BP curve illustrates an incident peak in January. Lastly, it is discernable from the curves that the months of November, December, and January in the HP curve all represent more cases than the peak in the BP curve, which occurs in January.

These curves represent the beginning of the epidemiological investigation into the occurrences of leptospira-induced abortions. The following chapters will include weather data for the fifteen years investigated in this study. The weather data will be analyzed to try to determine if such a dynamic shift in epidemiological curves between those years having large numbers of leptospiral abortions compared to those years having average numbers is related to temperature, precipitation, or a combination of both.

Serovars

The serovars detected by the LDDC can be viewed in Figure 2.18. The pie chart shows that 55% of the abortions are specific to serovar pomona. Another 11% of the abortions involve the serovar pomona and other *Leptospira* serovars. Serovar grippotyphosa is specific in 8% of the incidents, while serovar hardjo is specific in only 1% of the incidents. A serovar combination of icterohaemorrhagiae and grippotyphosa account for 2% of the incidents, and the remaining 23% of the incidents are caused by an undetermined serovar or serovar combination. Figure 2.18 shows that serovar pomona is the most often detected serovar.

The most often detected serovar in the occurrences of leptospira-induced abortions is *Leptospira interrogans* serovar pomona. Serovar pomona was detected in 55% of the total incidents of such abortions. It is notable that the 55% represented in Figure 2.18 only includes those incidents in which serovar pomona was the lone serovar detected. Interestingly, the high leptospira-induced abortion foaling years of 2001 and 2004 accounted for 46 out of 47 of the cases involving only serovar pomona (66.67 % of total cases). The baseline leptospira-induced abortion foaling years of 2000, 2002, and 2003 had one case involving only serovar pomona, (5.56 % of total cases).

In order to examine the total involvement serovar kennewicki had in leptospira-induced abortion cases for the five years being studied, combinations of serovars that were isolated from one incident were separated and treated as an individual identifications. For example, an incident involving the isolation of serovar pomona and serovar hardjo was broken down into two separate incidents, one involving serovar pomona and one involving serovar hardjo. The results of this examination are depicted in Figure 2.19.

Serovar kennewicki is incriminated in 82% (72) of the cases examined by the LDDC. Serovar grippotyphosa was incriminated in 16% (14) of the cases and serovar hardjo was incriminated in only 2% (2) of the cases examined by the LDDC. Remember that the number of cases used for the calculation of percentage of total cases is greater because serovar combinations have been separated. Because of the high traffic of broodmares into and out of Kentucky, it is not possible to conclusively state that these serovars are the responsible strains of *Leptospira* causing equine abortions in central Kentucky, but it is possible to ascertain that *Leptospira interrogans* serogroup Pomona serovar kennewicki is a likely causative agent of most abortions.

Thoroughbred Mare Data

Although the Jockey Club provided the specific information used to determine parity and the age of the mare at time of the abortion, it was a combination of Jockey Club data and LDDC data that provided the gestational age of the Thoroughbred fetus at the time of the abortion. The Jockey Club data confirmed that the mare suffering the abortion was a registered Thoroughbred mare and the LDDC data provided the gestational age of the fetus at the time of the abortion. The mean age of all mares at the time of leptospira-induced abortion was 9.3 years. The mean parity for all mares at the time of leptospira-induced abortion was 3.38. There was no statistical difference between the calculated averages in mare age or parity for the high-prevalence and the baseline-prevalence years.

In most cases the submitting veterinarian calculates the gestational age and the age is recorded on the LDDC accession sheet at the times of the aborted fetus' submissions. In most cases the gestational age can be regarded as accurate (Dr. David Bolin, University of Kentucky Livestock Disease and Diagnostic Center, personal communication, 2004).

Figure 2.22 indicates the average gestational age of the fetus at the time of abortion for the total year, the high-prevalence years, and the baseline-prevalence years. The average for the total years is approximately 9 months. The average for the high-prevalence years is approximately 8.5 months. The average for the baseline-prevalence years is almost a full month greater at approximately 9.5 months.

The average gestational age for the high-prevalence years and the average for the baseline-prevalence years were statistically compared to show a significant difference ($p < 0.005$). This finding suggests that the average gestational age of foals at the time of a leptospira-induced abortion during years of greater prevalence is significantly younger than the average gestational age of foals during years of baseline prevalence.

Gestational Age and Epidemiological Curve

As mentioned earlier, the peak of the epidemiological curve during the years of high-prevalence is during the month of December, while the peak during the years of baseline-prevalence is during January. This difference is statistically confirmed by the significance in the difference of gestational ages of the aborted fetuses during the years of high-prevalence and the years of baseline-prevalence. The gestational age of the aborted fetuses during the high-prevalence years is approximately one month younger, which corresponds to the shift in epidemiological curves from January to December.

Chapter 3: Temperature and Precipitation Analysis

Introduction

The data accumulated in Chapter One provided integral information to begin the epidemiological investigation of leptospira-induced abortions in horses in central Kentucky. Years of high-prevalence of such abortions were identified, as well as years having a baseline number of abortions. In this chapter the high-prevalence years average precipitation and temperature, the baseline-prevalence years average precipitation and temperature, and the 15-year average precipitation and temperature will be compared and contrasted.

The hypothesis of this investigation is that leptospira-induced abortions are more likely to occur when the weather consists of milder temperatures and heavier precipitation because those conditions provide optimum conditions for the survival of leptospires in the environment. The survival of more leptospires would suggest an increased risk of exposure and possibly result in an increased number of abortions. Previous work has supported such a claim.

Chapter one established that one of the most important serovars involved in the leptospira-induced abortions is kennewicki, a member of the Pomona serogroup. It has been found that serovar pomona can survive in neutral pH soil (6.7-7.2) under moist conditions (15.2 – 31.4%) for up to 74 days (Zaitsev et al., 1989). Furthermore, survival has also been established in standing rice-paddy water for 7-14 days while subjected to temperatures between 0 and 30 degrees Celsius (Ryu and Liu, 1967). The primary focus of this investigation is to determine if an increase in precipitation, milder temperatures, or a combination of both will increase the likelihood of leptospira-induced abortions in the equine population in central Kentucky.

Materials and Methods

Lexington Climate Data

The University of Kentucky Agricultural Weather Department was consulted, specifically Dr. Thomas Priddy, as to the best informational source for temperature and precipitation data for the bluegrass region of Kentucky. The counties of the Kentucky bluegrass region, Fayette County and the surrounding 6 counties, have the same general climate and experience few differences in temperature and precipitation (Dr. Tom Priddy, University of Kentucky College of Agriculture, Weather Department, via personal communication, 2004). Therefore, gathering data from one weather station, the Lexington Airport weather station in Fayette County, was satisfactory.

The information was accessed via the University of Kentucky Agricultural Weather Department's website at http://wwwagwx.ca.uky.edu. Once on the website the user may choose "climatology" by clicking on it. After the "Climatology" page loads, an option for "Kentucky Climate Data" is available approximately halfway down the page. Under this option "Variable Length Summary" was selected. This selection opens a query window. The station selected for the query was "Lexington" and the dates were for each foaling year used in this study. The interval chosen was for 7 days and "screen" output was designated. The "Submit Choices" button was then selected and the climate report was opened on the screen.

The climate reports were broken down into seven-day intervals beginning on July 1^{st} and ending on June 30^{th} the following calendar year (Foaling Year). A maximum and minimum temperature in degrees Fahrenheit and an average temperature in degrees Fahrenheit defined each week. The maximum, minimum, and average temperatures were also defined by a departure from the normal temperature for each category. The report also contains the total precipitation for the week and the departure from the normal precipitation.

LDDC Data for Dates of Abortion

As previously discussed, the LDDC accession sheets and spreadsheets were used to define the cases for all breeds of horses having a leptospira-induced abortion. The accession sheets also provided the date, which was used as the date of abortion in this study.

Information Processing

Microsoft Excel from Microsoft Office 2000 SR-1 Professional program was used to create spreadsheets of data collected and the graphs and charts that are included in this document.

<u>Results</u>

The following figures will have an x-axis titled "Weeks" and numbered 1 through 53. Table 3.1 indicates the dates for which each numbered week corresponds.

Table 3.1: Key for the Dates Comprising Each Week Numbered in Chapter Three's Figures

Week Number	Dates
1	July 1 thru July 7
2	July 8 thru July 14
3	July 15 thru July 21
4	July 22 thru July 28
5	July 29 thru August 4
6	August 5 thru August 11
7	August 12 thru August 18
8	August 19 thru August 25
9	August 26 thru September 1

10	September 2 thru September 8
11	September 9 thru September 15
12	September 16 thru September 22
13	September 23 thru September 29
14	September 30 thru October 6
15	October 7 thru October 13
16	October 14 thru October 20
17	October 21 thru October 27
18	October 28 thru November 3
19	November 4 thru November 10
20	November 11 thru November 17
21	November 18 thru November 24
22	November 25 thru December 1
23	December 2 thru December 8
24	December 9 thru December 15
25	December 16 thru December 22
26	December 23 thru December 29
27	December 30 thru January 5
28	January 6 thru January 12
29	January 13 thru January 19
30	January 20 thru January 26
31	January 27 thru February 2
32	February 3 thru February 9
33	February 10 thru February 16
34	February 17 thru February 23
35	February 24 thru March 2*
36	March 3 thru March 9
37	March 10 thru March 16
38	March 17 thru March 23
39	March 24 thru March 30
40	March 31 thru April 6
41	April 7 thru April 13
42	April 14 thru April 20
43	April 21 thru April 27
44	April 28 thru May 4
45	May 5 thru May 11
46	May 12 thru May 18
47	May 19 thru May 25
48	May 26 thru June 1
49	June 2 thru June 8
50	June 9 thru June 15
51	June 16 thru June 22
52	June 23 thru June 29
53	June 30

*Includes February 29th during leap years

Temperature

The results from the temperature query performed on the University of Kentucky's Agricultural Weather website are summarized in Figures 3.1 through 3.12. Figures 3.1 through 3.3 illustrate the average weekly temperatures for total average, maximum, and minimum temperatures for all 15 of the years included in the study. Figures 3.4 through 3.6 illustrate the same averages, but the years of high-prevalence are the only years included. Figures 3.7 through 3.9 correspond to the baseline-prevalence years. Contrasts of the total years, the high prevalence years, and the baseline-prevalence years are provided in Figures 3.10 through 3.12. An additional figure, Figure 3.13, has been added to provide a better illustration of differences in the 15-year, high-prevalence years, and baseline-prevalence years temperature curves.

Figure 3.1: Average Weekly Temperature for Foaling Years 1990 – 2004 in Degrees Fahrenheit

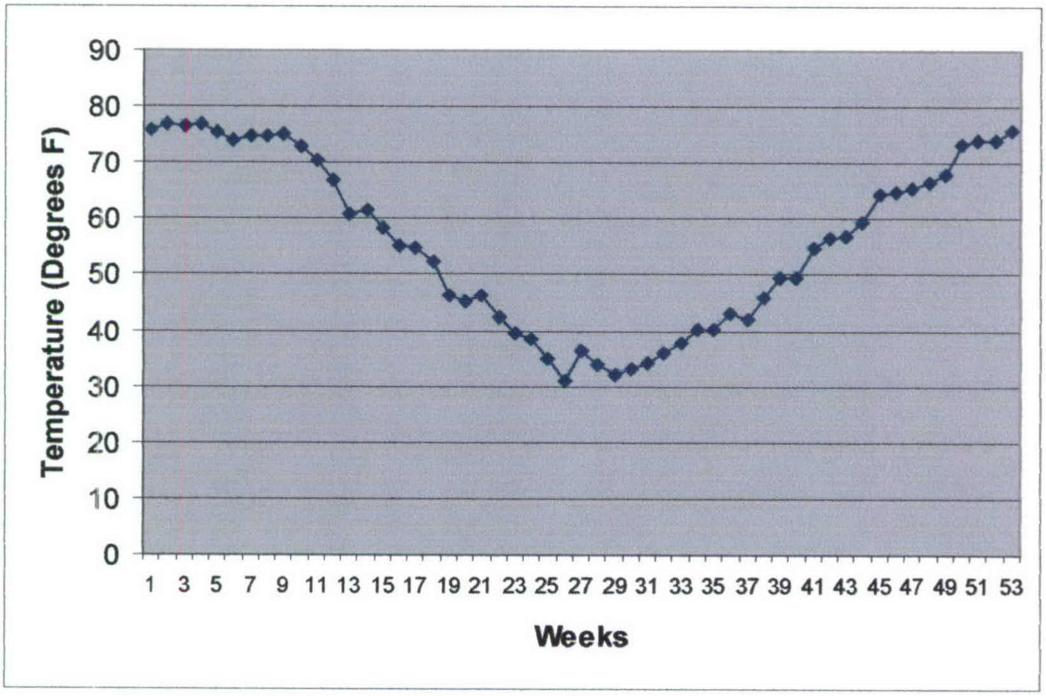


Figure 3.2: Average Maximum Weekly Temperature for Foaling Years 1990 – 2004 in Degrees Fahrenheit

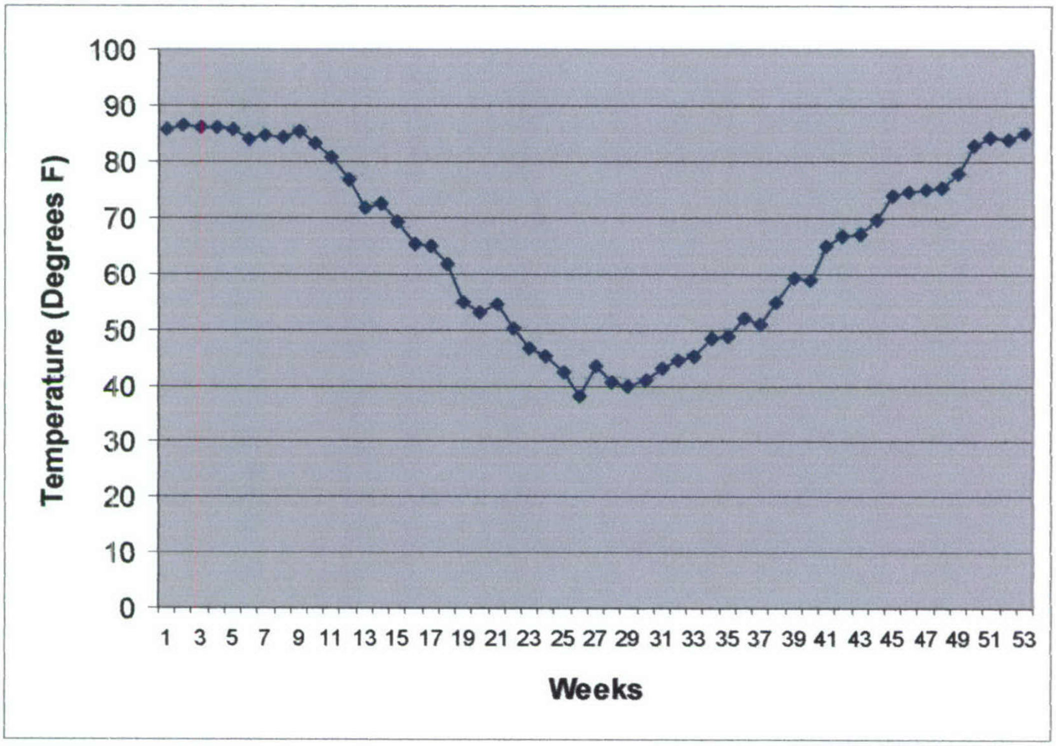


Figure 3.3: Average Minimum Weekly Temperature for Foaling Years 1990 – 2004 in Degrees Fahrenheit

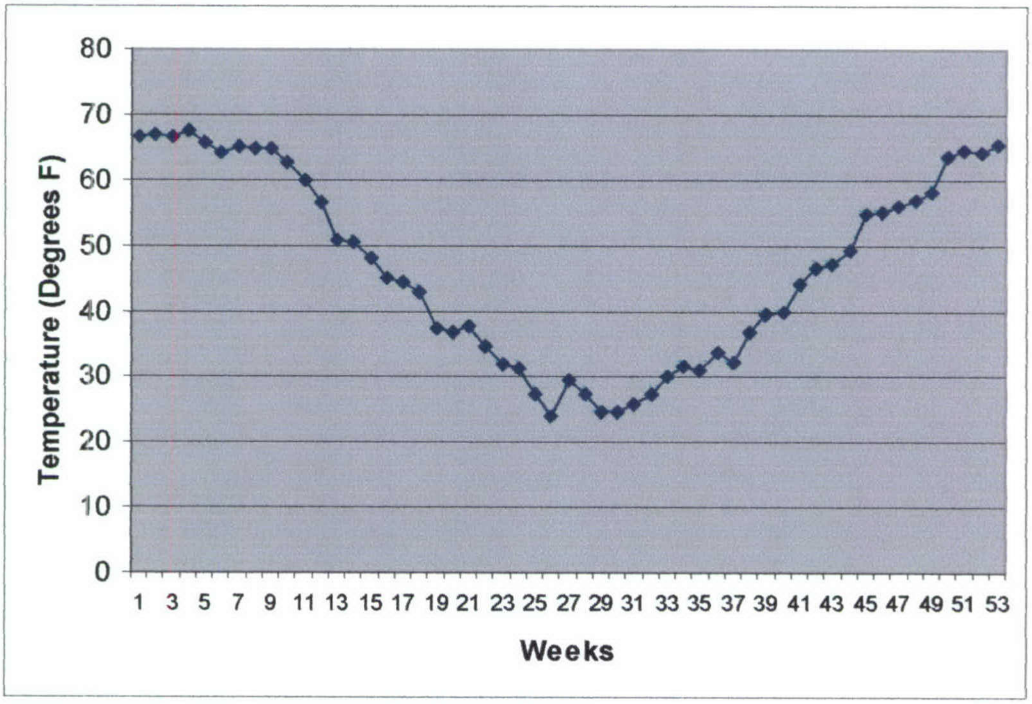


Figure 3.4: Average Weekly Temperature for High-Prevalence Foaling Years, 1990, 1992, 1993, 2001, and 2004, in Degrees Fahrenheit

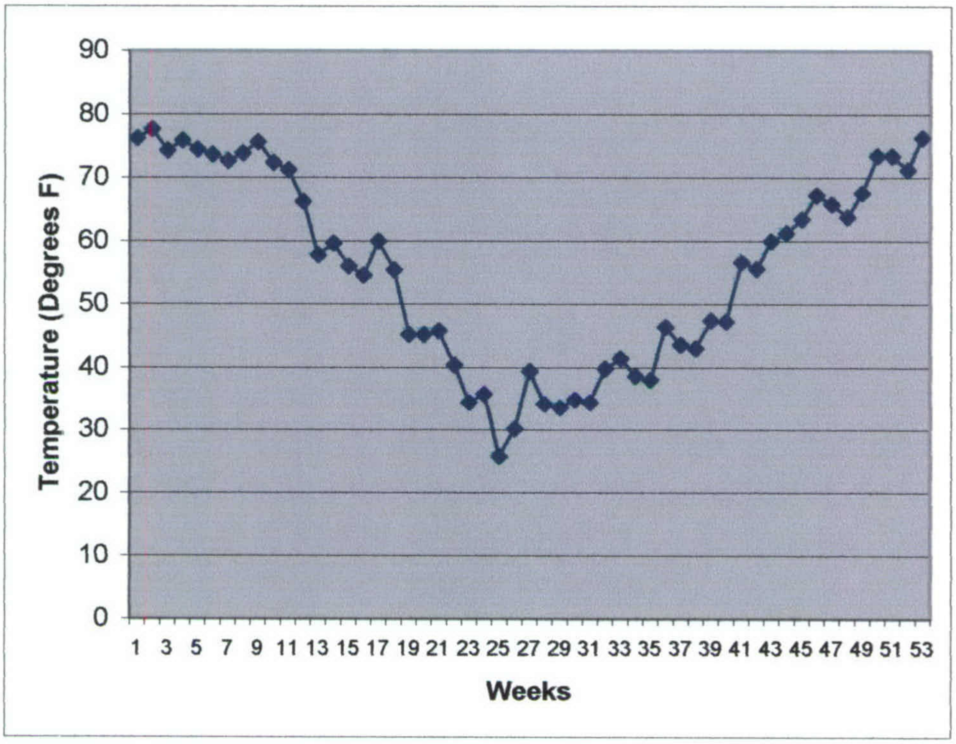


Figure 3.5: Average Weekly Maximum Temperature for High-Prevalence Foaling Years, 1990, 1992, 1993, 2001, and 2004, in Degrees Fahrenheit

