Equine Leptospirosis Serorelevance in the Central and Bluegrass Regions of Kentucky from 1993 - 2015

Charlene Siza

University of Kentucky

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Equine Leptospirosis Seroprevalence in the Central and Bluegrass Regions of Kentucky from 1993-2015

CAPSTONE PROJECT PAPER

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ABSTRACT

BACKGROUND

Leptospirosis is a worldwide zoonotic bacterial disease of significant importance for both human and animal health. There are many sources of infection and shedding of the bacteria, including horses. There is a known occupational hazard for leptospirosis, especially in occupations that work directly with animals or animal products. This study examined the prevalence of equine leptospirosis in Kentucky in relation to trends over time and geographical distribution.

METHODS

Data was obtained from the University of Kentucky Veterinary Diagnostic Laboratory (UKVDL) on equine leptospirosis from 1993 through September 2015. Overall seropositive prevalence and prevalence stratified by serovar were calculated. Furthermore, distribution of positive and negative tests by month, year, and season was depicted graphically. The relationship with season and seroprevalence was further analyzed using chi square and logistic regression. Geographical distribution of prevalence was explored as well as relationship with environmental factors.

RESULTS

Based on samples submitted to the UKVDL, the seroprevalence of equine leptospirosis in the central and bluegrass regions of Kentucky was 12.23%. Leptospira interrogans serovar Grippotyphosa was the most common serovar in these data, followed by serovar Pomona. Positive tests were most common in the winter months of December and January. Fall had the
greatest of odds of having a positive test (OR 3.88 [95% CI: 3.54, 4.25]). The highest prevalence of seropositive results in 23 years occurred in 2012. Positive prevalence was geographically limited to five counties, with Woodford County having the highest prevalence based on horse population. Six out of nine farms that had positive results were close in proximity and in the same hydrologic unit.

CONCLUSION

The prevalence of positive leptospirosis has increased over the last 23 years in Kentucky and there is a significant correlation with season. Geographical distribution is focused on four bordering counties. Further research should be done exploring the effect of management practices and environmental exposure on leptospirosis seroprevalence in the equine population in Kentucky.
INTRODUCTION

Leptospirosis is a worldwide zoonotic disease of significant importance for both public and veterinary health (Verma, Stevenson, & Adler, 2013; World Health Organization (WHO), 2003; Haake & Levett, 2015). Leptospirosis is caused by a bacterial spirochete from the genus *Leptospira*. The organism is shed in the urine of animal carriers and can contaminate the environment and survive for a prolonged period of time in wet conditions (Verma et al., 2013; WHO, 2003). This is why it thrives in tropical and subtropical regions with heavy rainfall and flooding (WHO, 2003; Haake & Levett, 2015). The organism more commonly gains entry into its host through cuts and breaks in the skin and mucous membranes or transplacentally (Verma et al., 2013; WHO, 2003; Haake & Levett, 2015). Common sources of infection and shedding are rodents, cattle, pigs and dogs, although many other animals, including horses, can spread the disease (WHO, 2003).

Occupational exposure is a major concern with leptospirosis, especially among veterinarians, farmers, abattoir workers, and other animal related fields (Verma et al., 2013; Haake & Levett, 2015). Because animals and humans can be carriers and shed the bacteria without showing any clinical signs or symptoms, it is difficult to provide surveillance and estimate the actual number of infected animals and people. This also makes it hard to identify possible sources of infection without random testing (WHO, 2003). In order to improve leptospirosis surveillance and intervention programs, it is important to understand the regional intricacies of the disease as there can be significant geographical variation.

In horses, leptospirosis can also be asymptomatic, but it is commonly associated with recurrent uveitis, reproductive issues such as placentitis, stillbirths, and abortions, and in more
severe cases, jaundice, renal failure, and pulmonary hemorrhage (Verma et al., 2013; Hamond, Martins, Lawson-Ferreira, Medeiros, & Lilenbaum, 2013; Hamond, Martins, Lilenbaum, & Medeiros, 2012; Hamond, Pinna, Martins & Lilenbaum, 2014a). The purpose of this study is to characterize equine leptospirosis in the central and bluegrass regions of Kentucky through three main objectives. The first objective is to ascertain the prevalence of positive laboratory tests and identify the most common serovars in the equine population based on samples submitted. The second objective is to determine variation in the prevalence of leptospirosis by month, season, and year in this part of Kentucky. The third objective is to evaluate geographic case distribution and associations between the number of positive cases and environmental factors.

**LITERATURE REVIEW**

The purpose of this review is to delineate the knowledge base concerning equine leptospirosis in the published literature. This will not only aid in understanding the organism and how it causes disease in humans and animals, but also, more specifically, what is known about its effects on the equine population and the zoonotic potential that arises from this species. The literature referenced in this review was collected from journals and reports from major health organizations. A majority of the journal articles were found through the database PubMed by using the key words or phrases “equine leptospirosis” and “equine leptospirosis zoonosis”. Additional references were found from reviewing bibliographies of relevant articles and searching the websites of health organizations such as the World Health Organization, World Organisation for Animal Health (OIE), and the American Veterinary Medical Association.
Characteristics of *Leptospira* spp.