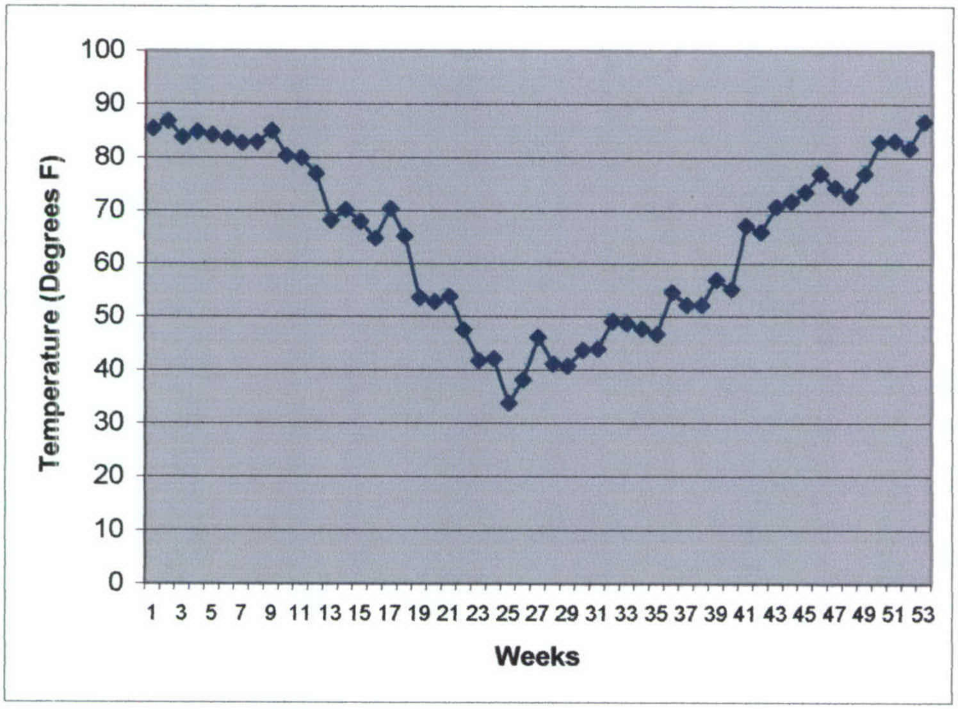


Figure 3.6: Average Weekly Minimum Temperature for High-Prevalence Foaling Years, 1990, 1992, 1993, 2001, and 2004, in Degrees Fahrenheit

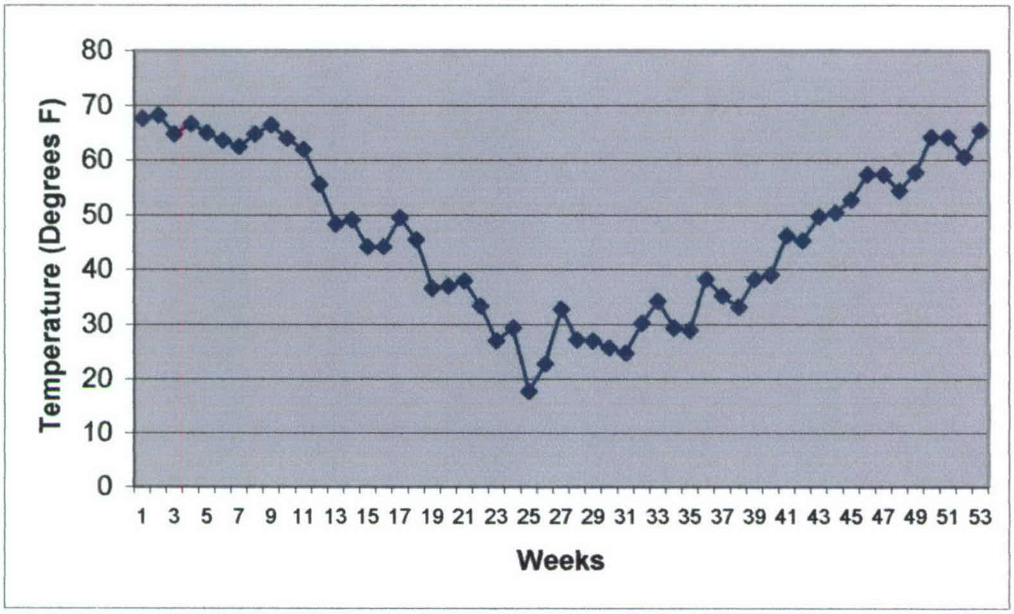


Figure 3.7: Average Weekly Temperature for Baseline-Prevalence Foaling Years, 1991, 1994-2000, 2002, and 2003, in Degrees Fahrenheit

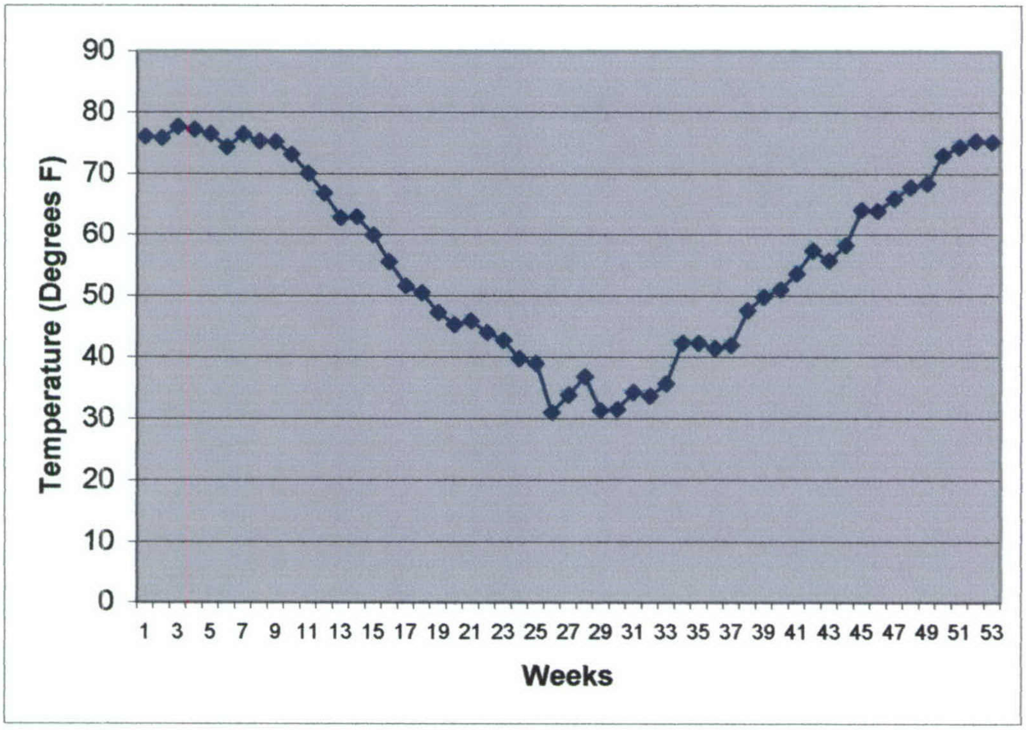


Figure 3.8: Average Weekly Maximum Temperature for Baseline-Prevalence Foaling Years, 1991, 1994-2000, 2002, and 2003, in Degrees Fahrenheit

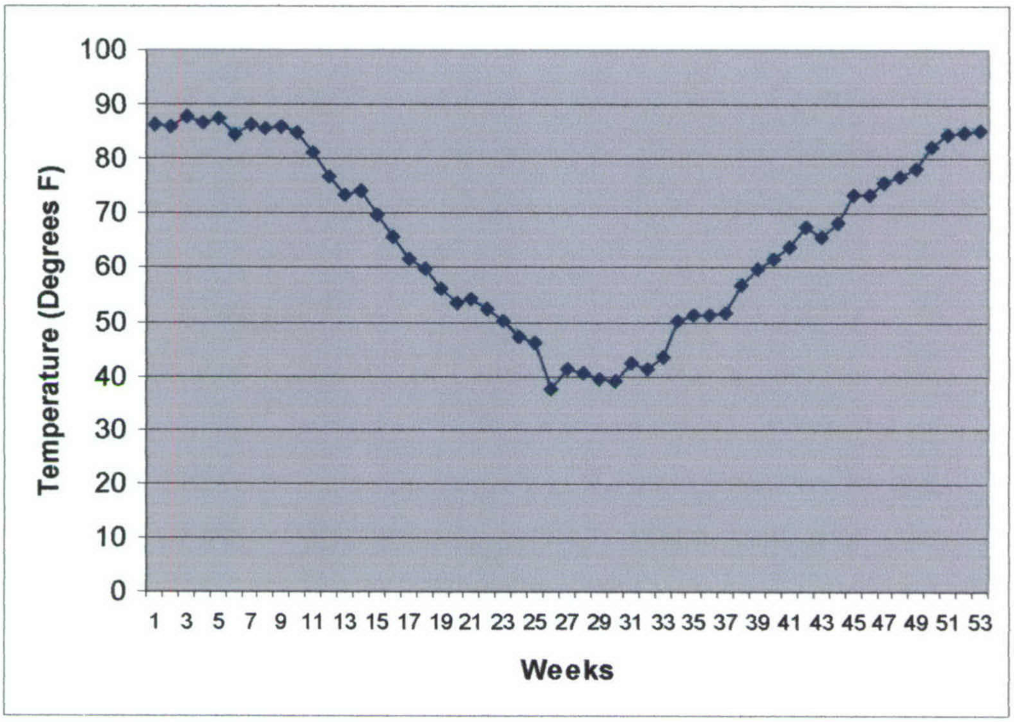


Figure 3.9: Average Weekly Minimum Temperature for Baseline-Prevalence Foaling Years, 1991, 1994-2000, 2002, and 2003, in Degrees Fahrenheit

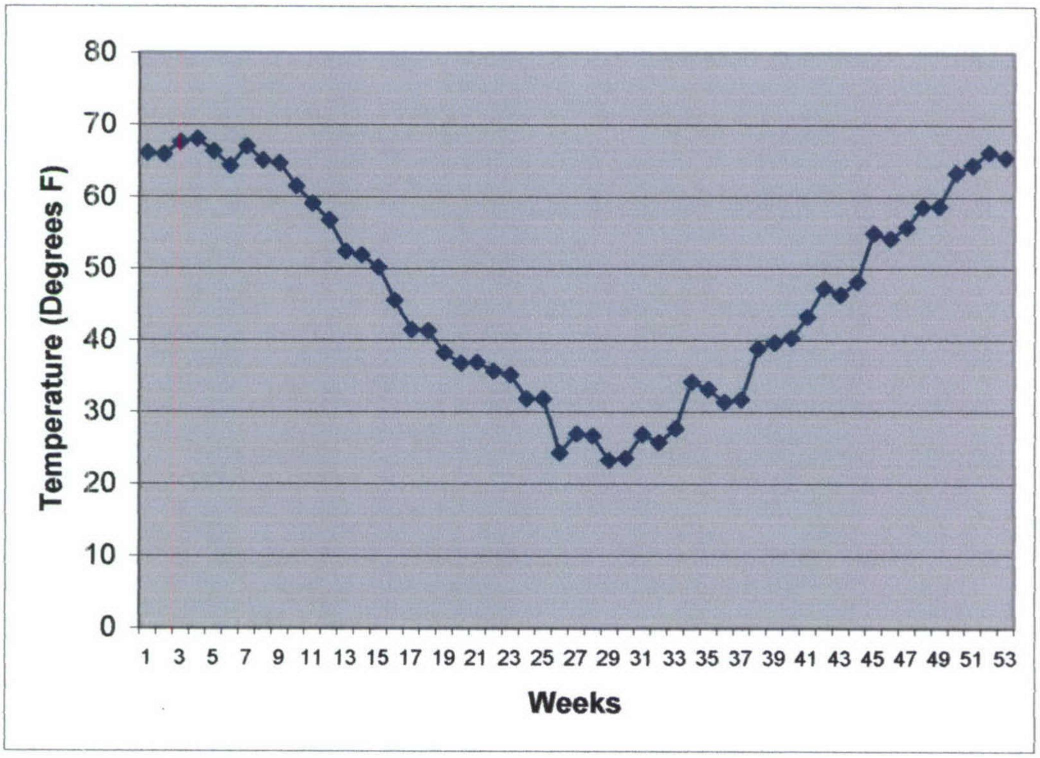


Figure 3.10: Combination of Average Weekly Temperature for 15 Year, High-Prevalence Years, and Baseline-Prevalence Years in Degrees Fahrenheit

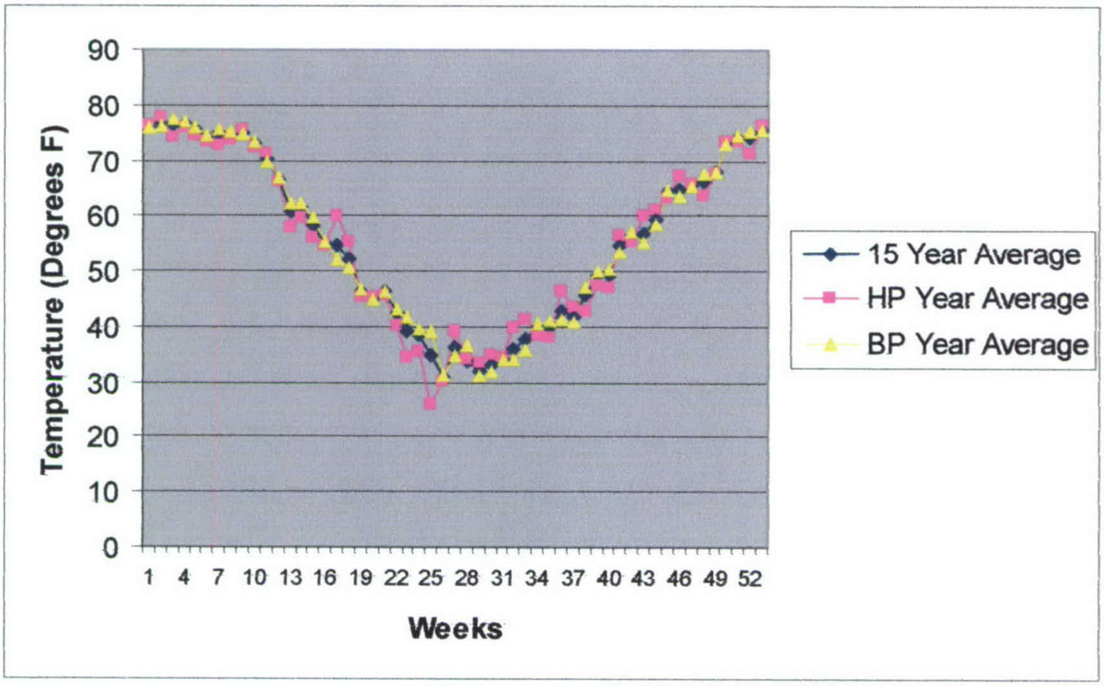


Figure 3.11: Combination of Average Weekly Maximum Temperature for 15 Year, High-Prevalence Years, and Baseline-Prevalence Years in Degrees Fahrenheit

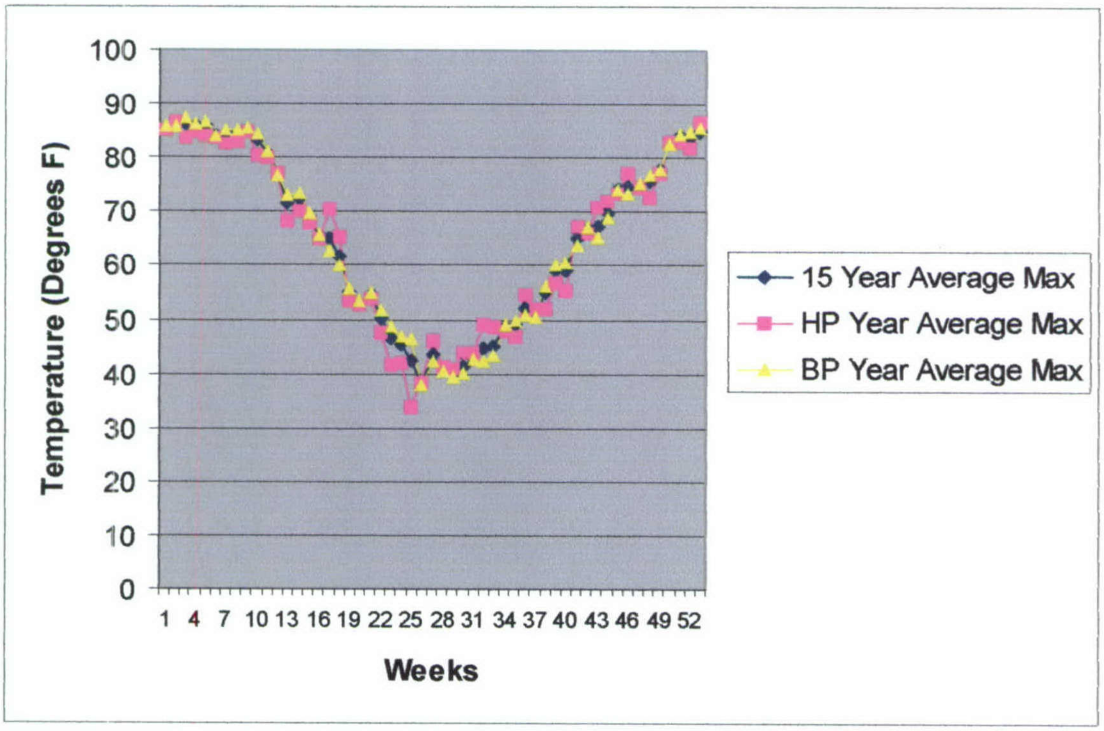


Figure 3.12: Combination of Average Weekly Minimum Temperature for 15 Year, High-Prevalence Years, and Baseline-Prevalence Years in Degrees Fahrenheit

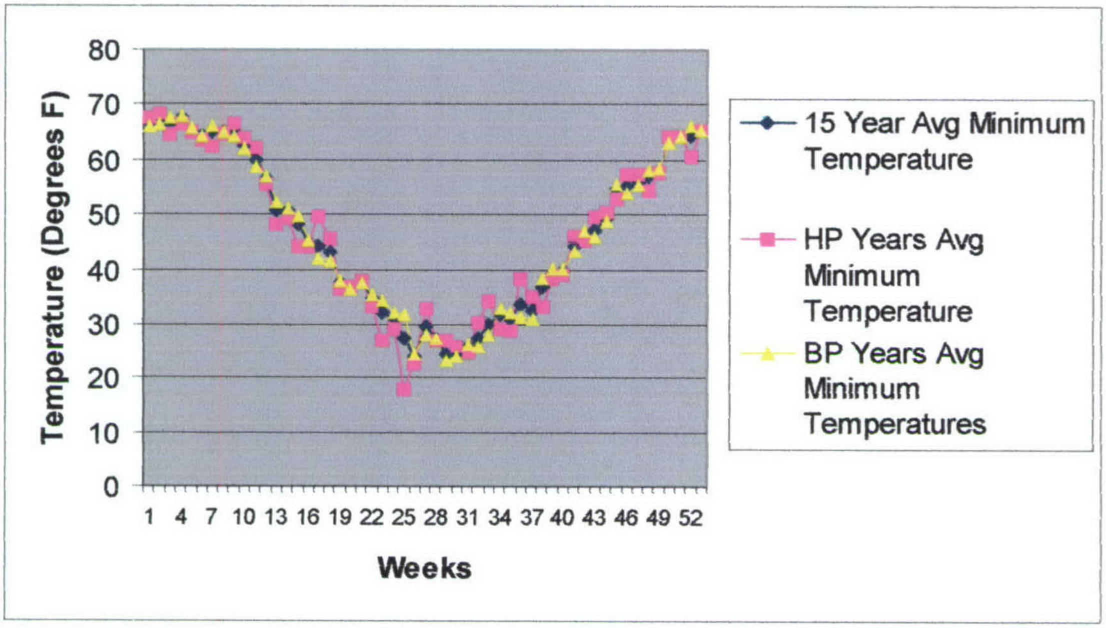
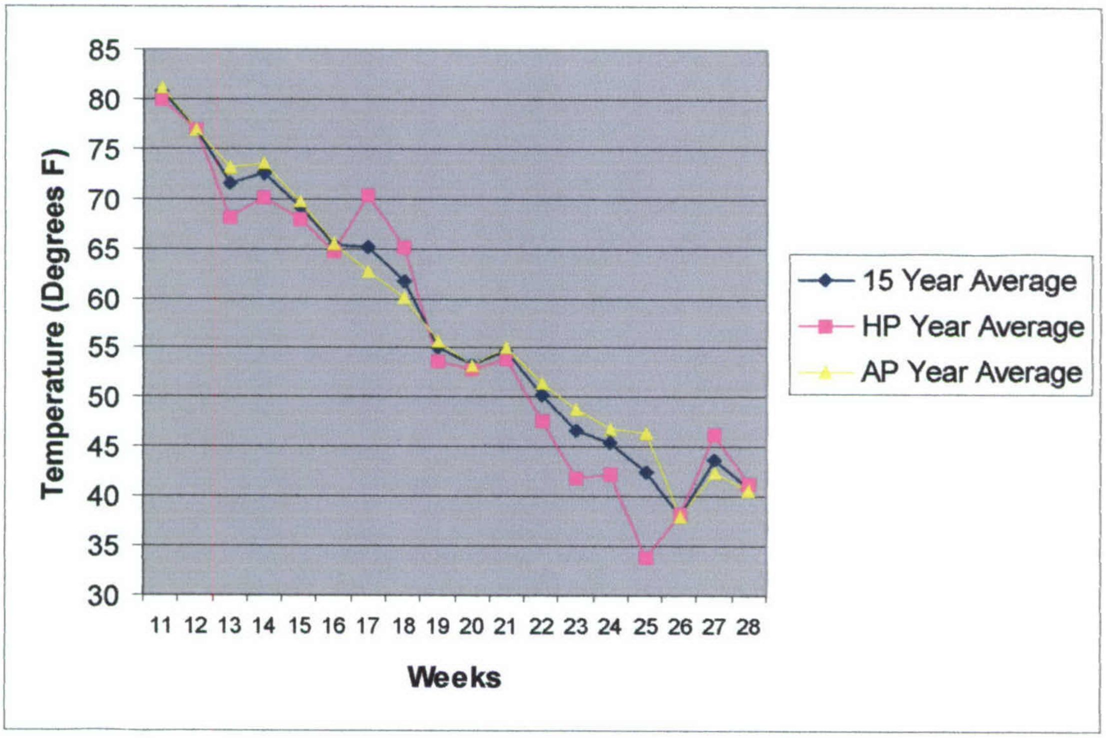


Figure 3.13: Combination of Average Weekly Temperature for 15 Year, High-Prevalence Years, and Baseline-Prevalence Years in Degrees Fahrenheit (Section Enlargement)



The hypothesis for this study is that more leptospira-induced abortions occur in the horse population in the bluegrass region of Kentucky if temperature remains milder than normal and the precipitation is higher than normal. The data collected and archived by the Lexington Airport Weather Station and accessed through the University of Kentucky's Agricultural Weather website contrasts in temperature between periods and trends during periods were analyzed.

The first aspect studied in temperature was the departure from an average weekly temperature for the 15-year period of the study (Figure 3.1). This was accomplished by comparing the average weekly temperature for the high-prevalence years (Figure 3.4) and for the baseline-prevalence years (Figure 3.7) with the 15-year average. The comparison can be seen in Figure 3.10.

Looking at Figure 3.10, there appears to be symmetry in all three lines plotted except for two points. The week of October 21st through October 27th marks an 8 degree Fahrenheit increase in temperature during the high prevalence foaling years compared to the baseline-prevalence foaling years, and a 5 degree Fahrenheit increase in temperature from the 15-year average. December 16th through December 22nd represents the second most noticeable difference in temperature plots. There is a 12.5 degree Fahrenheit decrease in temperature between the high-prevalence foaling years compared to the baseline-prevalence foaling years and an 8.5 degree Fahrenheit decrease in temperature from the 15-year average. Alone these points provide little information, but analysis of the temperatures 6 weeks prior to these specific points shows some subtle trends in temperature. The incubation time for leptospiral abortions in the horse has been estimated to be 6 weeks (Dr. Mike Donahue, LDDC, via personal communication, 2004) and thus, the six-week time frame.

Six weeks prior to the week of October 21st is the week of September 9th. September 9th through September 22nd show little deviation in temperature during the high-prevalence and baseline-prevalence years. The next five weeks, however, do show a difference in the temperature trends between the high-prevalence and baseline-prevalence years. As illustrated in Figure 3.13, the baseline-prevalence years average temperature steadily decreases (73 degrees Fahrenheit to 60 degrees Fahrenheit) through the September 23rd through November 3rd period. The high-prevalence years average remains between 65 degrees Fahrenheit and 70 degrees Fahrenheit during this same period. The temperature curves realign during the week of November 4th; however, November 25th through December 1st starts to show differences once again. The average temperature for the high-prevalence years are cooler than the average temperature for the baseline-prevalence years leading into the week of December 16th, the second point of interest.

Leading up to December 16th there are three weeks of cooler temperatures during the high-prevalence years compared to the baseline-prevalence years. The week of December 16th represents the peak difference in temperature, 12.5 degrees Fahrenheit cooler in the high-prevalence years. The weeks following December 16th represent a return to the 15-year average and little differences can be seen between the average temperatures of the high-prevalence years and the baseline-prevalence years as they both begin to increase into the summer months.

To summarize the findings of temperature differences between the high-prevalence and the baseline-prevalence years, the portion of Figure 3.10 containing the previously discussed trends has been enlarged in Figure 3.13. The temperature average maximums and average minimums plotted in Figures 3.4 through 3.9 are used to illustrate the continuity in the temperatures recorded in the average weekly temperature. For example, the week of October 21st has a higher average temperature during the high prevalence years. The same week shows a higher average maximum temperature (Figure 3.11) and a higher average minimum temperature (Figure 3.12) then the baseline-prevalence years. This indicates that it most likely a trend increase rather than one temperature high or low causing an extreme fluctuation.

Precipitation

The first look at average precipitation was done on a yearly basis (Figure 3.14). The yellow bar represents the 15 year average, the red bars represent the years of high-prevalence, and the blue bars represent the baseline-prevalence years. Since yearly precipitation did not appear to show any trends in amount of precipitation versus incidents of leptospiral abortions, the precipitation totals were also done on a weekly basis (Figures 3.15 through 3.18). Additionally, Figure 3.19 shows an enlargement of the precipitation totals for weeks 3 through 18. The weeks included in this figure have shown to have different temperature trends in the high-prevalence years and baseline prevalence years, and a closer look shows any differences in trends in precipitation totals.

Figure 3.14: Average Yearly Precipitation in Inches for Foaling Years 1990 – 2004 and a 15 Year Average Precipitation for the Same Foaling Years

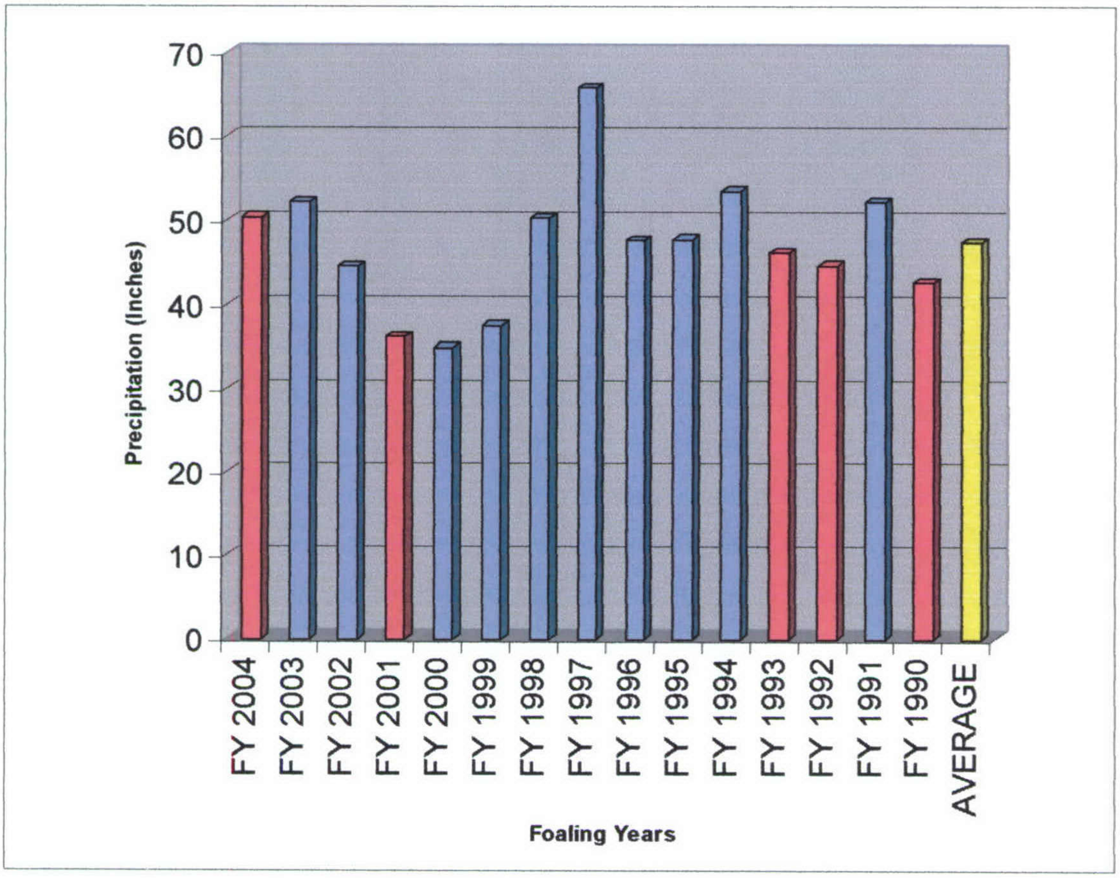


Figure 3.15: Average Weekly Precipitation in Inches for Foaling Years 1990 – 2004

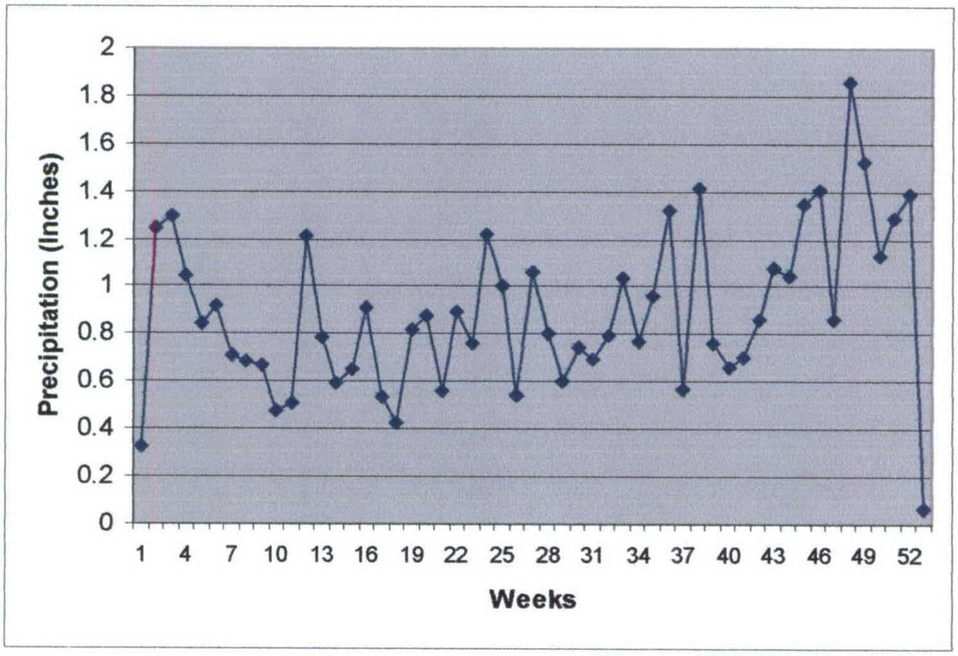


Figure 3.16: Average Weekly Precipitation in Inches for High-Prevalence Foaling Years 1990, 1992, 1993, 2001, and 2004

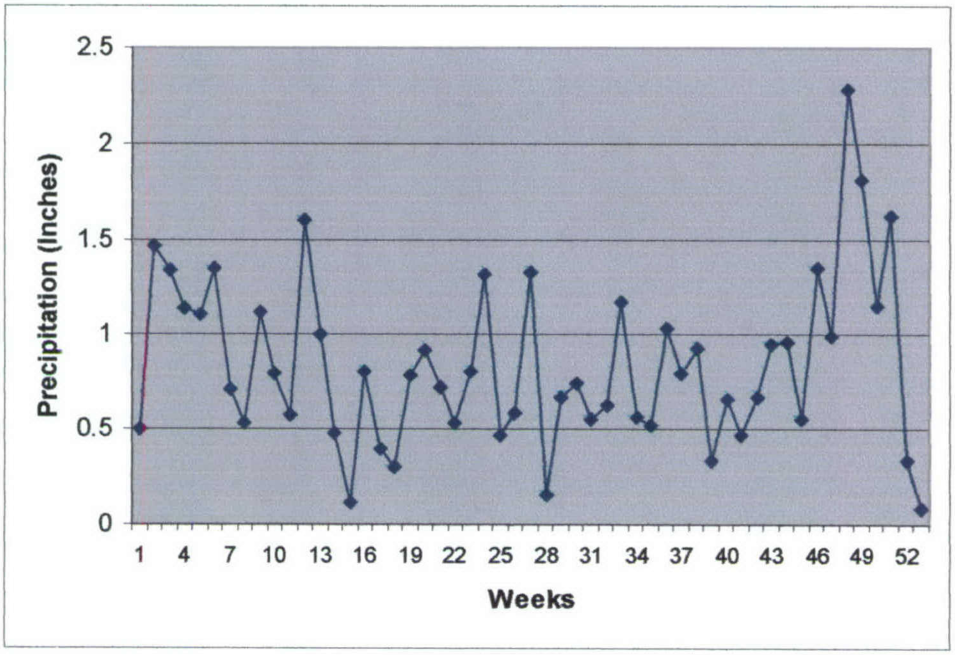


Figure 3.17: Average Weekly Precipitation in Inches for Baseline-Prevalence Foaling Years 1991, 1994 -2000, 2002, and 2003

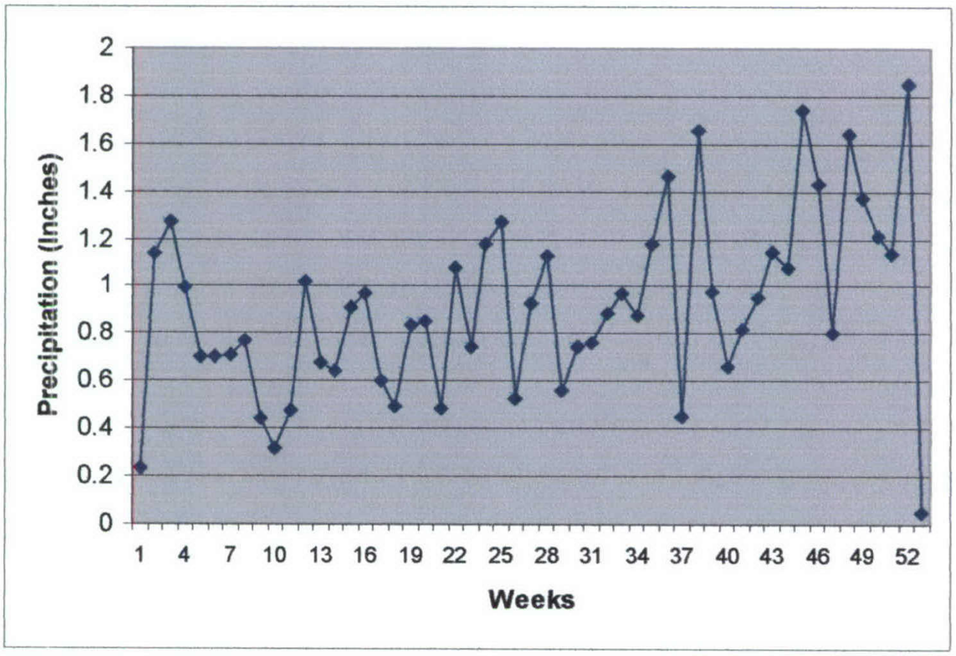


Figure 3.18: Comparison of Weekly Precipitation Totals for 15-Year Average, High-Prevalence Year Average, and Baseline-Prevalence Year Average

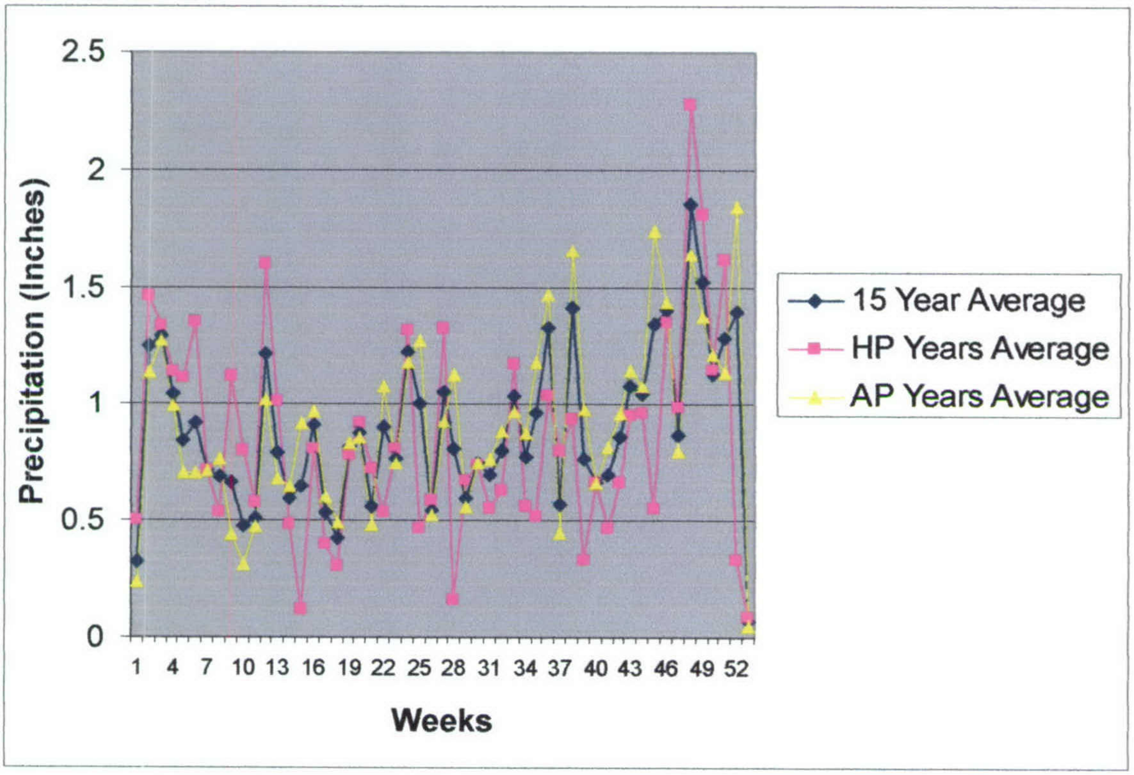


Figure 3.19: Comparison of Weekly Precipitation Totals for 15-Year Average, High-Prevalence Year Average, and Baseline-Prevalence Year Average (Section Enlargement)