Leptospirosis is an important zoonotic disease that is found worldwide. It is caused by a bacterial spirochete called *Leptospira*. The genus *Leptospira* includes nine pathogenic species as well as five intermediate and six saprophytic species. Within those species, over 300 serovars have been identified and categorized into 25 serogroups (Guerra, 2009; World Organisation for Animal Health (OIE), 2014; Båverud, Gunnarsson, Engvall, Franzén, & Egenvall, 2009; Spickler & Leedom Larson, 2013). *Leptospira* is a gram negative organism that displays high motility and characteristics of an obligate aerobe. It has a diameter of 0.2 μm and a length of 6-20 μm and a characteristic coiled appearance with hooked ends (Guerra, 2009; WHO, 2003). It is usually visualized with silver staining under a darkfield or phase contrast microscope (Guerra, 2009; OIE, 2014; Spickler & Leedom Larson, 2013; WHO, 2003).

An important characteristic of *Leptospira* is that it can live for long periods in wet environments and therefore poses a risk for water transmission in endemic areas (Park, Gordon, Bech-Nielsen, & Slemons, 1992; Verma et al., 2013; Guerra, 2009). In fact, infection from contact with contaminated water is one of the more common modes of transmission. The organism invades the host through cuts or abrasions on the skin or directly through mucous membranes or conjunctiva (Verma et al., 2013; Guerra, 2009; Spickler & Leedom Larson, 2013). Exposure may also occur from direct contact with urine from an infected animal, transplacentally and in rare occasions, through breast milk and sexual contact (Guerra, 2009; Hamond et al., 2014b; Spickler & Leedom Larson, 2013; Båverud et al., 2009; Verma et al., 2013).

Leptospirosis can occur in most animals as well as humans. It has been reported in over 180 species, including reptiles and birds (OIE, 2014; Guerra, 2009; Båverud et al., 2009). Despite
this, transmission can only occur through mammals (Guerra, 2009). Certain mammals tend to
serve as hosts for specific serovars of *Leptospira*. These animals are called *maintenance hosts*
and may exhibit little to no clinical signs of disease. In turn, however, they can harbor the
bacteria in their kidneys or genital tract and shed the organism into the environment for months
to years. For example, dogs are the reservoirs for serovar Canicola and cattle are the reservoir
for serovar Hardjo. Rodents are a major source of transmission and are reservoirs for serovars
Icterohaemorrhagiae and Copenhageni; rodents can be chronic shedders and shed the organism
for life (OIE, 2014; Guerra, 2009; Spickler & Leedom Larson, 2013).

Leptospires have been found in every continent except Antarctica; however, the various
serovars have a significant regional variation. Some serovars are widespread while others may
only be predominant in certain parts of the world. In general, leptospirosis is more common in
tropical and subtropical climates, which are warmer and more humid. Regions where flooding
and heavy rainfall occur are also favorable locations for the organism (Finger et al., 2014;

**Disease Characteristics**

Leptospirosis in humans can have a wide range of symptoms. The incubation period ranges
from 2 - 30 days but is usually 1-2 weeks. Most of the time, cases are subclinical or very mild
and may appear similar to symptoms of influenza with occasional rash and conjunctival redness.
If the disease progresses to the second phase, patients can have more severe signs. These are
divided into icteric and anicteric forms. Most cases fall under the anicteric category (~90%) and
consist of aseptic meningitis, which causes stiffness in the neck and severe headache. Patients
can also, on occasion, develop uveitis with this form. The icteric form is more severe and can
lead to dysfunction in the liver, kidneys and lungs, and cause hemorrhages. These symptoms can progress to full kidney failure and death. Another less common sequela is pulmonary hemorrhage. Fatality rates vary dependent on a host of factors, but have been reported as high as 20% for the icteric form (Guerra, 2009; Verma et al., 2013; Spickler & Leedom Larson, 2013; WHO, 2003). If a woman is pregnant while infected with the bacteria, fetal death, stillbirth, abortion and, on rare occasions, congenital leptospirosis may occur (WHO, 2003).

Leptospirosis in animals demonstrates a similar variation in signs as that of human leptospirosis. As mentioned previously, animals serving as maintenance hosts usually show either mild signs or no signs at all. In incidental hosts, the signs may be more severe but usually start with mild symptoms such as general malaise or decreased milk production in cattle and sheep. These can progress to more severe acute kidney and liver injury and meningitis. Chronically affected animals can demonstrate reproductive, renal, pulmonary, and ocular issues (OIE, 2014; Båverud et al., 2009; WHO, 2003).

In symptomatic horses, although respiratory, renal and hepatic issues may occur, it is more common to see stillbirths, abortions, weak foals, and recurrent uveitis (Pinna, Martins, Hamond, Lilenbaum & Medeiros, 2011; OIE, 2014; J. M. Donahue, Smith, Redmon, and J. K. Donahue, 1991; J.M. Donahue et al., 1992; Park et al., 1992; Finger et al., 2014; Båverud et al., 2009; Verma et al., 2013). The ability of the organism to cross the placenta is a major concern for abortions in mares (Verma et al., 2013). In a Central Kentucky study by Donahue et al. involving aborted or stillborn fetuses and deceased neonates, the authors found that 2.5% or 15/594 of samples tested positive for leptospirosis. Maternal serum samples were also collected for 14 of the fetal samples that tested positive. Amongst those mares, there was a 100% seroprevalence of
In a similar study by Donahue et al. the following year, the prevalence in the aborted specimens was 4.4% (32/726) (J.M. Donahue et al., 1992).

**Diagnostic Testing for Leptospirosis**

Leptospires grow slowly in culture, and recovery rates are low; therefore, isolation of the leptospires from human tissue or body fluids is not routinely done. Serologic tests are available in specialized laboratories. Three primary methods are used to diagnose leptospirosis or to test for the *Leptospira* organism in tissue or fluid. These include: Microscopic Agglutination Test (MAT); Polymerase Chain Reaction (PCR); and Fluorescent Antibody Test (FAT).

MAT is the standard for serological testing of leptospirosis. The process uses live antigens from various leptospiral serovars to test for reactions with antibodies in the serum of a patient. This process has a high specificity so cross reaction with other bacterial species is not likely to occur. However, cross reaction within *Leptospira* serovars and serogroups does occur. This usually presents as a sample being positive for several serovars, although the infecting serovar generally has a higher titer than the others. Therefore, to get more accurate results, it is important to use serovars known to be endemic in the areas in which the test is being conducted. In the United States, the tests typically include the serovars: L. Bratislava; L. Canicola; L. Grippotyphosa; L. Hardjo-bovis; L. Icterohaemorrhagiae; and L. Pomona. MAT cannot distinguish between antibodies that were obtained from vaccination versus those that were obtained from exposure to disease (Wisconsin Veterinary Diagnostic Laboratory(WVDL), 2015; OIE, 2014; Båverud et al., 2009). This is more of a factor in species that have an approved vaccine such as cattle. Historically, a specific vaccine for horses has not been approved, although the cattle vaccine has been known to be used off-label in horses (Verma et al, 2013).
November 2015, however, Zoetis released the first USDA approved equine leptospirosis vaccine that had efficacy towards *L. interrogans* serovar Pomona. To avoid problems interpreting MAT results, veterinarians and laboratory personnel generally look for either a four-fold increase in titers in acute and convalescent sera or one elevated titer result along with clinical signs in the horse to be diagnostic of acute leptospirosis (OIE, 2014; Båverud et al., 2009). Seropositivity has generally been defined as being reactive at the 1:100 dilution level or above (WVDL, 2015; OIE, 2014; Park et al., 1992; Finger et al., 2014; Båverud et al., 2009). MAT may not identify some cases due to the transient nature of bacteremia as well as the tendency for carriers to harbor the organism in the kidneys or genital tract (OIE, 2014).

FAT testing is typically done with tissue samples from the fetal placenta, kidneys, or liver. The tissue is fixed to a glass slide and stained so that antibodies fluoresce under special microscopy. Results may be classified as rare, few, moderate and numerous. The FAT method is problematic when tissue has begun to autolyze or if there is significant contamination (Erol et al., 2014).

PCR testing has recently come into more frequent use. The procedure has a good sensitivity and specificity as well as the potential for rapid, early diagnosis. Real time PCR (RT-PCR) is faster and has fewer issues with sample contamination. This method has been validated more in human testing than in animal testing; however, there have been a few studies comparing its use to other methods of diagnosis (Pinna et al., 2011; OIE, 2014; Erol et al., 2014; de Abreu Fonseca et al., 2006). In a 2014 study by Erol et al. in Kentucky, 339 equine fetuses were tested by RT-PCR, FAT and MAT for leptospirosis. Among the 339 samples, 6.19% or 21/339 tested positive for the organism. Of the 21 cases, only RT-PCR was able to detect all of them, while FAT detected 18 and MAT detected 19. This provided further support for PCR as a sensitive tool for
diagnosis of leptospirosis. One issue with conventional PCR testing is that it cannot identify the organism at the serovar level (Erol et al., 2014).

Isolation of leptospires can provide a definitive diagnosis and identify the infecting serovar. The patient’s blood, CSF, dialysate, and urine can be cultured on a semisolid bovine serum albumin medium that contains 5-fluorouracil for 13 to 26 weeks. The cultures are checked every week using dark-field microscopy for presence of the organism (OIE, 2014; S. Ahmad, Shah & F. Ahmad, 2005). This organism is very fragile and can require a long incubation period, which hinders use of this test in routine screening (Pinna et al., 2011). The specific serovar has been historically identified using cross-agglutination absorption, however, there are now faster methods for this procedure such as the use of monoclonal antibodies (OIE, 2014).