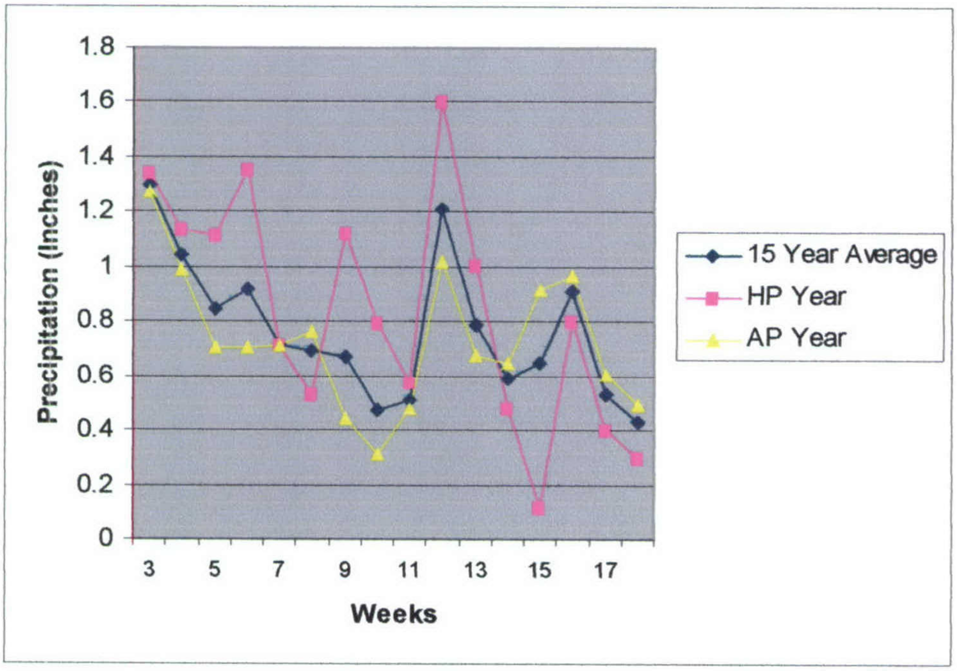


Figure 3.14 illustrates the yearly totals of precipitation in inches for the bluegrass region of Kentucky. Red bars indicate the high-prevalence years, the baseline-prevalence years by blue bars, and the lone yellow bar represents the fifteen-year average precipitation. Recalling that the hypothesis asserts higher than average precipitation as a contributing factor to the high prevalence years of leptospira-induced abortions, it is not evident in this chart. Four of the five high prevalence years have annual precipitation totals less than the 15-year average, with 2004 being the only year having a higher total precipitation. There is also no discernable difference between the precipitation totals of the high-prevalence years and the baseline-prevalence years. Both sets of years exhibit random totals of annual precipitation. These results required breaking down the totals of precipitation into the same weeks used to determine temperature trends.

Figure 3.18 compares the weekly precipitation totals for the high-prevalence years, baseline-prevalence years, and the 15-year average. As this chart makes evident, the precipitation totals vary from week to week, regardless of season or time of year. Like the temperature comparison, however, there are some notable points of interest. Figure 3.19 extracts the area of the chart pictured in Figure 3.18 found to be most intriguing.

Figure 3.19 depicts a vast difference in precipitation totals at several points. Before interpreting these points, however, it is important to identify any extreme total occurring in a single year, which could offset the average. Extreme precipitation totals were analyzed for both individual high-prevalence years and individual baseline-prevalence years. The examination of the extreme values in precipitation totals in relation to Figure 3.19 does not prevent the analysis of this figure. The extreme values, when included, only prevent the differences between the high-prevalence year precipitation totals and the baseline-prevalence year precipitation totals from being greater. Therefore it is possible to make the following interpretation of Figure 3.19.

The high-prevalence years' precipitation totals are marked by a week of significant precipitation followed by two or more weeks of decreasing precipitation. For example, the week of August 5th has a recorded total of 1.352 inches of precipitation. The following two weeks have totals of 0.714 inches and 0.534 inches. The next week, August 26th through September 1st, has a marked increase in average precipitation at 1.12 inches.

The baseline-prevalence years through this same weekly time period, August 5th through September 1st, have the weekly average precipitation totals of 0.705, 0.715, 0.768, and 0.441 inches. Therefore the same pattern of heavy precipitation followed by light precipitation is not as evident during the baseline-prevalence years.

Temperature and Precipitation

It is likely that if the prevalence of leptospira-induced abortions is influenced by temperature and precipitation and that the two act in tandem to cause the effect. As mentioned earlier, the most likely cause of death of a leptospire in the natural environment is desiccation. Therefore, it could be assumed that high heat and lack of water would shorten the lifespan of this spirochete. Conversely, it could be assumed that mild temperatures and an adequate supply of water could prolong the life of the organism. Based upon the information collected for temperature and precipitation in this study the following conclusion can be made.

In the months leading up and into October during the years of high prevalence, milder temperatures are seen, followed by a spike in temperature during the last two weeks of October (Figure 3.13). This spike is not particularly an increase in temperature, however, it is a prolonging of temperatures in the 65 to 70 degree Fahrenheit average range for a couple of weeks. Simultaneously, the weekly precipitation is following a cycle of heavy precipitation (greater than 1.0 inches) followed by two or more weeks of light precipitation (Figure 3.19). Meanwhile during the baseline-prevalence years the average weekly temperature has been steadily decreasing since early September (nearly 2.5 degrees Fahrenheit a week) and average weekly precipitation totals have been 1.0 inch or less since July (Figure 3.19).

Discussion

It is likely that the extension of the mild temperatures prolong the warmth of the soil containing the leptospires. The limestone in the bluegrass region soil helps to maintain a relatively neutral pH (Dr. Dennis Hancock, University of Kentucky College of Agriculture, via personal communication, 2004). Meanwhile the heavy precipitation moistens the soil and in some area could cause standing water, which would bring the leptospires to the surface for ingestion. These conditions could cause the earlier onset of the cases of leptospira-induced abortion and the increase in prevalence. If more organisms are surviving in the environment, more horses are likely to be exposed to leptospires.

The drop in temperatures during the month of December in high-prevalence years to temperatures below the baseline-prevalence years, coinciding with a close return to the

15-year average for precipitation during this time, could explain why the numbers of abortions do not continue throughout the foaling year. The leptospires are no longer able to survive as long in the environment.

Furthermore, the activity of reservoir species for leptospirosis might be related to the temperature and precipitation as well as the leptospires themselves. Prolonging warmer temperatures might increase the amount of time a reservoir species spends in horse populated areas. This increased amount of time could increase the chance of an area becoming contaminated with leptospires through the animal's urine. Additional studies would have to be done to determine the exact reservoir species and the effects of weather on those species.

Chapter Four: Near Real-Time Analysis of Foaling Year 2004

Introduction

The 2004 foaling year was a foaling year of high prevalence. There were 36 cases of leptospira-induced abortion diagnosed by the LDDC in all breeds of horses located in the bluegrass region of Kentucky. Thoroughbreds were the confirmed breed of horse in 24 of the cases. The prevalence rates established in Chapter Two for the 2004 foaling year were .0017 (Thoroughbreds only) and .0026 (all breeds).

Because the 2004 year was a high-prevalence year, further investigative strategies were employed. First, a questionnaire was designed and delivered to those farms participating in the study. While questionnaires can be confounded by recall bias, this questionnaire was delivered during the same year that the abortion occurred thus limiting the need for long-term recall. Also, most of the farms examined in this study were major commercial operations that painstakingly tracked the horses on the farm and surveyed the land regularly.

In addition to the questionnaire, farm visits were accomplished to those farms participating in the study. For the first time in an investigative study into leptospira-induced abortions in equines, a global positioning system (GPS) receiver was used to mark the pasture that the mare was in 6 weeks prior to her abortion by latitude and longitude. The coordinates were than uploaded into geographical information system (GIS) software to analyze patterns of occurrence and the potential for leptospire transmission through water. Major waterways were examined as well as watershed data. Watershed data indicates the natural flow of water over an area of land as precipitation falls onto that area or when flooding occurs.

Materials and Methods

Questionnaire

A leptospirosis questionnaire (Appendix B) was created using similar techniques applied when the University of Kentucky Gluck Equine Research Center created a questionnaire concerning Mare Reproductive Loss Syndrome (MRLS) in the 2001 foaling year (http://www.uky.edu/Agriculture/VetScience/mrls/2001/questionnaire.htm). The MRLS questionnaire was quite long (11 pages) because the cause of syndrome was unknown. Because previous research has been accomplished on leptospirosis a more concise questionnaire was created (3 pages). The conciseness of the questionnaire was designed for maximum cooperation from the farm managers in this study.

The farms in the bluegrass region of Kentucky that had an incident of leptospira-induced abortion during the 2004 foaling year confirmed by the LDDC were contacted and asked to participate in the study. The farms were contacted through a systematic process. First, the farm veterinarian was reached via telephone and permission was requested to include his/her client's farm in the study. If the veterinarian found the study acceptable, the farm manager was then contacted. If the farm manager agreed to participate in the study, a copy of the questionnaire was mailed or faxed to the farm manager and/or the farm veterinarian. The questionnaires were collected by mail or by facsimile. In some cases the questionnaires were collected when the farms were visited in the summer of 2004.

Farm Visits

Of the 25 farms with leptospira-induced abortions detected by the LDDC for FY 2004, 22 farms were located in the bluegrass region. Visits to each farm agreeing to participate in the study out of the 22 located in the bluegrass region were conducted during the summer of 2004 (June through August). This time period was selected for the visits because it was the least intrusive into the farms' daily schedules and there were only two major Thoroughbred sales events during this period (occurs in early August). The primary breeding season was coming to an end or over in most cases and sales preparation of Thoroughbred yearlings was in progress.

The farm visit provided the opportunity to review the questionnaire with the farm manager, to gather further information, and to tour the farm in order to view and record an accurate depiction of the layout. The farm layout was analyzed in terms of water flow, wooded areas, and areas of standing water. These environmental factors were being investigated as potential factors increasing the risk of exposure to leptospires by horses.

The most important aspect of the farm visitation was the use of a Global Positioning System (GPS) to determine the exact latitude and longitude of the field that the mare was in 6 weeks prior to her abortion. If the mare was in several fields during this 6-week period, a reading was taken in each field. However, there was only one farm that had multiple pastures (2) for the same mare.

Global Positioning System (GPS) and Geographical Information Systems (GIS)

During the visits conducted to each farm a Global Positioning System (GPS) receiver (Garmin International Inc, Olathe, Kansas, model Rino 110) was employed. The pasture(s) that the mare was/were located in six weeks prior to the abortion were established and the farm manager determined the approximate center of the pasture. The GPS receiver was taken to this location and the latitude and longitude was noted. This process was completed on each participating farm.

Following the compilation of geographical points for the pastures, the GPS receiver was taken to the University of Kentucky College of Agriculture where the data collected was uploaded into Geographical Information Software (GIS) (ESRI, Redlands, California, ARC GIS Version 8.1 and Garmin International Inc, Olathe, Kansas, GPS Utility Version 4.10.5) by Dr. Dennis Hancock, an extension associate for precision agriculture at the University of Kentucky College of Agriculture.

The software takes the geographic points collected by the GPS unit and applies them to accurate geographical representations of the state of Kentucky. The projection map that was used in this study was a representation available on the internal computer services at the University of Kentucky College of Agriculture and is titled "Kentucky State Plane North". This projection allows the geographical points collected at each farm to be accurately depicted on a map of the bluegrass region of Kentucky that includes outlines of the counties involved.

In six cases the GPS receiver malfunctioned and the address of the farm was used to create a geographical point on the map. The geographical point was double-checked and moved to the correct pasture, when possible, using satellite photos from www.terraserver.com and comparing those satellite photos to the maps drawn at the time of the farm visit.

One of the primary objectives of the GPS process was to determine the proximity of water to the pastures where the mares experiencing leptospira-induced abortions were located. Using the "Kentucky State Plane North" representation of the points created previously and applying overlays of hydrography data obtained from the Kentucky Office of Geospatial Information (University of Kentucky College of Agriculture's Access) the major and minor waterways, and the watershed data could be analyzed in relation to pasture location.

Confidentiality Agreements

This investigation relied heavily on data collected from the participating farms in the form of surveys, visits, and the use of a global positioning system (GPS). Confidentiality agreements (Appendix C) were distributed to farms participating in this study. This agreement states that the data collected will be used only for scientific research and is exempt from disclosure pursuant to KRS 61.878 (1)(b).

Results

Questionnaire

The questionnaire (Appendix B) was created and disseminated to 22 farms in the bluegrass region of Kentucky that experienced a leptospira-induced abortion during the 2004 foaling year. The equine cases confirmed by the LDDC were included in the survey. Twenty-two farms met the eligibility to complete the survey, all farm veterinarians agreed to the study, and of those 22 farms, 16 (73%) returned the completed survey to the researcher. Farm visits were completed on 14 of the 16 farms that returned the survey, with two farm managers that never returned the survey despite numerous reminders. Therefore farm visits were conducted on approximately 73% of the farms eligible, however, two of the farms are different than those in the 73% that returned the questionnaire. Every attempt was made to contact farms that did not return the survey nor allow for farm visits. The omission of these farms from this study are the result of unreturned phone calls, chronic rescheduling of appointments, and the inability to make contact with the farms.

The average number of mares that aborted on each farm returning the questionnaire was 1.5 with a median of 1.0, and a range of 1 to 3. Mare's urine was tested for *Leptospira* shedding on 4 of the 16 (25%) farms following a leptospirosis diagnosis. No information was provided concerning when the testing was conducted nor the length of time the shedding of the spirochete persisted.

Analysis of the questionnaire indicated that 20 pastures were involved on the 16 different farms and 50% of these pastures contained some form of water. Four pastures were identified as having areas of standing water only; three pastures had streams flowing through them; one pasture had a small pond within its perimeter; and one pasture had a stream, a small pond, and areas of standing water on it. Additionally, one pasture had a wooded area within the fence perimeter; eight pastures had wooded areas outside of the fencing and in close proximity; while 2 pastures had wooded areas within and in close proximity outside of the fencing perimeter. These responses were confirmed, when able, during farm visitations. Six farms had more than one incident of leptospira-induced abortion on the farm. Three of the multi-abortion farms kept the mares in separate fields during the 6-week incubation period previously established and three farms kept multiple leptospira-induced abortion having mares in same field 6 weeks prior to the abortion.

Rodent problems in the stables were indicated by only one farm (6%), while rodent problems in the feed area and the stables were indicated by two farms (12.5%). One farm manager replied that other horses on the farm did test positive for leptospirosis, but no forms indicated leptospirosis in other species on the farm. Fourteen of the 16 farms (87.5%) treated the mare with medication as a response to a leptospirosis diagnosis either prior to or after the abortion. Oxytetracycline was used as the medication on 3 of the farms (21.43%), tetracycline was used as the medication on 5 of the farms (35.71%), and the other 6 farms (42.86%) did not specify the medication administered. Fourteen of the farms (63.64%) isolated the mare after leptospirosis diagnosis. The median time of isolation was 4 weeks with a range of 1 week to 12 weeks. Eight of the farms used preventative measures on the other horses on the farm to protect them leptospirosis: seven of the farms administered medication to the other horses and one farm administered medication and tested the water sources.

Of the 23 mares on the 16 farms, 22 were bred in the 2005 foaling year; 21 of the 22 (95%) mares were pregnant as of the questionnaire return in August 2004. None of the farms had tested foals for *Leptospira* shedding in their urine if born to a mare that tested positive for leptospirosis in previous years. None of the farms administered the cattle vaccine as a preventative measure for *Leptospira* infection.

Among the farms that returned the survey, the presence of wildlife was determined by using the replies "sometimes" (S), "often"(O), and "never" (N). Table 4.1 indicates the presence of wildlife on the farms as indicated by the farm managers. The farm managers, or those completing the survey, made additional comments that supported the results seen in Table 4.1.

Table 4.1: Presence of Wildlife on 16 Central Kentucky Farms as Reported by Farm Managers in the 2004 Foaling Year

Mice and Rats	O=2, S=8, N=6
Raccoons	O=7, S=6, N=2
Coyotes	O=2, S=11, N=3
Deer and Elk	O=1, S=7, N=8
Waterfowl	O=8, S=6, N=2
Bats	O=2, S=6, N=8
Opossums	O=2, S=12, N=2
Skunks	O=4, S=10, N=2
Foxes	O=2, S=11, N=3

Global Positioning System (GPS) and Geographical Information System (GIS)

The first, and most important, thing that a Global Positioning System (GPS) receiver does for the researcher is help to pinpoint where the cases occurred. The coordinates were than uploaded from the GPS receiver into the Geographical Information System (GIS) software. The GIS software created a map of the bluegrass region of Kentucky with the latitude and longitude of each case marked by a green dot on the map. The green dots become larger as the incident occurs later in the gestational period for the foaling year of 2004. The map is pictured in Figure 4.1.

Analysis of pasture in relation to waterways was also mapped. Figure 4.2 shows the GIS representation of the locations of the incidents as they occurred through time in their proximity to the major waterways in the bluegrass region of Kentucky.

Figure 4.1: Geographical Information System (GIS) Map Indicating Locations of Leptospira-Induced Equine Abortions Occurring in the Bluegrass Region of Kentucky During the Gestational Period for the 2004 Foaling Year (7/1/2003 through 6/30/2004).

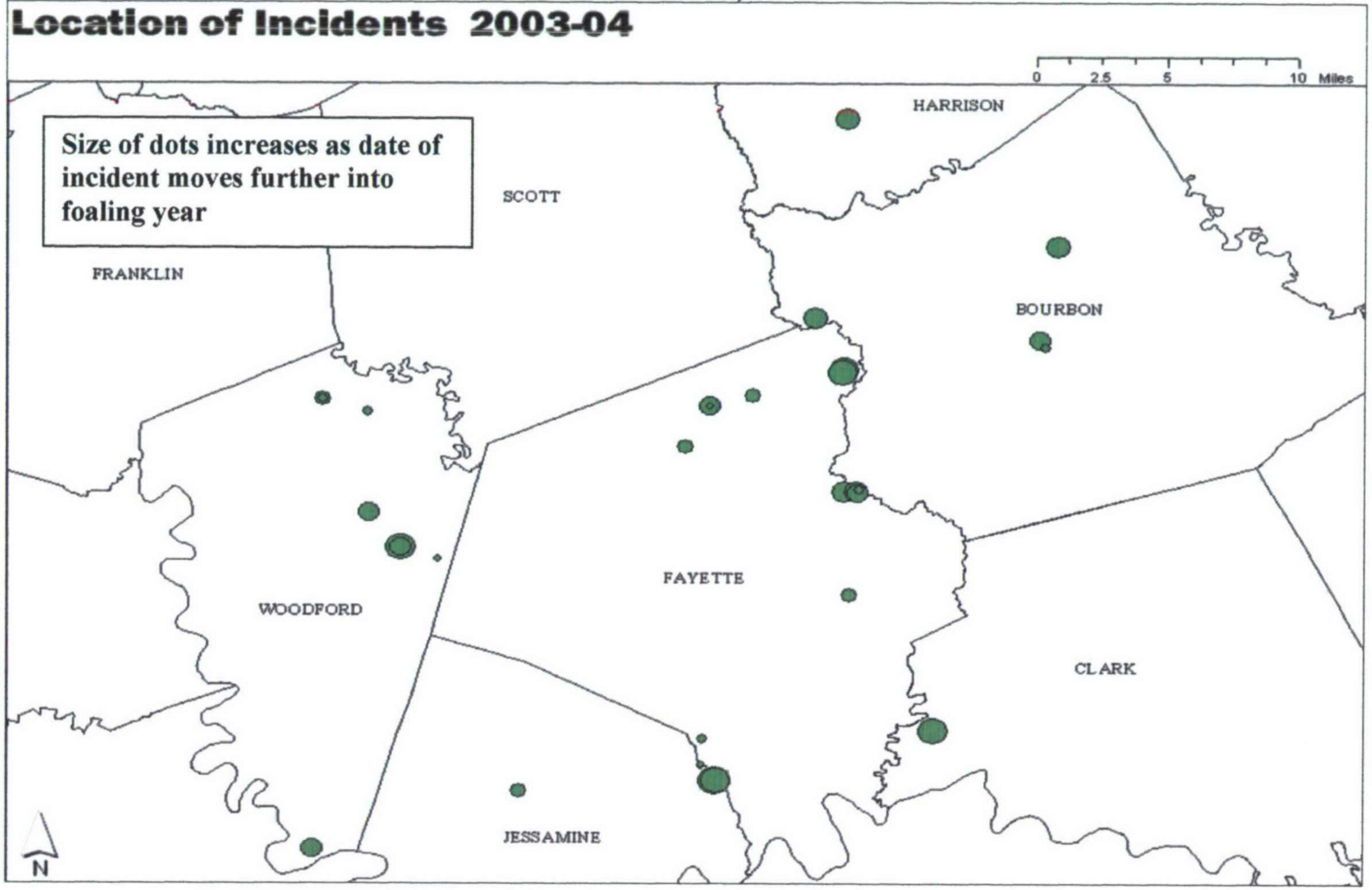


Figure 4.2: Geographical Information System (GIS) Map Indicating Locations of Leptospira-Induced Equine Abortions Occurring in the Bluegrass Region of Kentucky During the Gestational Period for the 2004 Foaling Year in Relation to Major Waterways.

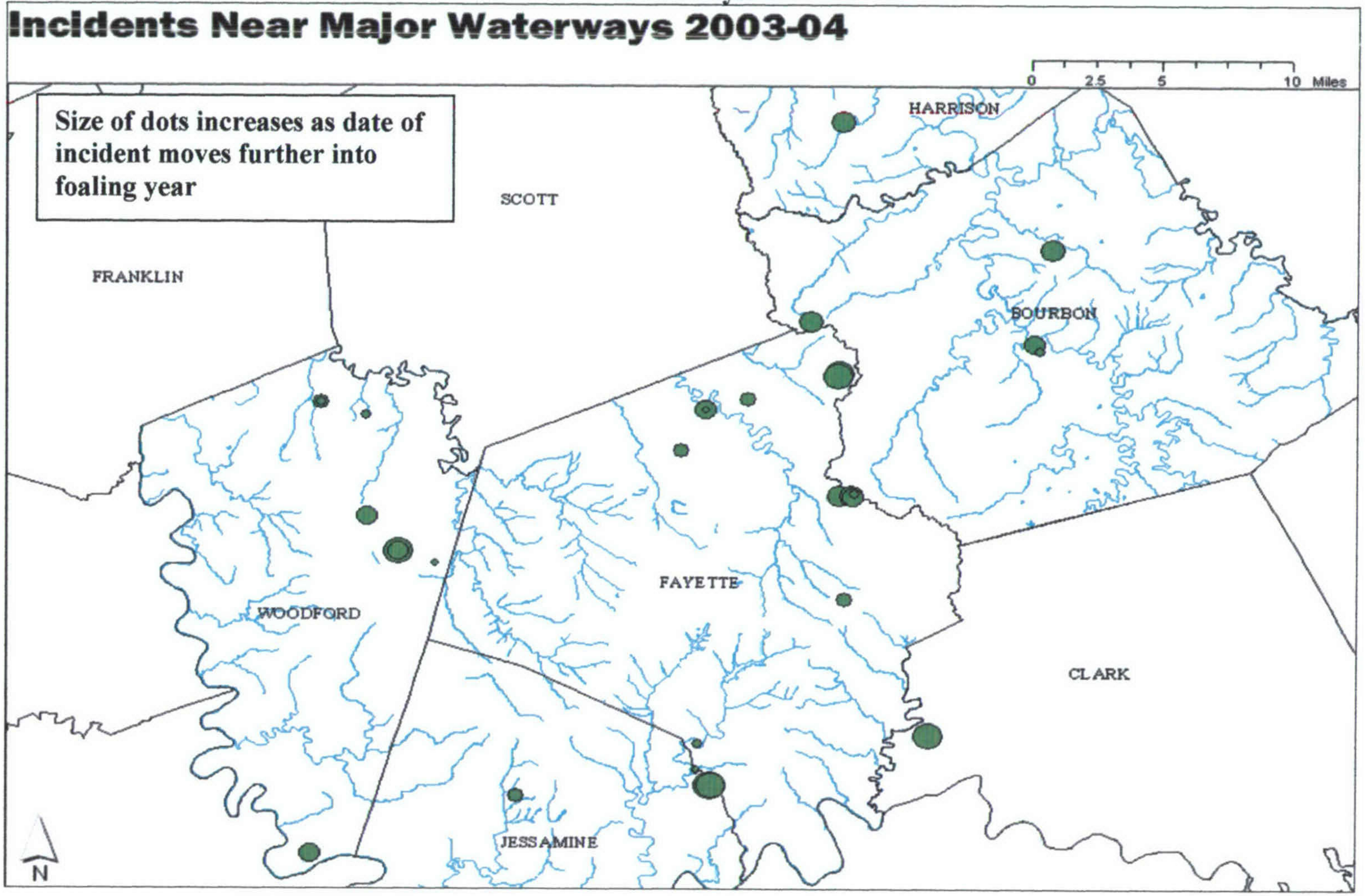


Figure 4.3: Geographical Information System (GIS) Map Indicating Locations of Leptospira-Induced Abortions Occurring in the Bluegrass Region of Kentucky During the Gestational Period for the 2004 Foaling Year in Relation to Major Waterways and Minor Watershed Areas in the Region.

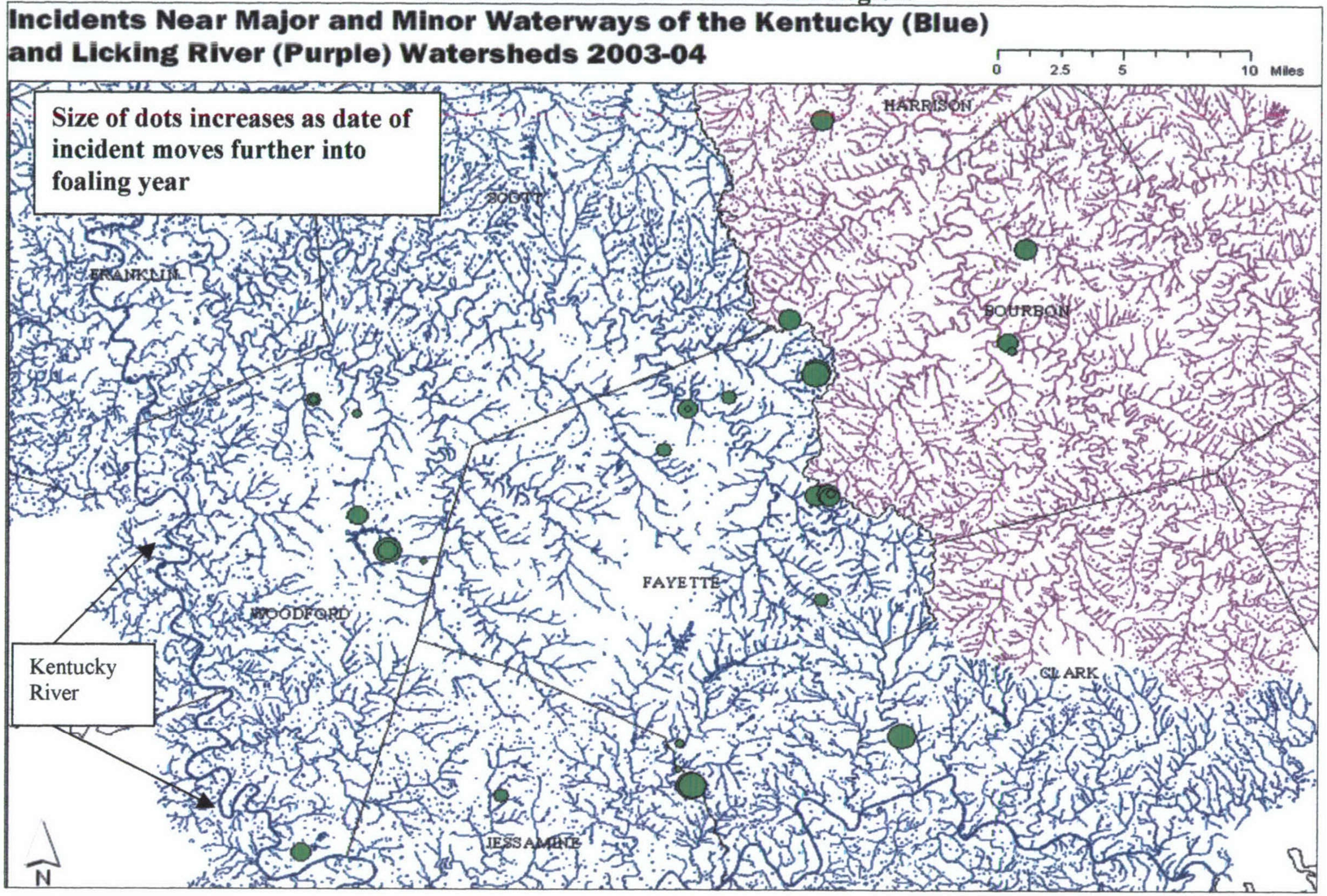


Figure 4.3 adds the watershed data for the bluegrass region of Kentucky to the major waterway data and the location of the incidents through time. Watershed data indicates the flow, or run-off, of water toward major waterways following rainfall. The Kentucky watershed data, in blue, moves toward the Kentucky River (southwest). The Licking River watershed data, in purple, flows westward toward the Licking River. Therefore the boundary line where the two watershed areas meet can be seen as a separation point for direction of watershed. Because the representation of watershed data is detailed, the map has been separated into six sections and enlarged (Figures 4.4 through 4.8).

Figure 4.4: Geographical Information System (GIS) Map Indicating Locations of Leptospira-Induced Abortions Occurring in the Bluegrass Region of Kentucky During the Gestational Period for the 2004 Foaling Year in Relation to Major Waterways and Minor Watershed Areas in the Region – Southern Fayette County.

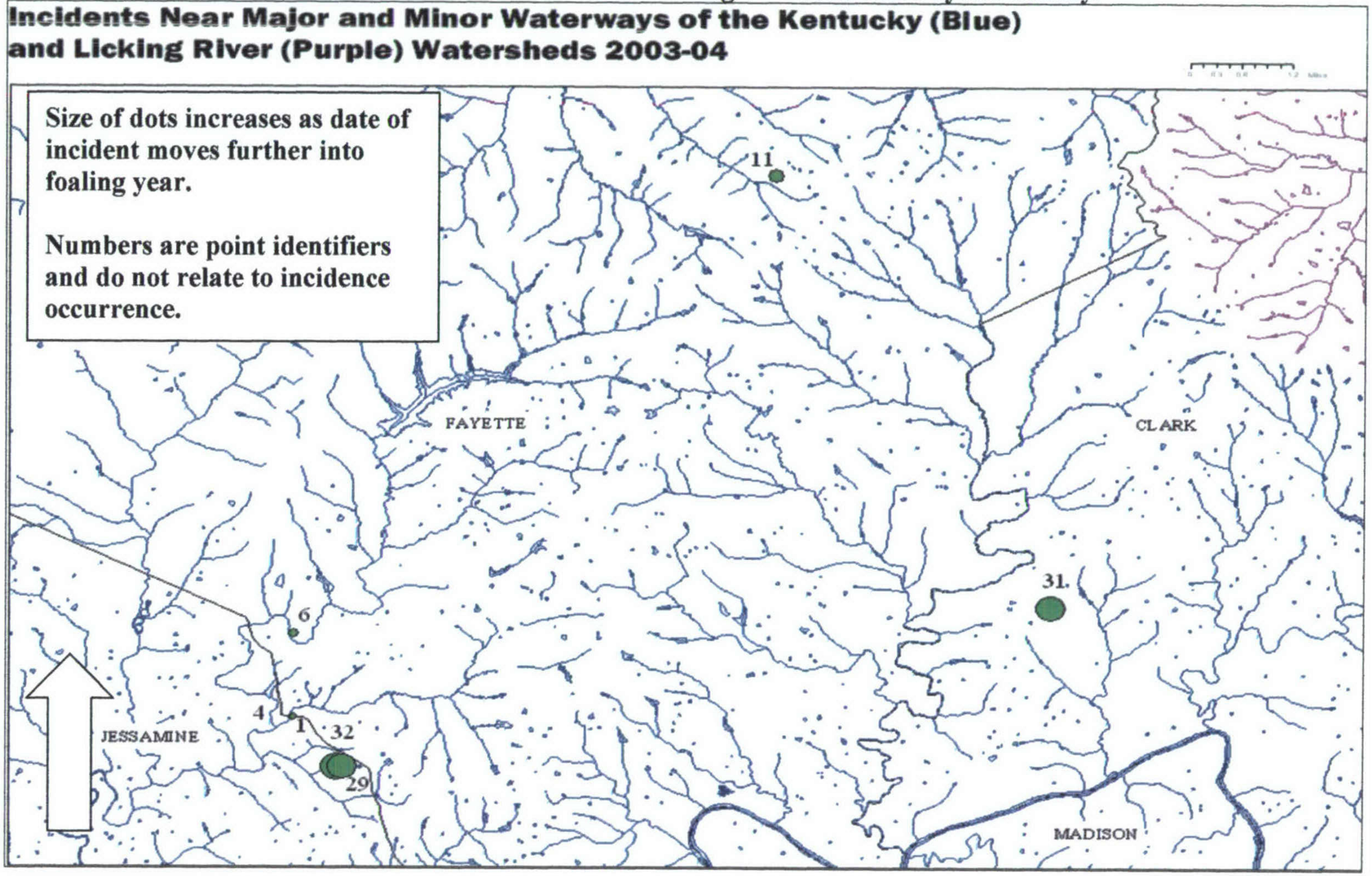


Figure 4.5: Geographical Information System (GIS) Map Indicating Locations of Leptospira-Induced Abortions Occurring in the Bluegrass Region of Kentucky During the Gestational Period for the 2004 Foaling Year in Relation to Major Waterways and Minor Watershed Areas in the Region – Northern Woodford County.

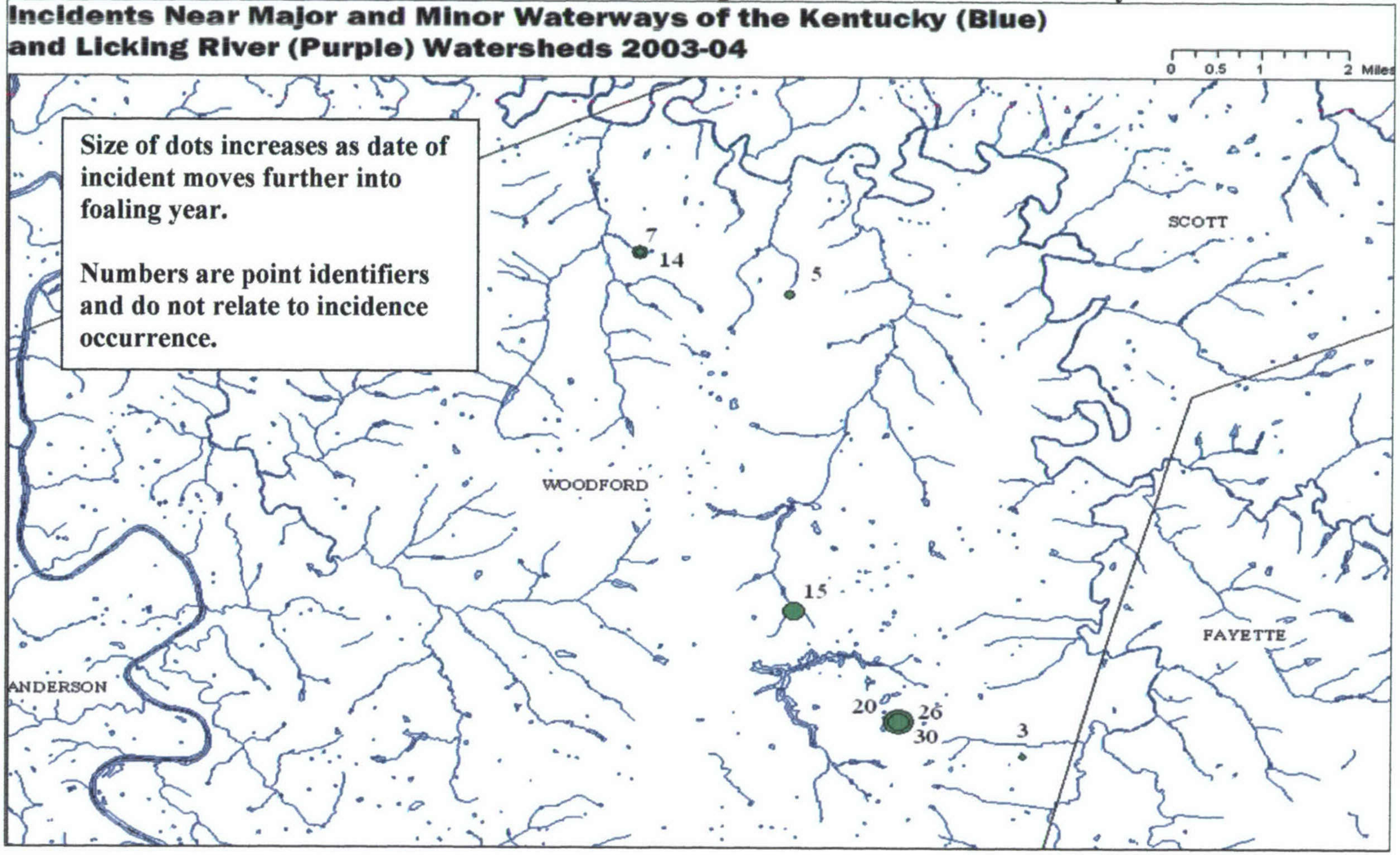


Figure 4.6: Geographical Information System (GIS) Map Indicating Locations of Leptospira-Induced Abortions Occurring in the Bluegrass Region of Kentucky During the Gestational Period for the 2004 Foaling Year in Relation to Major Waterways and Minor Watershed Areas in the Region –Northern Fayette County.