**Occupational Risk of Leptospirosis**

Human exposure to Leptospires occurs environmentally and can be affected by weather conditions. Humans are also exposed through carrier or host animal species, in particular, through their occupation (Finger et al., 2014). In a 2009 report by Guerra, farmers, veterinarians, and abattoir workers were found to have increased risk for leptospirosis through animal contact. Outdoor workers, such as agricultural workers, miners, and fishermen, also had greater exposure to infected wildlife or contaminated water (Guerra, 2009). Tangkanakul et al. list rice-farmers, fishermen, sugar cane workers, sewer workers, and military personnel as having an increased risk for leptospirosis (Tangkanakul et al., 2000). These occupations may be more at risk in tropical areas with endemic leptospirosis. According to the Centers for Disease Control and Prevention (CDC), human leptospirosis was a reportable disease from 1947 until 1994 and then, just recently, became reportable again in 2014. As the disease was only reinstated as reportable
in the last couple of years, the most recent data available is from 1994 and there were 38 cases of human leptospirosis in the United States during that year (CDC, 2016). Therefore, changes in prevalence rates for human disease over the past 20 years cannot be determined.

In an epidemiologic outbreak investigation by Campagnolo et al. in 1998, nine cases of human leptospirosis were found in workers exposed to an infected swine herd at the University of Missouri- Columbia (Campagnolo et al., 2000). In a study by Park et al., samples collected from Ohio State University horses showed a seropositive prevalence of 20.6% (42/204). Samples collected from a nearby farm where cases of clinical leptospirosis had been found showed a prevalence of 72.7% (16/22) (Park et al., 1992). The high prevalence of equine leptospirosis on these farms along with reports of horses shedding the bacteria in their urine for weeks presented a potential occupational risk to farm workers and others in contact with these animals (Donahue et al., 1992; Guerra, 2009; Verma et al., 2013).

**METHODS**

**Dataset**

This is a cross sectional study utilizing data collected by the University of Kentucky Veterinary Diagnostic Laboratory (VDL) from 1993 through September of 2015. The VDL stores all records of routine testing done at the laboratory since 1993 in two database systems. The older database system is from 1993 through July of 2009 and the new system starts in mid-2009. For the purpose of this study, data was captured from both systems on leptospirosis testing among equines from 1993 to 2015. Although the database included leptospirosis tests on other species, this study focused only on equines. The data query from both systems produced 290,920
test results and included variables for analytical procedure (MAT, RT-PCR, FAT), test result, species, breed, serovar, specimen, animal ID, date, age of animal, gender, and zip code.

The basic inclusion criteria for this study including being of the equine species, having a leptospirosis test result, and having a recorded location and testing date. Primary exclusion criteria were not having a test result and not being from a Kentucky zip code. The original dataset of 290,920 measurements was further reduced by removing duplicate values (n= 56,323), entries with no result (n= 96), and entries that did not have a Kentucky zip code (n= 1537). This produced a dataset of 232,964 measurements. Another important step was to reduce the large number of tests per horse when an individual horse was tested for multiple serovars at the same time. Each serovar test was placed on a separate data line such that an individual horse might be tested for five different serovars and thus would have five separate lines in the dataset. This was simplified by narrowing the test count to include the horse as having a positive test if there were any positives and having a negative test if there were no positive results. This brought the final sample size 70,009 (Figure 1).

Some variables had many missing values. Age and gender had greater than 85% missing values and were excluded from the analysis. Specimen had 76% missing values and was excluded from the analysis due to lack of relevance to study objectives and because it was highly correlated with the type of test being done.

Variables were reclassified or created for statistical analysis. A binary outcome variable was created based on the test results. The data contained multiple methods of testing for leptospirosis including: MAT; RT-PCR; and FAT. With FAT, those that were categorized as rare, few, moderate, numerous and positive were coded as a “1”. The negative FAT results were coded as
a “0”. RT-PCR results were described as positive, having a numerical value, negative, not detected, and undetected. The results that were positive or had a numerical value were coded as “1” and the negative, undetected, and not detected were coded as “0”. MAT results are given as titers; there are different interpretations as to what dilution signifies a positive result. According to the World Organisation for Animal Health, or OIE, the titer of 1:100 is the cut-off for international trading purposes; the literature generally used a 1:100 cut off for studies as well. For the purpose of this study, a titer result of 1:100 and above was considered a positive and those <1:100 were considered negative.

Another variable that was reclassified was date. The original variable included day, month, year, and occasionally time, in the same cell. The date was broken up into a month column and a year column for ease of analysis for seasonality and year by year trend analysis. The four seasons were categorized using the Northern Meteorological Season classification, defining spring as March 1st-May 31st; summer as June 1st-August 31st; fall as September 1st-November 30th; and winter as December 1st-February 28th or 29th.

**Statistical Analysis**

The first study objective was to assess the prevalence of positive laboratory tests and identify the most commonly reactive serovars. The positive prevalence was calculated by dividing positive results by the total number of tests (8564/70,009). This calculation accounted for horses that were tested for multiple serovars at once by counting only one test for that specific date; it did not account for horses that were tested separate times in the same year.

Information on serovar was only available for MAT testing since conventional RT-PCR and FAT do not identify serovar. For this analysis, the original dataset minus exclusion criteria and
duplicates was utilized (n= 232,964). With testing of multiple serovars per horse, there was the potential for cross reactivity between serovars. Generally, the infecting serovar will react the most in agglutination tests and produce a higher titer than the others that are simply cross reacting (OIE, 2014). In order to ascertain major reacting serovars, the results were reclassified based on whether they were the highest titer in that batch or a secondary reactor. If a horse had two serovars that had the highest titer, both were classified as the major reactor. The distribution of serovars based on level of reactivity was obtained using these counts. Overall positive prevalence stratified by serovar was obtained by dividing the total number of positive results in each serovar by the total number of tests (n=232,964), regardless of level of reactivity.

The second objective of the study was to ascertain correlation between a positive or negative test result and month, season and year. For the distribution of seroprevalence by month, a simple stacked horizontal bar graph was created that depicted the testing counts for negative and positive tests over 12 months. Distribution by year from 1993 through September of 2015 was represented in a stacked horizontal bar graph.

Data on rainfall levels in Kentucky were used to determine which seasons had the most rainfall. These data were obtained from the National Oceanic and Atmospheric Administration (NOAA) database for Kentucky for the years 1993 through 2015 (National Oceanic and Atmospheric Administration, 2016). The amount of rain in inches was obtained by month and added to get totals for each three-month season. According to these calculations, the spring season had the most rainfall followed by summer, winter and fall, respectively. Chi square was performed to determine if the distribution of positive leptospirosis tests was independent of season. Next, simple logistic regression was performed with the binary result being the outcome and season being the explanatory variable. Spring was chosen as the reference month as it had
the most rainfall. The test produced estimated odds ratios between spring and the other three months as well as 95% confidence limits for each value. The analysis was done using SAS Version 9.4, SAS Institute, Cary, NC.

**Geospatial Analysis**

To evaluate the geographical distribution of positive leptospirosis tests in the central and bluegrass regions of Kentucky the zip code associated with the sample was used. Unfortunately, the zip code in the database could only be clearly assigned to the farm for 16,452 (23.5%) of the samples.

Using this smaller dataset, the owner zip code variable was used to create a county variable. Data for total horses and ponies by Kentucky county was available from the Agriculture Census which is conducted every 5 years. Since the study data ranged from 1993 to 2015, the census information from 1997, 2002, 2007, and 2012 was obtained, and the total horse and pony inventory counts for each five-year period were averaged to obtain a 20-year average by county. This allowed county prevalence rates to be calculated based on total number of horses by county.

Of the 16,452 records available with owner zip code, only 6,016 included farm name; these data covered 19 different farms. The latitude and longitude coordinates were obtained for each farm address in order to provide farm location on a map. The point data was layered over a 11-digit hydrologic unit shapefile that was obtained from the Kentucky Geography Network. County boundaries were added to aid in visualization of data. Data on bodies of water and streams were obtained from the Kentucky Geological Survey in order analyze association with surface water and the location of farms where positive leptospirosis samples were obtained.
Geospatial analysis was performed using Geographic Information System Version 10.3, Esri, Redlands, CA.

RESULTS

The final dataset contained 70,009 measurements, of which 8564 were positive. This yielded a period prevalence of 12.23% for this population between 1993 and 2015. The distribution of positives by serovar is illustrated in Table 1. Among the major reactors based on MAT, serovars Grippotyphosa and Pomona represented a majority of the results with 50.64% and 40.96%, respectively. The least reactive serovar was Hardjo with only 0.35% prevalence in the major reactor category.

Correlation between testing and year is described in Figure 2. The number of total tests remained relatively stable from 1993 through 2006 as well as the number of positives. There was a testing spike in 2007, however, the number of positive tests remained low. In 2009, the proportion of positive tests began to increase along with the total tests being performed. In 2012, the number of tests doubled and the proportion of positives was 2.7 times greater than the previous year. Those levels steadily decreased in subsequent years.

The distribution of testing by month is demonstrated in Figure 3. There was an obvious pattern in testing, with the majority of tests having occurred in December or January. There was a greater proportion of positive tests in December and January, as well, when compared to months in the middle of the year such as June, July and August.

Figure 4 shows how testing was distributed by season. Winter had the greatest number of total tests as well as the greatest number of positives. Summer had the lowest total test and positive test count. Analysis with chi square and logistic regression demonstrated a significant
(p = 0.0001) difference between all seasons when compared to spring as the reference, as shown in Table 2. A test had 3.88 times the odds of being positive in the fall as compared to the spring, followed by winter (OR 3.35) and then summer (OR 2.28).

Geospatial analysis revealed that the distribution of positive tests encompassed only 5 counties out of the 68 represented. The dataset used for this analysis included 1699 positive tests out of 16,452 measurements, which accounted for 19.8% of the 8564 positive tests from the original dataset. Table 3 describes the distribution of positives in those counties as well as the number of horse farms and individual horses present based on census data. Bourbon, Fayette, Lincoln, Scott, and Woodford counties accounted for 100% of the positive tests, 12.6% of the farms and 30.9% of the horses represented among the 68 counties in the dataset. In Map 1, the highest prevalence of positive tests by county total testing is Scott County, followed by Woodford County. In contrast, Map 2 shows that the county with the highest prevalence of positive tests by county total horse population is Woodford, followed by Fayette County.