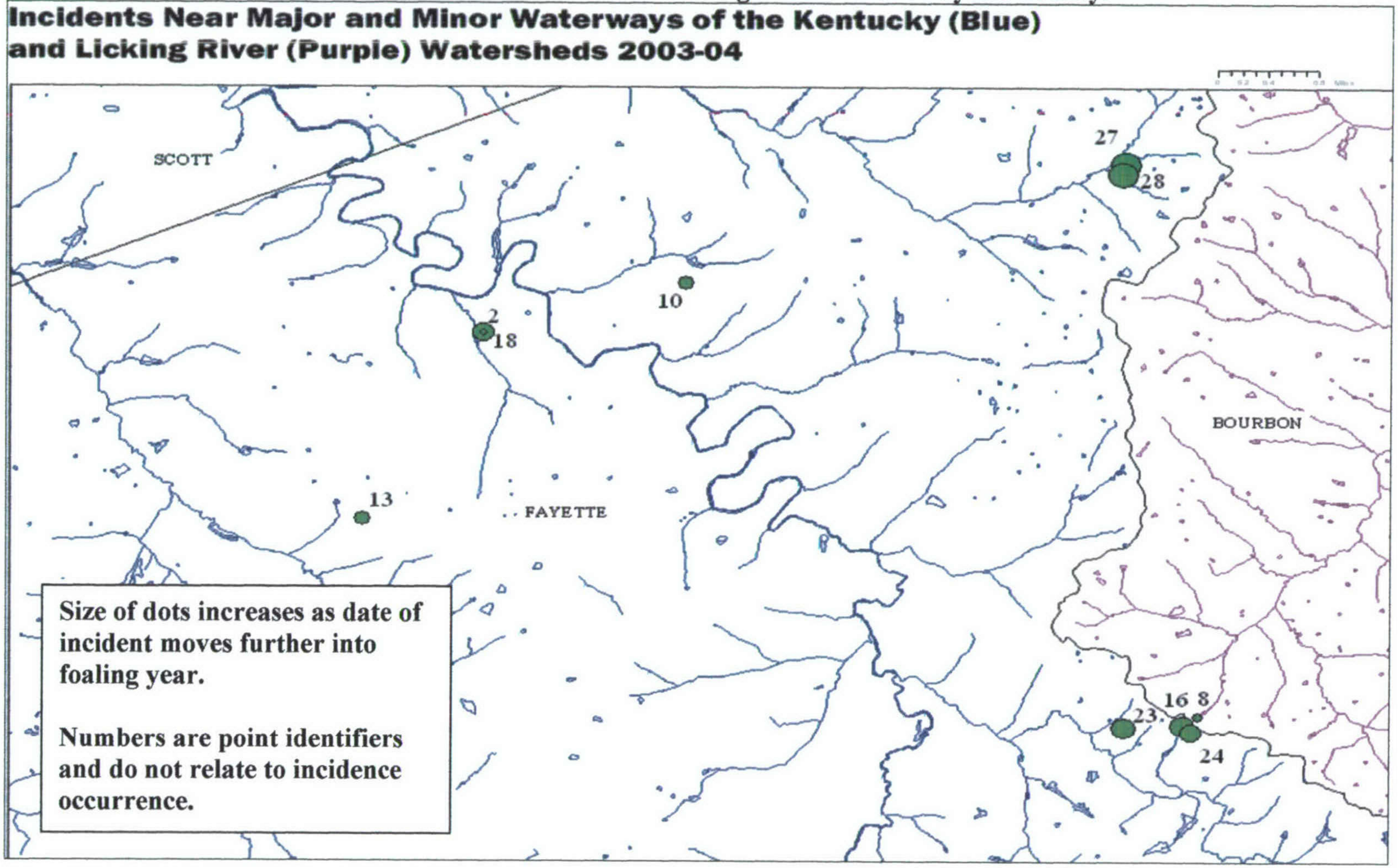


Figure 4.7: Geographical Information System (GIS) Map Indicating Locations of Leptospira-Induced Abortions Occurring in the Bluegrass Region of Kentucky During the Gestational Period for the 2004 Foaling Year in Relation to Major Waterways and Minor Watershed Areas in the Region – Southern Woodford and Jessamine Counties.

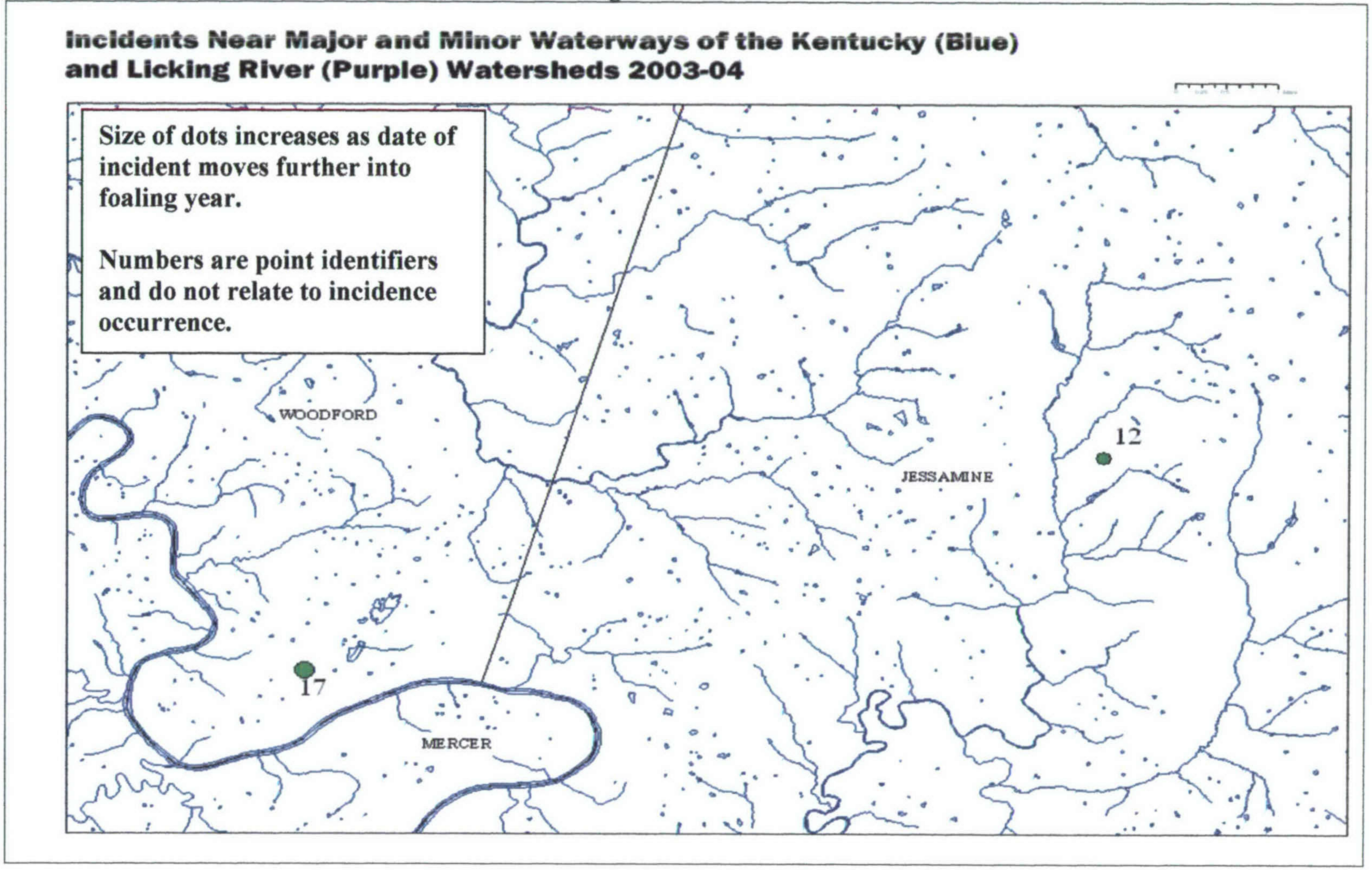
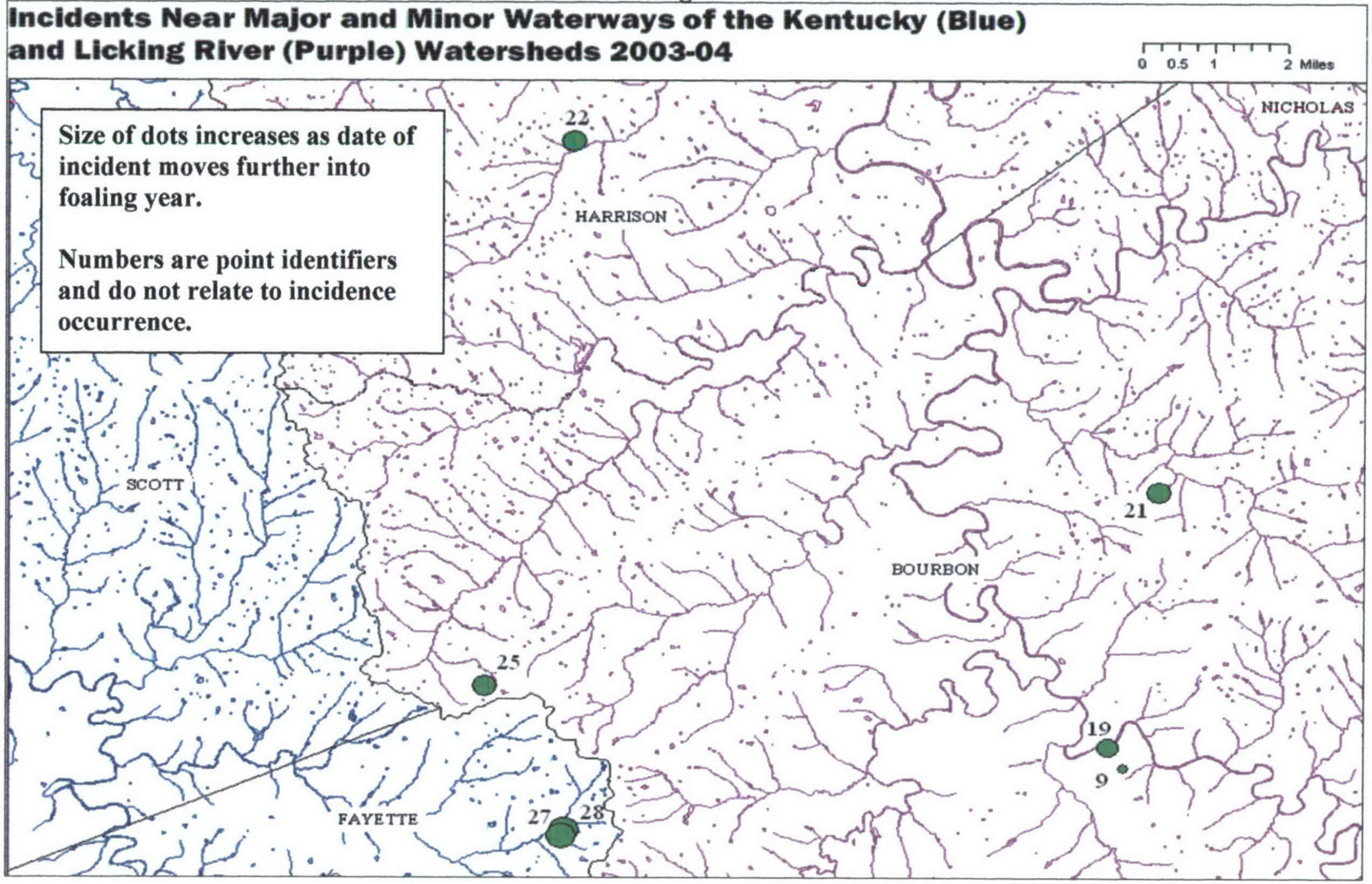


Figure 4.8: Geographical Information System (GIS) Map Indicating Locations of Leptospira-Induced Abortions Occurring in the Bluegrass Region of Kentucky During the Gestational Period for the 2004 Foaling Year in Relation to Major Waterways and Minor Watershed Areas in the Region – Harrison and Bourbon Counties.



Discussion

One primary hypothesis for this study was that milder temperatures and periods of increased precipitation contribute to higher prevalence of a leptospira-induced abortion in the bluegrass region of Kentucky. While the majority of the study was done retrospectively, the high occurrence of the abortions during the 2004 foaling year allowed for a more real-time look at the pasture condition and environment.

There was a high percentage of cooperation among farm veterinarians (100%) and farm managers (82%) concerning this study. This cooperation allowed for more accurate assessments to be made concerning the farm environment because of the questionnaire return. Furthermore, without access to the pastures on the farms, the GPS part of the investigation would not have been possible.

One of the most important questions on the survey was the identification of water sources located on the pastures where mares were kept six weeks prior to the abortion. As noted on questionnaires and personal visits, only 10 (50%) of the pastures were identified as having a source of water within the fencing. Interestingly, 4 of these 10 pastures were identified as only having standing water after a period of heavy precipitation. This means that out of 20 pastures only 6 (30%) had a daily environmental water source. This would suggest that, although a water source could contribute to the spread of leptospirosis, it is certainly not the only method of transmission that occurred during the 2004 foaling year.

Another response that was of great interest was the breeding status of the mare for the 2005 foaling year. Mares that aborted during the 2004 foaling year because of a leptospiral infection showed little trouble in conceiving during the 2005 foaling year. As of September 2004, all of the study mares experiencing a leptospira-induced abortion in foaling year 2004 that were bred were pregnant except one.

The presence of wildlife on the farms was important to establish in order to answer some questions concerning the transfer of leptospira from other animal species to horses. The answers to the survey suggest that the farms have large numbers of raccoons, waterfowl, and skunks on the property. Raccoons and skunks have been identified as reservoir species for leptospires, however, the serovar most commonly associated with raccoons is grippotyphosa and skunks are possibly the maintenance carriers for serovar kennewicki (Ellis et al., 1983; Wohl, 1996; Brown et al., 1996; Bolin, 1996; Birnbaum et al., 1998; Pilgrim and Threllfall, 1999; Mitchell et al., 1999; Adin and Cowgill, 2000; Donahue and Williams, 2000; Sykes, 2001; Ward, 2004). The farms that reported above average populations of raccoons and skunks did not have a mare abort in which the serovar grippotyphosa was involved. In fact, there was only one case in the 2004 foaling year in which the serovar grippotyphosa was involved and that farm reported average populations of raccoons and skunks. In addition, the mare experiencing the abortion related to infection by serovar grippotyphosa had recently arrived from Florida and was, most likely, exposed to the leptospires prior to arriving in central Kentucky.

Every farm, other than the farm experiencing a case due to serovar grippotyphosa, had serovar kennewicki incriminated by the LDDC as the causative agent. If the skunk is the maintenance carrier of serovar kennewicki, it is probable that urinary shedding of leptospires by skunks might increase the risk of equine exposure to leptospirosis and increase the rate of leptospira-induced abortions.

Birds are not identified as carrier species of leptospirosis. In fact, birds cannot contract the disease in nature and are not known to excrete leptospires in their urine (Davis, 1939). However, a bird could possibly transfer leptospires from one pasture to another if its feathers or body is contaminated.

Recent studies have indicated that the use of a geographical information system (GIS) technology to analyze the cases of leptospirosis in dogs in a given area helps to identify topographical features that may help contribute to the spread of the disease (Ward, 2004). The locations of cases in dogs were indicated on a map of Indiana in a similar manner to the marking of locations of leptospira-induced abortions in horses in the bluegrass region of Kentucky. The use of a GPS receiver and GIS software provides great benefits in analyzing the occurrence of incidents over time and any relationship to topographical data.

As seen in Figure 4.1 the incidents of leptospira-induced abortion appear to be completely random as they occurred over time in the bluegrass region of Kentucky. If a specific point of origination was responsible for the incidents, the map would show clustering of small green dots in one area and green dots of gradually increasing size as the distance increased from the original cluster. There is no evidence of a single origination point on Figure 4.1.

A particular interest in the location of the leptospirosis cases was the proximity of the cases to water. Indirect transmission of the leptospires can occur through waterways, such as lakes, rivers, and streams (Faine, 1994c). This type of indirect transmission can occur by different means, such as an animal in the carrier stage of leptospirosis urinating in the waterway, or wading birds transferring the leptospires from different environments through contamination of their feet (Faine, 1994c). Therefore it is important to look at the location of the cases of leptospira-induced abortions through time and the proximity of those cases to waterways.

Figure 4.2 adds the major waterways to the same map used in Figure 4.1. Although there is no single origination point, an analysis can now be made to determine if leptospires could be transferred from one point to another via a waterway in close proximity. The flow of the waterways on the map can be explained by treating the blue waterway depictions of branches from a tree. The smallest limbs (streams) are the terminal blue lines on the map. These small limbs flow into a much larger branch (small river or large stream), which in turn, flows into the trunk of the tree (major river). Therefore the water flows from small blue lines to larger blue lines.

If the transfer of leptospires along the major waterways were the main method of transmission, the map would depict small green circles at the end of the small blue lines, and green circles of increasing size along the waterways flowing toward a large river. This is not the case in this depiction. Again the occurrence of the abortions appears to be completely randomized in a geographical sense.

Figures 4.3 through 4.8 add more hydrography data to the geographical analysis of the locations of leptospira-induced abortions. The same description of water flow used for Figure 4.2 applies to these figures. Because the diagram in Figure 4.3 becomes more crowded with the addition of the watershed data, Figures 4.4 through 4.8 depicts all of the points in increased resolution. Interestingly, there appears to be no association with the flow of water in the bluegrass region of Kentucky to the occurrence of leptospira-induced abortion in the same region. The result is a completely random occurrence of the incidents in the region over time.

Chapter 5: Conclusions

This 15-year retrospective study in central Kentucky is the first investigation done on leptospira-induced abortions in horses that analyzes over five years of data. Previous studies done in central Kentucky have used data from 1, 2, 4 and 5 years (Poonacha et al., 1990; Hong et al., 1993; Giles et al., 1993; Donahue et al., 1995). There are two primary reasons that the location of the study, central Kentucky, is important. The first is that there is a large equine population in the region. The second is that the University of Kentucky Livestock Disease Diagnostic Center (LDDC) is likely the only the laboratory in the country that does testing for leptospires on all aborted equine fetuses (Dr. Mike Donahue, LDDC, via personal communication, 2004).

Having the cases diagnosed by the LDDC over the 15 year period in this study allowed for the establishment of foaling year prevalence of leptospirosis, which allowed for further comparisons between 5 high-prevalence and 10 baseline-prevalence years. Prevalence was established by dividing the number of Thoroughbred fetuses diagnosed by the LDDC as leptospira-induced abortions by the number of Thoroughbreds born alive in Kentucky and registered at the Jockey Club. Previous studies have established prevalence based upon the number of aborted fetuses submitted to the LDDC that were diagnosed as leptospirosis versus the number of aborted fetuses that were submitted to the LDDC that were not diagnosed as leptospirosis-related incidents (Poonacha et al., 1990; Hong et al., 1993; Giles et al., 1993; Donahue et al., 1995). This study used a different method, because the necropsy records for 15 years would have been very numerous and there was great concern for confidentiality of the LDDC records. Furthermore, this is the first study to look at more than one year of high prevalence of leptospiral abortions since the incidence of the disease was found to be of greater importance in central Kentucky in the 1988 and 1989 foaling years (Poonacha et al., 1990; Hong et al., 1993).