Of the 1699 positive test results in the dataset, farm location was available for 1689 or 99.4%. Farm locations were mapped over hydrologic units and streams in Kentucky. Map 3 depicts the distribution of farms and shows that 6 out 9 farms with positive results are located close to where Scott, Fayette and Woodford county lines meet. These farms are all in the same hydrologic unit and relatively close to streams in the area. The 6 farms account for 1440 or 85.3% of the positive tests over the 23-year period in the study.

**DISCUSSION**

The prevalence of leptospirosis among horse populations can vary for a variety of factors such as climate and proximity to water or maintenance hosts. In a study by Hamond et al in Brazil, the seroprevalence among horses exposed to flooding was 47.8% (Hamond et al., 2013).
The tropical climate and rainy conditions in Brazil are favorable to having endemic leptospiral infection in the animal population. Studies specific to Kentucky found a prevalence of leptospirosis induced abortions of 2.5 to 4.4% (Donahue et al., 1991; Donahue et al., 1992). This study found a seroprevalence of 12.23% over a period of 23 years. This value might be misleading because when stratified by individual year, seroprevalence was as high as 34.2% in 2013 or as low as 0% in 1993. In contrast to previous studies in Kentucky, this study looked at seroprevalence among all ages tested and not just abortions specifically. This study did not control for serial testing on the same horse as it would have altered the prevalence analysis by month and season and possibly introduced a bias. Over the 23 years in this dataset, 22% of the horses were tested multiple times for leptospirosis either within the same year or over multiple years.

Stratifying the test results by year showed a distinct pattern. Overall testing remained relatively stable from 1993 to 2008, with the exception of a small increase in 1996 and larger increase in 2007. Both times, the testing level returned to the lower stable level the following year. In 2009, however, not only did the overall testing begin to increase, but the proportion of positives also began to increase. There was a dramatic increase in 2012, and although levels began to decrease in subsequent years, they were still above the average levels observed since 1993. There are several possible explanations for this sudden rise in positive tests. Surveillance bias could account for the change in seroprevalence if there was an increased demand for leptospirosis testing in the area, particularly among suspect horses. The serial testing seen in this region of Kentucky might overestimate the prevalence of positives as leptospiral antibodies may persist in the blood long after infection. Another potential explanation may have been improvement in diagnostic tests over time. In this dataset, a majority of tests were done using
MAT, which is the gold standard for screening. While improvements have been made in tests such as Real Time PCR, those tests do not account for enough of the dataset to have made a significant difference in the outcomes presented. The increased proportion of positive test results could also have been the result of a true upsurge in leptospirosis cases. This would not be able to be confirmed without additional data on clinical signs and medical history for each horse.

Analysis of testing by month demonstrated that testing was largely occurring in November, December and January. Most of the positive tests occurred in those months as well. Since 72% of the horses in this sample were identified as Thoroughbreds, this may have been a byproduct of the breeding season. The Thoroughbred industry set a universal birthday of January 1st for all foals registered into the breed. As such, the breeding season is set up to deliver foals as close to January 1st as possible. Leptospirosis induced abortions generally occur later in gestation (>6 months) in horses and could potentially take place in the fall or winter if the breeding season begins in February (Divers, 2015; Donahue et al., 1991; Donahue et al, 1992). This is not always the case, however, and it also does not account for leptospirosis testing submissions that are unrelated to abortions or stillbirths.

When analyzed by MAT, the most prevalent serovar in this population was *L. interrogans* serovar Grippotyphosa. Pomona was the second most reactive serovar. Previous studies in Kentucky found that serovar Pomona occurred more frequently, followed by Grippotyphosa (Donahue et al., 1991; Donahue et al., 1992). There can be significant geographical variation in predominant serovar, however. Grippotyphosa was also the most prevalent serovar in the equine species in studies in Tabriz, Iran and eastern Poland (Hassanpour, Monfared, Abdollahpour, and Satari, 2009; Wasinski et al, 2012). In contrast, two studies in Rio de Janeiro and Curitiba, Brazil showed serovar Icterohaemorrhagiae and serovar Copenhageni, which is in the
Icterohaemorrhagiae serogroup, to be the most common reactor in their study population (Finger et al, 2014; Hamond et al, 2013). Furthermore, a national study in Sweden and a study in Ohio found serovar Bratislava as the most common serovar (Båverud et al, 2009; Park et al, 1992). Serovar Bratislava has historically been thought of as host adapted to horses, however, there is still debate on whether it can be significantly implicated in disease. Understanding this variation and identifying prevalent serovars is important for prevention of the disease. A new equine leptospirosis vaccine was recently released in the United States for *Leptospira interrogans* serovar Pomona. This vaccine would not be helpful in controlling for other serovars and may, therefore, be less effective in reducing the burden of disease in certain regions where Pomona does not predominate. Although Grippotyphosa was a more prevalent test result in Kentucky, it may not necessarily mean it caused the most clinical disease or abortions. In fact, serovar Pomona tests tended to produce higher titers with greater than 71.8% reacting at 1:800 or greater. Grippotyphosa results at 1:800 or greater accounted for only 40% of its tests. Further research is necessary to understand how much impact serovar Grippotyphosa has on morbidity and mortality in the equine population and whether production of a bivalent vaccine should be pursued.