A foaling year begins on July 1st and ends on June 30th the following calendar year. For example foaling year 1990 would encompass the dates from July 1, 1989 through July 30, 1990. Foaling years were used in this study because most Thoroughbreds are on the same foaling schedule with January 1st being designated by the Jockey Club as a horse's official birthday. One of the reasons for this is so the Thoroughbred weanlings will be ready for sale at the appropriate times throughout the year. Using the foaling year allowed investigation of one period of gestation a year, rather than the end of one gestation period and the beginning of another gestation period as would be in the calendar year. The foaling years that were designated as high-prevalence foaling years were 1990, 1992, 1993, 2001, and 2004. The prevalence rates for these years can be seen in Table 2.2.

Previous studies have indicated that the highest incidence of abortion occurs in fall, with the highest incidence occurring in November or December (Donahue et al., 1995; Levett, 2001; Ward, 2004). The epidemiological curves shown in Figures 2.2 through 2.16 demonstrate the seasonal incidence of leptospira-induced abortion. Because this study is the first to compile 15 years worth of data and encompass more than one year of high prevalency in leptospira-related abortions in the horse, a combined year comparison was able to be made between the high-prevalence and baseline-prevalence years. Figure 2.17 depicts the two separate curves throughout a foaling year.

Figure 2.17 shows a one-month shift in years of high prevalency versus the years of baseline prevalency. This suggests that in a year of more incidents of leptospira-induced abortions that the abortions will occur earlier in the foaling year and peak in December, rather than the January peak incidence occurring in a baseline year. The data represented in Figure 2.22 provides further evidence that a one-month shift occurs between the high-prevalency and baseline-prevalency years. Figure 2.22 depicts the difference in gestational age of the fetus at the time of abortion in the two categories of years. The average gestational age during the years of high-prevalence was 8.5 months compared to the average gestational age of 9.5 months during the baseline-prevalence years. The difference between the two years was statistically significant ($p < 0.005$) and therefore supported the epidemiological curve depicting the one-month shift in incidence.

An attempt to explain the shift in incidence was made using temperature and precipitation data. It is commonly thought that mild temperatures and increased precipitation promote the risk of exposure to leptospires in temperate climates (Faine, 1994d; Harkin and Gartrell, 1996; Bolin, 1996; Brown et al., 1996; American Public Health Association, 2000; Levett, 2001; Ward, 2004). Therefore the hypothesis of this study was that in the years of high prevalency the temperatures were milder and the precipitation totals would be above normal. The comparisons between years were done by weekly average temperatures and weekly average precipitation in inches (http://wwwagwx.ca.uky.edu).

Other studies (Carroll and Campbell, 1987; Miller et al., 1991; Barwick et al., 1997, 1998; Adin and Cowgill, 2000; Ward, 2002; Meites et al., 2004) have researched the importance of precipitation on leptospire survival and/or incidence of leptospirosis.

Although the studies conducted involved different species, the results were similar. Periods of higher than normal precipitation were associated with a higher prevalence of leptospirosis. None of these studies, however, combined the effects of temperature and precipitation.

Figure 3.10 shows three separate lines. The blue line represents the 15-year average temperatures. The red line represents the average temperatures of the five years of high leptospira-induced abortion prevalency. The yellow line represents the average temperatures of the 10 years of baseline leptospira-induced prevalency. There are two periods where the average temperatures during the high-prevalence years differ from the average temperatures during the 15-year mean and the baseline prevalence years. These temperatures are depicted more closely in Figure 3.13. The average temperature remains above normal from October 14th through November 3rd during the years of higher prevalence. The average temperature then falls below normal from November 25th through December 22nd during the years of higher prevalence. These temperature differences are the only notable changes between the high-prevalence and baseline-prevalence years.

Figure 3.14 indicates the average foaling year precipitation in inches and designates the high-prevalence years in red and the baseline-prevalence years in blue. The 15-year mean precipitation is indicated by a yellow bar. This figure indicates that the incidence of leptospira-induced abortions is random in occurrence when compared to annual rainfall, and therefore suggested a more in depth look at weekly precipitation totals.

Much the same way as determining the difference in average temperatures, the average weekly precipitation was plotted for a foaling year, Figure 3.18. The color designations were the same as mentioned in the previous paragraph. There were some points of keen interest and each of these points was analyzed to make sure that it was not one specific precipitation total for a particular year, skewing the results. Upon completion of this analysis, the most intriguing section of the precipitation totals was determined to be the section shown in Figure 3.19. The combination of the data described in Figures 3.13 and 3.19 created the following scenario.

In the months leading up to and into October during high prevalence years, milder temperatures are seen, followed by a spike in temperature during the last two weeks of October (Figure 3.13). This spike is not an increase in temperature. However, it is a prolonging of temperature in the 65 to 70 degree Fahrenheit average range for 2 weeks. Simultaneously, the weekly precipitation is following a cycle of heavy precipitation (greater than 1.0 inches) followed by two or more weeks of light precipitation (Figure 3.19). Meanwhile during the baseline prevalence years the average weekly temperature steadily decreased since early September (nearly 2.5 degrees Fahrenheit a week) and average weekly precipitation totals were 1.0 inch or less since July (Figure 3.19). It is likely that the extension of the mild temperatures prolong the warmth of the soil containing the leptospires during the high prevalence years.

Furthermore, it has been determined (Karaseva et al., 1979) that pathogenic leptospires can survive up to 15 days in moist (74.3%), neutral pH (6.5-7.5) soil compared to 12 hours in slightly moist (16.5%) slightly acidic (pH 6.2) soil. A similar study showed that serovar pomona was able to retain its pathogenicity and viability up to

74 days in soil having a pH of 6.7 to 7.2 and moisture content of 15.2 to 31.4% (Zaitsev et al., 1989). The limestone in the bluegrass region soil helps create a relatively neutral pH (Dennis Hancock, University of Kentucky College of Agriculture, via personal communication, 2004). Meanwhile the heavy precipitation moistens the soil and in some areas could cause standing water, which would bring the leptospires, if present in the soil, to the surface for ingestion. These conditions could cause the earlier onset of the cases of leptospira-induced abortion and increase the risk of exposure to leptospires. If more organisms are surviving in the environment, more horses are likely to be exposed to the leptospires.

The drop in temperatures during the month of December in high prevalence years to temperatures below the average prevalence years average, coinciding with a close return to the 15-year mean for precipitation during this time, could explain why the number of abortions do not continue throughout the foaling year. The leptospires are no longer able to survive as long in the colder environment.

This study did not make an attempt to determine the serovars responsible for the cause of the leptospira-induced abortion. Dr. Mike Donahue and Dr. Neil M. Williams (2000) made such an effort using fetuses aborted because of a leptospiral infection during many of the calendar years covered in this study (1989-1999). The LDDC uses the fluorescent antibody test (FAT) as the confirming test for a leptospiral abortion. Serologic testing is then performed by the LDDC on the fetal pericardial fluid and mare sera by the microscopic agglutination test (MAT). The MAT will diagnose the serogroup responsible for the infection. Dr. Donahue and Dr. Williams (2000) found that fetal fluids tested positive for only one, and most likely the causative, serogroup in 120 of 123

(97.6%) samples, while sera from the mare usually tested positive for one or more serogroups. They went one step further in their study and cultured the serovar responsible for the infection. This procedure takes at least six months to culture and identify the bacteria and is very costly. Due to these factors it is accomplished infrequently.

The LDDC uses the MAT to identify the serovars (kennewicki, grippotyphosa, or hardjo) responsible for an equine leptospira-induced abortion. There was no determination made between mare sera or fetal pericardial fluid. Therefore, in many cases, there was more than one serovar identified (Figure 1.19). To make matters easier, each positive identification for a particular serovar was treated as its own incident and broken down into incriminating serovars determined by the LDDC (Figure 1.20). Similar to what Dr. Donahue and Dr. Williams found in their study, serovar kennewicki was present in the majority of the cases (82%), serovar grippotyphosa was second most prevalent (16%), and serovar hardjo was identified in only 2% of the cases. These findings support Dr. Donahue and Dr. Williams' (2000) findings that the serovar most often responsible for an equine abortion is kennewicki.

Much research has indicated that the horse is a maintenance carrier for serovar bratislava (Ellis et al., 1983; Ellis, 1999). This research, however, has been carried out in Northern Ireland and not North America. One would suspect if the horse in North America were a maintenance carrier for serovar bratislava that the serovar would have been identified in more than 2% of the cases. The other case to be made is that being a maintenance carrier of serovar bratislava may offer a protective effect from other leptospiral infections and thus decreases the chance of abortion. The only way to determine the later is to test the healthy mares and foals on random central Kentucky farms and determine the prevalency of serovar bratislava.

Lastly, the serological findings of this study, and previous studies, indicate that the horse is an incidental carrier of serovar kennewicki, as well as serovars grippotyphosa, and hardjo (Donahue et al., 1991, 1992, 1995). This means that at some point a maintenance carrier of these serovars must come into contact with the horse, or contaminate an area where the horse may be stabled, fed, watered, or pastured.

Interestingly, the foaling year in which this study was being conducted (FY 2004) was a year of high prevalence. This allowed for real-time investigation of the leptospirosis epidemic in central Kentucky. The investigation of the FY 2004 was accomplished by submitting surveys (Appendix B) to the farms in central Kentucky that had experienced a leptospira-induced abortion during that year, and by visiting the farms and taking latitude and longitude coordinates of the pastures where the aborting mare was kept six weeks prior to the abortion for use in geographical information system (GIS) analysis.

The survey was submitted to 22 farms and returned by 16 for an excellent response rate of 73%. There are some limitations to the data received by a survey and the most significant is memory bias. The survey was sent to the farms and farm visits conducted at the end of the foaling year (May 2004 through August 2004). In some cases the farm manager (or other farm personnel) was recalling information that occurred almost a year prior to the visit. However, the commercial operations kept meticulous records where the mares were stalled and pastured which limited the recall bias in this study. Only one farm was not a commercial operation and only one pasture was used for horses on the farm.

Another limitation of the survey is the subjectiveness of some of the answers, especially those concerning wildlife. Although the determination of wildlife on a farm might be a subjective answer, it is very important data. As established previously, the horse is most likely an incidental host for a leptospiral infection. Thus it must contract the infection from a maintenance carrier by direct or indirect transmission. It has been determined that raccoons and opossums are the maintenance carriers for serovar grippotyphosa and skunks and pigs the maintenance hosts for serovar kennewicki (Ellis et al., 1983; Bolin, 1996; Brown et al., 1996; Wohl, 1996; Birnbaum et al., 1998; Mitchell et al., 1999; Pilgrim and Threllfall, 1999; Adin and Cowgill, 2000; Sykes, 2001). Therefore it is important to establish how frequently these mammals are spotted on a horse farm.

Skunks were seen often on 4 out of 16 farms, sometimes on 10 out of the 16 farms and never seen on two of the farms. Opossums were often seen on 2 of the 16 farms, sometimes seen on 12 of the 16 farms, and never seen on 2 of the farms. Raccoons were often seen on 7 of the 16 farms, sometimes seen on 6 of the farms, never seen on two of the farms, and no response was marked on one of the farms. Pigs were not included in the questionnaire, but during the farm visits, no evidence of pigs was seen on any of the farms. These responses indicate that the animals considered to be the maintenance hosts for the pathogenic strains of leptospira causing abortion in equines were present on most of the farms in the study. One farm did keep cattle in the field next to the pasture of a mare that aborted, but cattle are most likely the maintenance host for serovar hardjo (Faine, 1994e) and the serovar incriminated in the aborted fetus was kennewicki.

The survey also indicated that 20 pastures were involved on the 16 different farms and 50% of these pasture has some source of ground water on them. This is an important determination because flooding has been associated with outbreaks of leptospirosis in humans and animals, thus incriminating water as a viable source of indirect transmission of the organism (Rentko et al., 1992; American Public Health Association, 2000; Herbert, 2004; Ward et al., 2004).

The determination of the location of flowing water in proximity to the location of the pasture that the mares experiencing a leptospira-induced abortion were kept was made through the use of global positioning system (GPS) and geographical information system (GIS) technology. The reason this was possible was because the farms that had more than one pasture maintained impeccable records that tracked the horses on the farm. Out of the 16 farms that were visited, all were sure of the location of the mare 6 weeks prior and leading up to the abortion.

Although this is the first time that GPS and GIS technology has been used in a leptospirosis study involving horses, it is not the first time this type of study has been accomplished. Dr. Michael Ward (2004) used this technology to identify landscape features that could be associated with leptospirosis in dogs. Dr. Ward used a GIS analysis known as overlay analysis. Overlay analysis was also accomplished in this study in order to determine clustering of cases and the proximity of pastures to flowing water.

The maps created by the overlay analysis can be seen in Figures 4.1 through 4.8. The first conclusion is that the cases are completely random geographically and there is no clustering (Figure 4.1). Major waterways were overlaid upon the geographical points to determine the likelihood of leptospiral transmission through these larger flowing water routes (Figure 4.2). Again the cases were completely geographically random. Finally, minor watershed data was added (Figures 4.3 through 4.8). Watershed data shows how water naturally flows following rainfall. Because this data is so intricate, areas of the map were magnified to show greater detail (Figures 4.4 through 4.8). However, once again, the geographical points collected through GPS technology are completely random in geographical occurrence. These findings would strongly suggest that the likely mechanism of transmission is not flowing water.

GPS and GIS technology proved to be very useful in this study and was able to answer a major question concerning the transmission of leptospirosis among horses. This technology will certainly be used in future studies to strengthen association between geographic and environmental factors and the distribution of disease (Glass et al., 1995; Ward, 2004). This study faced a limitation of satellite photos being almost eight years old (Dennis Hancock, University of Kentucky College of Agriculture, via personal communication, 2004). A follow-up study accomplished when the photos are updated would provide further geographical evidence concerning forested area, hydrography, and developed land in relation to leptospirosis occurrence.

Analysis of 15 years of data was done to determine individual mare factors and the effects of temperature and precipitation on the prevalence of leptospira-induced abortions. The results were that a mare having the average age of 9 years will most likely abort her foal between 8 and 10 months of gestation if she contracts a leptospiral infection. This abortion will not affect the mares' ability to produce further offspring or her ability to conceive. In this study, no mare had a leptospira-induced abortion more than once in the 15 year time period examined. However, with the frequent transportation of mares into and out of central Kentucky, a more in-depth study of the reproductive histories of the mares would be needed.

The infection most likely occurs through indirect transmission of leptospires by mammal maintenance hosts. Since serogroup Pomona serovar kennewicki is most often attributed to the abortion of a foal, the maintenance host could be skunks and opossums. Both mammals were frequently seen on farms returning the questionnaire and both animals are nocturnal, making their spotting more difficult. These animals might shed the organism through urination allowing the organism to be absorbed into the soil.

Temperature and precipitation likely work together to keep the leptospires alive in the soil of the farm. The moist soil prevents the desiccation of the organism and allows it to retain its pathogenicity for a longer period of time. A short period of heavy precipitation during mild temperatures likely brings the organism to the surface. The soil then likely reabsorbs the organisms (dead or alive) unless indirect transmission of an incidental host has occurred.

If the first case of leptospira-induced abortion occurs in August, it does not mean that the year is going to be a high-prevalence year. If the first case appears in August and the temperatures remain milder into the fall, and precipitation follows a pattern of a week of high moisture followed by weeks of low moisture, then the risk of leptospiral exposure to a pregnant mare is higher.

Future studies should certainly employ the GPS/GIS technology. These tools are very valuable in determining the spread of disease over large geographical regions. More interest should be placed on the proximity of wooded areas to the pastured mares. One method of accomplishing this analysis would be to take a pasture reading, as done in this study, and then take a reading at the edge of the nearest wooded area.

Additional studies could be accomplished to determine the likely survival period for leptospires in soil similar to the soil found in Kentucky and under the weather conditions established in this study. Such an experiment would provide accurate data on the length of time leptospires survive in the central Kentucky environment and help make the period of exposure to the organism more precise.

In conclusion, the results of this study support the following recommendations. First, farms should fence off flooded areas and ground water areas, where practical, to decrease the risk of mare exposure to leptospires. Next, mares should not be fed hay and grain off the ground, especially in wet conditions. Finally, wildlife activity on the farm should be monitored closely and the increased presence of opossums, raccoons, and skunks should be especially noted.

Appendix A: Accession Sheet

ACCESSION SHEET

UK UNIVERSITY OF KENTUCKY
College of Agriculture
Livestock Disease Diagnostic Center
1429 Newtown Pike
P.O. Box 14125
Lexington, Kentucky 40512-4125
Phone: (859) 253-0571 Fax: (859) 255-1624

Accession # ____
Date ____
Species ____
Breed ____
Sex ____ Age ____
Color/Weight ____

Animal Name or # ____

Veterinarian: ____
Clinic: ____
Address: ____
City: ____ State: ____
Zip code: ____ County: ____
Telephone: ( ) ____

Owner/Mgr: ____
Farm Name: ____
Address: ____
City: ____ State: ____
Zip code: ____ County: ____
Telephone: ( ) ____

Have Other Specimens Been Submitted for this Problem? When: ____ **Case No.:** ____

History: Date/Time of Death ____ Duration of illness ____
No. of Animals in Affected Group ____ No. Sick ____ No. Dead ____
Vaccination History ____
Nutrition (type and quality of feed, water, supplement, mineral, etc) ____
Case History and Clinical Signs: ____

____ (continue history on back)

Treatment ____
Euthanasia ____ No ____ Yes ____ How? ____
Clinical Diagnosis ____
Specimens Submitted ____
Tests requested: Write in names of desired tests that are not listed. **Veterinarian's Signature** ____
http://www.ca.uky.edu/lddc **Veterinarian's License Number** ____

Contact the Livestock Disease Diagnostic Center if you need additional information

Rabies
___Human exposure
___Animal exposure
___No exposure

Pathology
___Necropsy and related testing
___Histopathology (other testing if needed)
____

Bacteriology
___Aerobic culture & sensitivity
___Mastitis culture
___Fungal culture
___Johne's culture
____

Clinical Pathology
___Cytology
___Complete fluid analysis
___CBC (EDTA & 2 slides)
___Chemistry panel (serum)
___Protein electrophoresis
___Urinalysis
___Stone analysis
____

Parasitology
___Routine
___Cryptosporidia
____

Serology
___Anaplasmosis
___Blastomycosis
Bluetongue
___Brucellosis (K-9)
___Histoplasmosis
___Johne's Disease
___Leptospirosis
___Leukosis (BLV)
___Neospora
___Toxoplasmosis
____

Toxicology
___Copper (serum)
___Selenium (serum)
___Nitrate (forage)
____

Virology
___Virus Isolation
___Virus Isolation for BVD
___Rotavirus latex test
Canine Parvovirus Antigen
___BVD Serology
___BRSV Serology
___IBR Serology
___Eq herpes 1 Serology
___Equine influenza Serology
___Potomac Horse Fever
___PRRS Serology
____

Molecular Biology
___Nucleic Acid Testing (PCR assay)
____

REVERSE SIDE MUST BE COMPLETED FOR INSURANCE CASES AND ALL EQUINE SUBMISSIONS

LABORATORY USE ONLY
Specimens received ____
Rab Nec His Bact Cl Path M Bio Ser Vir Tox Other

History (continued):

____

____

____

____

____

____

____

INFORMATION REQUIRED FOR INSURANCE CASES AND EQUINE CASES

1. Identification by species, breed, sex, age and animal name/number **MUST BE COMPLETED ON THE FRONT OF THIS FORM.** Please list below further information such as tattoo numbers: ______________________

____

____

2. Is this animal insured? Yes __________ No ____________

   Name of insurance company: ______________________

3. Can information related to this case be released by the Livestock Disease Diagnostic Center to the insurance company? Yes __________ No __________

____
REQUIRED SIGNATURE OF OWNER OR REPRESENTATIVE   DATE

____
Title

Information related to this case will not be released to an insurance company without the signature of the owner or his representative.