Climate was another significant contributor to the prevalence of leptospirosis. Distribution of positive test results was analyzed by season in order to ascertain any correlation with weather and testing positive. Spring, which had the most rainfall out of all seasons, had the lowest odds of having a positive test result. In contrast, fall had the greatest odds of having a positive test result, although it had the least rainfall. This result may be affected in part by the increased testing in the fall months. Further investigation into the environmental conditions and farm management practices may be beneficial in narrowing the cause of increased positive results in
the fall as compared to other seasons. Factors may include housing of horses, exposure to rodents, and travel between farms.

The geospatial analysis of seroprevalence of leptospirosis in Kentucky highlighted five counties where positive cases were found. Bourbon, Scott, Fayette, and Woodford counties bordered each other. Lincoln County was completely separate geographically from these four counties, but only 1 (2.3%) out of 43 samples from this county was positive. As the data used for geographical distribution accounted for only 23.5% of the original dataset, it may not provide an accurate representation of seroprevalence in the Central and Bluegrass regions of Kentucky. Despite not representing approximately three fourths of the testing data, it likely does correctly identify counties--Woodford, Bourbon, and Fayette--having potential for problems with leptospirosis. These counties not only have large equine populations in general, but also tend to own and work with expensive racehorses. Farms may be more apt to test for organisms that affect reproduction with those horses due to the high value of their offspring.

Out of the 19 farms in which farm location was known, only nine farms in Scott, Bourbon, Woodford, and Fayette counties accounted for all 1689 positive leptospirosis tests. Positive test results were mapped at the farm level to compare their location with hydrologic unit or watershed boundaries. Six of the farms were found in one watershed that covered the area where Scott, Woodford, and Fayette county lines met. The locations of these farms also appeared to coincide with nearby streams. Even though only a small portion of the data was used in this analysis, it may have shown a possible relationship between positive test results and hydrologic features near these farms that should be explored further. There may have been external factors that were confounding these results such as exposure to wildlife in areas near streams or woodlands.
Limitations

There were several limitations to this study. Although the dataset accounted for a large period of time and a large sample size, there were very few variables with adequate information for analysis. For example, the large amount of missing values for gender and age precluded these variables from statistical analysis. Information pertaining to farm environment, clinical signs and treatment was not available and would have been useful in interpreting results of serological testing. Serial testing and surveillance bias may have affected prevalence estimates in this region as well as affected when tests were performed. The lack of information on owner and origin of horses may have affected the geospatial analysis and caused a bias as to where leptospirosis prevalence was the highest. Finally, the potential cross reactivity between leptospiral serovars with MAT may have affected the prevalence of certain serovars over others.

Conclusion

Based on diagnostic laboratory submissions, exposure to Leptospira spp. in the equine species was prevalent in the central and bluegrass regions of Kentucky. Seropositivity had increased since 2009 for unknown reasons, although increased surveillance and serial testing may have affected this value. Serovars Grippotyphosa and Pomona accounted for the most seroreactivity among the equine population in this region, which was consistent with previous reports. Among the population represented in this sample, the positive tests for leptospirosis were concentrated in Woodford, Fayette, Scott and Bourbon counties, possibly on only a few farms. These results warrant further investigation into whether leptospirosis in Kentucky is truly concentrated in these counties on a few farms, and what factors including farm size, county horse
density, farm management practices and environmental characteristics such as hydrology and exposure to pests and wildlife, influence the transmission of this disease.
REFERENCES


APPENDIX

FIGURE 1: Data Management and Organization Algorithm.

- **Original Dataset**: 290,920 Test Results
  - 56,323 Duplicates
  - 234,597 Test Results
  - 96 Missing Results
  - 234,501 Test Results
  - 1537 No KY Zip Code
- **Dataset for Serovar Analysis**: 232,964 Test Results
  - 162,955 Extra Serovar Tests
- **Dataset for Time Analysis**: 70,009 Test Results
  - 53,557 No Owner Zip Code
- **Dataset for Map 1 & 2**: 16,452 Test Results
  - 10,436 No Farm Name
- **Dataset for Map 3**: 6,016 Test Results
TABLE 1: Distribution of Seroprevalence by Serovar for All MAT Tests from 1993-2015

<table>
<thead>
<tr>
<th>Serovar (MAT)</th>
<th>Major Reactor (%)</th>
<th>Minor Reactor (%)</th>
<th>Negative (%)</th>
<th>Total Tests</th>
<th>Overall Positive Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grippotyphosa</td>
<td>4472(50.64)</td>
<td>1439(71.49)</td>
<td>56617(25.49)</td>
<td>62528</td>
<td>2.54</td>
</tr>
<tr>
<td>Pomona</td>
<td>3617(40.96)</td>
<td>274(13.61)</td>
<td>58335(26.26)</td>
<td>62226</td>
<td>1.67</td>
</tr>
<tr>
<td>Icterohaemorrhagiae</td>
<td>338(3.83)</td>
<td>176(8.74)</td>
<td>30119(13.56)</td>
<td>30633</td>
<td>0.22</td>
</tr>
<tr>
<td>Canicola</td>
<td>231(2.62)</td>
<td>86(4.27)</td>
<td>35036(15.77)</td>
<td>35353</td>
<td>0.14</td>
</tr>
<tr>
<td>Hardjo</td>
<td>31(0.35)</td>
<td>38(1.89)</td>
<td>35560(16.01)</td>
<td>35629</td>
<td>0.03</td>
</tr>
<tr>
<td>No Classification (FA, PCR)</td>
<td>142(1.61)</td>
<td>--</td>
<td>6453(2.91)</td>
<td>6595</td>
<td>0.06</td>
</tr>
<tr>
<td>Grand Total</td>
<td>8831</td>
<td>2013</td>
<td>222120</td>
<td>232964</td>
<td>4.65</td>
</tr>
</tbody>
</table>

FIGURE 2:

Distribution of Equine Leptospirosis Testing\(^1\) by Year in Kentucky from 1993-2015\(^2\)

1. Data includes MAT, FAT and PCR testing.
2. For 2015, data is only available from January through September
Distribution of Equine Leptospirosis Testing\textsuperscript{1} by Month in Kentucky from 1993-2015\textsuperscript{2}

1. Data includes MAT, FAT and PCR testing.
2. For 2015, data is only available from January through September
FIGURE 4:

Equine Leptospirosis Testing\(^1\) by Season in Kentucky from 1993-2015\(^2\)

1. Data includes MAT, FAT and PCR testing.
2. For 2015, data is only available from January through September

TABLE 2: Distribution of Positive Equine Leptospirosis Tests and Estimated Odds Ratios with 95% Confidence Limits Stratified by Season.

<table>
<thead>
<tr>
<th>Season</th>
<th>Count of Positive Tests (% of Total, n = 70,009)</th>
<th>Odds Ratio</th>
<th>95% Wald Confidence Limits</th>
<th>Significance((\chi^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>596(0.85)</td>
<td>ref</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td>Summer</td>
<td>515(0.74)</td>
<td>2.28</td>
<td>(2.02, 2.58)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Winter</td>
<td>4363(6.23)</td>
<td>3.35</td>
<td>(3.07, 3.66)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fall</td>
<td>3090(4.41)</td>
<td>3.88</td>
<td>(3.54, 4.25)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total</td>
<td>8564(12.23)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 3: Distribution of Positive Leptospirosis Tests Compared with Farm and Horse Population by County in Kentucky

<table>
<thead>
<tr>
<th>County</th>
<th>Positive Tests (Total Tests)</th>
<th>Percent of Tests</th>
<th>Number of Farms(^1)</th>
<th>Number of Horses(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bourbon</td>
<td>118(918)</td>
<td>12.9</td>
<td>326</td>
<td>6769</td>
</tr>
<tr>
<td>Fayette</td>
<td>617(8677)</td>
<td>7.1</td>
<td>437</td>
<td>12627</td>
</tr>
<tr>
<td>Lincoln</td>
<td>1(43)</td>
<td>2.3</td>
<td>252</td>
<td>1400</td>
</tr>
<tr>
<td>Scott</td>
<td>132(648)</td>
<td>20.4</td>
<td>292</td>
<td>4286</td>
</tr>
<tr>
<td>Woodford</td>
<td>831(5210)</td>
<td>16.0</td>
<td>325</td>
<td>8541</td>
</tr>
<tr>
<td>All Others</td>
<td>0 (956)</td>
<td>0.0</td>
<td>11370</td>
<td>75137</td>
</tr>
<tr>
<td>Total</td>
<td>1699(16452)</td>
<td>10.3</td>
<td>13002</td>
<td>108760</td>
</tr>
</tbody>
</table>

\(^1\) Number of horses and farms is based on the Agriculture Census for 1997, 2002, 2007, & 2012.
1. A case is defined as testing positive on FAT or PCR or having a titer of 1:100 or greater on MAT.
1. A case is defined as testing positive on FAT or PCR or having a titer of 1:100 or greater on MAT.
MAP 3:

DUE TO CONFIDENTIALITY REASONS, THIS MAP HAS BEEN REMOVED
ACKNOWLEDGEMENTS

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BIOGRAPHICAL SKETCH

Charlene Renee Ballew Siza was raised in Cruzeiro do Sul, Acre, Brazil and moved to Mayfield, KY in 2000. She attended Murray State University in Murray, Kentucky where she majored in Pre-Veterinary Medicine and minored in Chemistry. She received her Bachelor of Science degree in 2009. She attended veterinary school at Auburn University College of Veterinary Medicine and received her Doctor of Veterinary Medicine degree in 2013. Upon completion of her DVM, she worked in clinical practice for one year before coming to the University of Kentucky to obtain a Master of Public Health in Epidemiology. Following graduation from UK, she will begin a two-year Epidemic Intelligence Service Fellowship with the Centers for Disease Control and Prevention.