General Information for submitters

The following lists identify the assays which comprise specified panels:

CHEMISTRY

LARGE ANIMAL PANEL

BUN
Calcium
Phosphorus
Magnesium
Total Protein
Albumin
Globulin
SCOT
CPK
GGT
Alk. Phos.
Glucose
Creatinine
Total Billrubin
Cholesterol
Sodium
Potassium
Chloride

SMALL ANIMAL PANEL

BUN
Calcium
Phosphorus
Total Protein
Albumin
Globulin
SGPT
Alk. Phos.
Amylase
Glucose
Creatinine
Total Billrubin
Cholesterol

SEROLOGY

ABORTION PANELS

Bovine
Brucella
Leptospira
IBR
BVD
Neospora

Porcine
Brucella
Leptospira
PRV
PRRS
PPV

Appendix B: Leptospirosis Questionnaire

CONFIDENTIALITY AGREEMENT: Survey information from horse farms has been provided to the University of Kentucky with the express understanding that any and all such information shall be considered records confidentiality disclosed to an agency and compiled and maintained for scientific research, and shall be exempt from disclosure pursuant to KRS 61.878(1)(b).

Leptospirosis Questionnaire for Cases Occurring Between the Fall of 2003 and the Spring of 2004

Date of Interview: _______________(mm/dd/yy) Farm ID: ____________________
Person performing interview: ____________________

1. For each mare that aborted a fetus due to leptospirosis please answer the following:

	Mare Name	2003 Last Date Bred	Year of Birth	Parity (If Known)
a.	__________	____________________	____________	________________
b.	__________	____________________	____________	________________
c.	__________	____________________	____________	________________
d.	__________	____________________	____________	________________
e.	__________	____________________	____________	________________

2. Have any of the horses that have been diagnosed with leptospirosis been tested for chronic shedding of the spirochete in their urine?
   Yes: _____ No: _______ Other: ________

3. Were the mares diagnosed with leptospirosis pastured in a field with:
   a. Stream: ____
   b. Lake: _____
   c. Farm Pond: _____
   d. Standing Water: _____
   e. Other Form of Water: ________

4. If more than one case of leptospirosis abortion on the farm occurred from 2003 breedings, which pastures were involved? (Please list name of Pasture or Pasture ID)
   a. ____
   b. ____
   c. ____
   d. ____
   e. ____

5. Were the horses diagnosed with leptospirosis, kept in the same or different fields on the farm?
   Same: ________ Different: ________

6. Did the farm have a rodent problem in the feed store areas, or within the stables?
   (Feed) Yes: _______ No: ________
   (Stables) Yes: _____ No: ________

7. Has any other animal on the farm been diagnosed with leptospirosis, including pets?

Yes: _________ No: ________
If yes please list: ____

8. Once a horse is diagnosed with leptospirosis what is the farm's standard treatment?
a. Medicine: __________ (length of treatment) __________
b. Isolation: Yes: _________ No: __________
c. Other: ____

12. If the horse is isolated, at what point is the horse reintroduced to the other horses?

One Week: _______ Two Months: _________
Two Weeks: ______ Three Months: ________
Three Weeks: ______ Four Months: __________
Four Weeks: _______ Other: ________

13. When a horse is diagnosed with leptospirosis on the farm, what preventative measures are taken?
a. Medication (to other horses): ________
b. Diet Change: _______
c. Testing water sources: ________
d. Leptospirosis immunization of other horses: _______ (product?)_________

14. Was the mare that aborted from leptospirosis bred in 2004?
Yes: ______ No: _______
If yes, what is the mare's current status?
Not Checked_______ Pregnant_____ Open_____ Other__________

15. If a mare was diagnosed with leptospirosis and successfully delivered a foal, has that foal ever been tested for shedding of leptospires?
Yes: ________(the results?)____
No: _________

18: Have the horses on the farm ever been vaccinated against leptospirosis using the cattle vaccine?
Yes: __________ No: __________
If yes, please indicate which years: _____________________________________

19. Is there a wooded area within the pasture perimeter?
Yes: _______ No: _________
Outside the pasture perimeter?
Yes: ________ No: __________

20. This next question refers to wildlife or evidence of their presence including tracks, scat, carcass, or damage. In the Fall of 2003 though the Spring of 2004, did you often, sometimes, or never see the following wildlife or evidence thereof on the horse premises or in the immediate vicinity? (Please circle answer.)

a. Mice or Rats	Often	Sometimes	Never

b. Raccoons	Often	Sometimes	Never
c. Coyotes	Often	Sometimes	Never
d. Deer or Elk	Often	Sometimes	Never
e. Water Fowl (geese or ducks)	Often	Sometimes	Never
f. Bats	Often	Sometimes	Never
g. Opossums	Often	Sometimes	Never
h. Skunks	Often	Sometimes	Never
i. Foxes	Often	Sometimes	Never

21. Has the presence (or absence) of these species of wildlife significantly changed over previous years? If so, how?

____
____
____
____

22. Please take a moment to provide any theory that you may have concerning the increased numbers of leptospirosis induced abortions in certain years compared to years having a lower number of leptospirosis induced abortions?

____
____
____
____
____

Appendix C: Confidentiality Agreement

UNIVERSITY OF KENTUCKY

College of Agriculture
Veterinary Science
GLUCK EQUINE RESEARCH CENTER
Lexington, KY 40546-0099
(859) 257-4757
www.uky.edu
FAX: (859) 257-8542
Writers Direct Dial Number

CONFIDENTIALITY AGREEMENT

____
farm name/address

____

____

____

Information regarding the above-referenced farm has been provided to the University of Kentucky with the express understanding that any and all such information shall be considered records confidentially disclosed to an agency and compiled and maintained for scientific research, and shall be exempt from disclosure pursuant to KRS 61.878(1)(b).

____
Form representative signature

____
Print name

____
Date

code #__________

Literature Cited

Adin, C.A. and Cowgill, L.D. 2000. Treatment and outcome of dogs with leptospirosis: 36 cases (1990-1998). Journal of the American Veterinary Association 216: 371-375.

American Public Health Association. 2000. Leptospirosis. In: Beneson, A.S., ed. *Control of communicable diseases manual.* Washington, D.C.: American Public Health Association.

Babudieri, B. 1958. Animal reservoirs of leptospirosis. Annals of New York Academy of Science 70: 393-413.

Baker, C.B. 1995. Studies of factors limiting reproductive success in horses and derivation of investigative strategies for identifying their causes. Doctoral Dissertation, University of Kentucky, 1995, 126.

Barwick, R.S., Mohammed, H.O., McDonough, P.L., and White, M.E. 1997. Risk factors associated with the likelihood of leptospiral seropositivity in horses in the state of New York. American Journal of Veterinary Research 58: 1097.

Barwick, Rachel S., Mohammed, H.O.., McDonough, P.L., and White, M. E. 1998. Epidemiologic features of equine *leptospira interrogans* of human significance.

Preventative Veterinary Medicine 36: 153-165.

Bernard, William V., Bolin, Carole, Riddle, Thomas, Durando, Mary, Smith, Barbara J., and Tramontin, Robert R. 1993a. Leptospiral abortion and leptospiruria in horses from the same farm. Journal of American Veterinary Medical Association 202: 1285-1286.

Bernard, W.V.,Williams, D., and Tuttle, P.A. 1993b. Hematuria and leptospiruria in a foal. Journal of American Veterinary Medical Association 203: 276.

Bernard, William V. 1993c. Leptospirosis. Veterinary Clinics of North America: Equine Practice 9: 435-444.

Birnbaum, N., Center, S.A., and Schermerhorn, T. 1998. Naturally acquired leptospirosis in 36 dogs: serological and clinicopathological features. Journal of Small Animal Practice 39: 231-236.

Bolin, C.A. 1996. Diagnosis of leptospirosis: a reemerging disease of companion animals. Seminars of Veterinary Medicine Surgery Small Animals 11: 166-171.

Brown, C.A., Roberts, W., and Miller, M.A. 1996. *Leptospira interrogans* serovar *grippotyphosa* infection in dogs. Journal of the American Veterinary Medical Association 209: 1265-1267.

Carpio, M.M., G. Wobesor, and Iverson, J.O. 1979. *Leptospira interrogans* serotype *pomona* in Saskatchewan: Isolation from a naturally infected striped skunk. Canadian Journal of Microbiology 23: 1654.

Carroll, A.G. and Campbell, R.S.F. 1987. Reproductive and leptospiral studies in beef cattle in central Queensland. Australian Veterinary Journal 64: 1-5.

Center for Disease Control. 2004. Leptospirosis. www.cdc.gov/ncidod/dbmd/diseaseinfo/leptospirosis_t.htm.

Chang, S.L., M. Buckingham, and Taylor, M.P. 1948. Studies on *Leptospira icterohaemorrhagiae*. IV. Survival in water and sewage: destruction in water by halogen compounds, synthetic detergents, and heat. Journal of Infectious Diseases 82: 256-266.

Chin, James ed. 2000. Control of Communicable Diseases Manual. American Public Health Association, Washington, D.C.: 293-296.

Crawford, Richard P., Heinemann, Jack M., McCulloch, William F., and Diesch, Stanley L. 1971. Human Infections Associated with Waterborne Leptospires, and Survival Studies on Serotype *pomona*. Journal of American Veterinary Medical Association 159: 1477-1484.

Donahue, J.M. and Williams, N.M. 2000. Emergent causes of placentitis and abortion. Veterinary Clinics of North America: Equine Practice 16: 443-456.

Donahue, James M., Smith, Barbara J., Poonacha, Kockanda B., Donahoe, Judy K., and Rigsby, Cindy L. 1995. Prevalence and serovars of leptospira involved in equine abortions in central Kentucky during the 1991-1993 foaling seasons. Journal of Veterinary Diagnostic Investigation 7: 87-91.

Donahue, James M., Smith, Barbara, Donahoe, Judy K., Rigsby, Cindy L., Tramontin, Robert R., Poonacha, Kockanda B., and Wilson, Mark A. 1992. Prevalence and serovars of leptospira involved in equine abortions in central Kentucky during the 1990 foaling season. Journal of Veterinary Diagnostic Investigation 4: 279-284.

Donahue, James M., Smith, Barbara J., Redmon, Kevin J., and Donahue, Judy K. 1991. Diagnosis and prevalence of leptospira infection in aborted and stillborn horses. Journal of Veterinary Diagnostic Investigation 3: 148-151.

Ellis, W.A. 1999. Equine leptospirosis. Equine Infectious Disease VIII: Proceedings of the Fifth International Conference. University Press of Kentucky. Lexington, KY: 168.

Ellis, William A. and O'Brien, John J. 1988. Leptospirosis in Horses. Equine Infectious

Diseases V: Proceedings of the Fifth International Conference: 168-171.

Ellis, W.A., Bryson, D.G., O'Brien, J.J., and Neill, S.D. 1983a. Leptospiral infection in aborted equine foetuses. Equine Veterinary Journal 15: 321-324.

Ellis, W.A.,O'Brien, J.J., and Cassells, J.A. 1983b. Leptospiral infection in horses in Northern Ireland: Serological and mircrobiological findings. Equine Veterinary Journal 15: 317.

Everard, J.D., and C.O.R. Everard. 1993. Leptospirosis in the Carribean. Review of Medical Microbiology 38: 114-122.

Faine, Solomon. 1994a. Sources, Transmission, and Spread of Leptospirosis. In: *Leptospira* and Leptospirosis. CRC Press, Inc., Boca Raton, FL. pp 243-265.

Faine, Solomon. 1994b. Clinical Leptospirosis in Animals. In: *Leptospira* and Leptospirosis. CRC Press, Inc., Boca Raton, FL. pp 215-227.

Faine, Solomon. 1994c. Sources, Transmission, and Spread of Leptospirosis. In: *Leptospira* and Leptospirosis. CRC Press, Inc., Boca Raton, FL. pp 244-245.

Faine, Solomon. 1994d. Sources, Transmission, and Spread of Leptospirosis. In: *Leptospira* and Leptospirosis. CRC Press, Inc., Boca Raton, FL. pp 248-249.

Faine, Solomon. 1994e. Sources, Transmission, and Spread of Leptospirosis. In: *Leptospira* and Leptospirosis. CRC Press, Inc., Boca Raton, FL. pp 248.

Faine, S., B. Adler, C. Bolin, and P. Perolat. 1999. Leptospira and Leptospirosis, 2nd ed. MedSci, Melbourne, Australia.

Giles, R.C., Donahue, J.M., Hong, C.B., Tuttle, P.A., Petrites-Murphy, M.B., Poonacha, K.B., Roberts, A.W., Tramontin, R.R., Smith, B., and Swerczek, T.W. 1993. Causes of abortion, stillbirth, and perinatal death in horses: 3,527 cases (1986 – 1991). Journal of American Veterinary Medical Association 203: 1170-1175.

Glass, G.E., Schwartz, B.S., and Morgan, J.M. 1995. Environmental risk factors for Lyme disease identified with geographic information systems. American Journal of Public Health 85: 944-948.

Hanson, L.E. 1977. Immunology of bacterial disease, with special reference to leptospirosis. Journal of American Veterinary Medical Association 170: 991-994.

Harkin, K.R., and Gartrell, C.L. 1996. Canine leptospirosis in New Jersey and Michigan: 17 cases (1990-1995). Journal of American Animal Hospital Association 32: 495-501.

Hartskeeri, R.A., and W.J. Terpstra. 1996. Leptospirosis in wild animals. Veterinary Quarterly 18 (Supplement 3): S149-S150.

Hellstrom, J.S. and Marshall, R.B. 1978. Survival of *Leptospira interrogans* serovar pomona in an acidic soil under simulated New Zealand field conditions. Research in Veterinary Science 25: 29-33.

Hodgin, E.C., D.A. Miller, and Lozano, F. 1989. *Leptospira* abortion in horses. Journal of Veterinary Diagnostic Investigation 1: 283.

Hong, C.B., Donahue, J.M., Giles, R.C., Petrites-Murphy, M.B., Poonacha, K.B., Roberts, A.W., Smith B.J., Tramontin, R.R., Tuttle, P.A., and Swerczek, T.W. 1993a. Equine abortion and stillbirth in central Kentucky during 1988 and 1989 foaling seasons. Journal of Veterinary Diagnostic Investigation 5: 560-566.

Hong, C.B., Donahue, J.M., Giles, R.C., Petrites-Murphy, M.B., Poonacha, K.B., Roberts, A.W., Smith, B.J., Tramontin, R.R., Tuttle, P.A., and Swerczek, T.W. 1993b. Etiology and pathology of equine placentitis. Journal of Veterinary Diagnostic Investigation 5: 56-63.

Jackson, R.S., E.E. Jones, and Clark, D.S. 1957. Abortion in mares associated with leptospirosis. Journal of American Veterinary Medical Association 131: 564.

Johnson, R.C., and S. Faine. 1984. Family II. *Leptospiraceae*. Bergey's Manual of Systematic Bacteriology, volume 1. Williams and Wilkins. Baltimore, MD.

Karaseva, E.V., Chernukha, Y.G., and Piskunova, L.A. 1973. Results of Studying the Time of Survival of Pathogenic Leptospira Under Natural Conditions. Journal of Hygiene, Epidemiology, Microbiology, and Immunology 17: 339-345.

Kentucky Thoroughbred Farm Manager's Club. 2003. Kentucky Thoroughbred Farm Manager's Club 2003 Directory. Frances and Associates. Lexington, KY.

Levett, Paul N. 2001. Leptospirosis. Clinical Microbiology Reviews 14: 296-326.

Meites, Elissa, Jay, Michele T., Deresinski, Stanley, Shieh, Wun-Ju, Zaki, Sherif R., Tompkins, Lucy, and Smith, D. Scott. 2004. Reemerging Leptospirosis, California. Emerging Infectious Diseases 10:3-15.

Miller, D.A., Wilson, M.A., and Beran, G.W. 1991. Relationships bewteen prevalence of Leptospira interrogans in cattle, and regional climatic and seasonal factors. American Journal of Veterinary Research 52: 1766-1768.

Park, Y.G., Gordon, J.C., Bech-Nielsen, S., and Slemons, R.D. 1992. Factors for seropositivity to leptospirosis in horses. Preventative Veterinary Medicine 13:

121-127.

Poonacha, K.B., Smith, B.J., Donahue, J.M., Tramontin, R.R., Tuttle, P.A., Hong, C.B., Petrites-Murphy, M.B., Swerczek, T.W., and Giles, R.C. 1990. Leptospiral abortion in horses in central Kentucky. Proceedings of the Annual Convention of the American Association of Equine Practitioners 36: 397-402.

Ratnam, S. 1994. Leptospirosis an indian perspective. Indian Journal of Medical Microbiology 12: 228-239.

Rentko, V.T., Clark, N., and Ross, L.A. 1992. Canine leptospirosis: a retrospective study of 17 cases. Journal of Veterinary International Medicine 6: 235-244.

Roberts, S.J., C.J. York, and Robinson, J.W. 1952. An outbreak of leptospirosis in horses on a small farm. Journal of American Veterinary Medical Association 121: 237.

Rocha, T., Ellis, W.A., Montgomery, J., Gilmore, C., Regalla, J., and Brem, S. 2003. Microbiological and serological study of leptospirosis in horses at slaughter: first isolations. Research in Veterinary Science 76: 199-202.

Rosner, Bernard. 2000. Fundamentals of Biostatistics, 5th Edition. Brooks and Cole. Pacific Grove, CA: 757.

Ryu, E. and Liu, C.K. 1967. The viability of leptospires in the summer paddy water. Japanese Journal of Microbiology 10: 51-57.

Sheoran, Abhineet S., Nally, Jarlath E., Donahue, James M., Smith, Barbara J., Timoney, John F. 2000. Antibody isotypes in sera of equine fetuses aborted due to *Leptospira interrogans* serovar *pomona*-type kennewicki infection. Veterinary Immunology and Immunopathology 77: 301-309.

Smith, D.J.W., and H.R.M. Self. 1955. Observation on the survival of *Leptspira australis* A in soil and water. Journal of Hygiene 53: 436-444.

Sykes, J.E. 2001. Canine leptospirosis – a re-emerging disease. In: Proceedings of the 19th Annual Meeting of the ACVIM: 763-765.

Twigg, G.J., Cuerden, C.M., Hughes, D.M., and Medhurst, P. 1969. The leptospirosis reservoir in British wild mammals. Veterinary Record 84: 424-426.

Ward, Michael P., Guptill, Lynn F., Wu, Ching Ching. 2004. Evaluation of environmental risk factors for leptospirosis in dogs: 36 cases (1997-2002). Journal of American Veterinary Medical Association 225 (1): 72-77.

Ward, Michael P. 2002. Clustering of reported cases of leptospirosis among dogs in the

United States and Canada. Preventative Veterinary Medicine 56: 215-226.

Wilkie, Ian W., Prescott, John F., Hazlett, Murray J., Maxie, M. Grant, van Dreumel, Anthony A. 1988. Giant Cell Hepatitis in Four Aborted Foals: A Possible Leptospiral Infection. Canadian Veterinary Journal 29: 1003-1004.

Williams, Deborah M., Smith, B.J., Donahue, J.M., and Poonacha, K.B. 1994. Serological and microbiological findings on 3 farms with equine leptospiral abortions. Equine Veterinary Journal 26: 105-108.

Wohl, J.S. 1996. Canine leptospirosis. Complete Small Animals 18: 1215-1224.

Zaitsev, S.V., Chernukha, Y.G., Evdokimova, O.A., and Belov, A.S. 1989. Survival rate of *Leptospira pomona* in the soil at a natural focus of leptospirosis. Journal of Microbiology, Epidemiology, and Immunobiology 2: 64-68.

Vita

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