Serological Evidence of Potential Occupational Exposure to Leptospirosis in Kentucky Horse Farm Workers and Veterinarians

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Abstract

Sera from employees and horses residing on farms affected (AF) by outbreaks of leptospirosis in Central Kentucky since 2006 were compared to farms unaffected (UF) by these outbreaks during this same time period. A total of six farms, three farms from each group, were compared (4 human subjects and 4 horses) for a total of 24 horses and 24 humans. Sera from a second group, veterinarians specializing in equine medicine (EV), were also analyzed. This group contained 24 veterinarians with a range of exposure levels from no known past exposure to individuals exposed five days previous to collection of sera. Each farm manager completed a survey assessing farm management of horses. In addition, veterinarians completed a survey regarding their equine medicine experience. Enzyme-linked immunosorbent assays (ELISA) showed an increased risk of seroconversion to Leptospira in 5 (22%) of 23 equine veterinarians compared to zero (0%) of 24 farm workers (p=0.05). Antibody levels for both sphingomyelinase 2 (Sph2) and leptospiral immunoglobulin-like protein A (LigA) were significantly increased (p=0.03 and p=0.05 respectively) in veterinarians. Indirect hemoagglutination (IHA) and microscopic agglutination (MAT) titers were negative for all individuals participating in the study. Risk for seroconversion did not appear to be influenced by the period of time from last exposure nor age of the individual. There is an increased risk for seroconversion to Leptospira, without the appearance of clinical disease, in EV compared to individuals working on Central Kentucky horse farms.

Introduction

Leptospirosis is a zoonotic infection of worldwide importance. Once thought to be contained it has emerged as one of the most widespread zoonotic disease in the world (1,2,3). It is readily spread from animal to animal and animal to man; however human-to-human transmission is considered rare. *Leptospira spp.* are found in over 160 mammals worldwide including dogs, cats, cattle, horses and sea lions (5,6). Leptospirosis is a major public health concern in South America and South east Asia, and is now considered a re-emerging disease in most developed countries (4). *Leptospira spp.* are found in over 160 mammals worldwide and vaccines at this time only exist for cattle, swine and dogs making infection in other mammals including humans a major health concern in many countries (5,6).

Horses are incidental hosts for several serovars of *Leptospira* (7). The bacterium is easily transmitted via urine and fetal membranes/fluids from horse to horse. In North America, transmission to horses is believed to be through contact with wildlife, or through food, water, soil infected with leptospiral bacteria. Those animals infected with leptospires will shed the organism in their urine contaminating ground water and soil (5, 6, 7). The disease is maintained in nature through chronic infection, shed in the animal's urine, which can last for months to years. Veterinarians, farmers, slaughterhouse workers, butchers and sewer workers are at risk for contracting the disease either through direct contact (urine or body fluids) or indirect contact (contaminated water or soil) (8). Individuals working on farms are generally considered at highest risk of contracting the disease.

The disease can be confused with common occurring infections and humans can remain asymptomatic, therefore misdiagnosis is common. Individuals infected with *Leptospira* often experience typical viral symptoms such as fever, headache, nausea, vomiting and abdominal pain (5,7). Those that seroconvert are unaware that they have been exposed and in individuals where the disease progresses, liver and kidney failure, and/or respiratory complications can occur. Mortality can be as high as 25%.

MAT is considered the standard method for diagnosis of leptospirosis (5,6). IHA is also widely used in human medicine, but test results can vary. Both are useful in detecting only active clinical cases as titers diminish rapidly post exposure (5,9). This study was carried out to determine the risks of seroconversion in farm workers and veterinarians on Central Kentucky farms comparing MAT, IHA and a non-commercial ELISA tests.

Methods

Data Collection

Six Farms
Central Kentucky horse farm
Three Affected (AF)
Three Unaffected (UF)
24 people (12 from each group)
24 horses (12 from each group)
Demographics and survey

Equine Veterinarians
Practice limited to horses only
Exposure from no known to recent
24 veterinarians (EV)
Demographics and survey

Positive Control Samples-Human
Positive MAT

Analysis

Human Samples
IHA*
MAT-Pomona, Grippotphosa**
ELISA-Lig A, Sph2, Pomona Lysate***

Equine Samples
MAT-Pomona, Grippotyphosa**

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Results

Survey results regarding demographic characteristics and farm management did not differ greatly between groups (Table 1). EV demographics showed a large percentage surveyed (95.8%) were involved in mare reproductive medicine, and only one individual had not been exposed to leptospirosis in the previous 3 years (Table 2).

Table 1 Farm Demographics and Management

Category	Unaffected Farms	Affected Farms
Average Age	27.4	34.4
Age Range	24-39	23-46
Female	5 (41.7%)	2 (16.7%)
Male	7 (58.3%)	10 (83.3%)
White	6 (50%)	8 (66.7%)
Other	6 (50%)	4 (33%)
Horses Traveled	Yes-all	Yes-all
New Horses on Farm	Yes-all	Yes-all
Topsoil/Bedding Changes	No-all	No-all
Water Source	City-all	City-all
Access to creeks or ponds	All	1 farm after heavy rain
Other animals	Cattle-1 farm	Cattle-2 farms
Wildlife	All types	All types
Number of foals born	Average 70 Range 46-90	Average 64 Range 30-95
Mares foal in stall	Yes-all	Yes-all

Table 2 Equine Veterinarian Demographics

Category	Equine Veterinarians
Average Age	41.7
Age Range	28-70
Female	8 (33.3%)
Male	16 (66.7%)
Caucasian	22
Other	2
Number of years in equine practice	Average-15.1 Range 1.7-47
Practice mare reproductive medicine	95.8% yes
Percentage of practice limited to mare reproductive medicine	Average 69.5% Range 0-100%
Exposed to leptospirosis in previous 3 years	95.8% yes
Diagnosed with renal disease	None

All study individuals, regardless of occupational group, had titer levels considered negative for both MAT and IHA (<1:100 and <1:50, respectively). ELISA results for all farm workers were considered negative. There was no significant difference between antibody levels to the Pomona lysate for veterinarians and farm workers (p=0.16). LigA data verged on significance at p=0.06, and differences for Sph2 were significant at p=0.03. Fisher's exact test between farm workers and veterinarians comparing seroconversion was significant (p=0.02, 95%CI 1.645 to 3.309). Results are listed in Table 3.

Table 3 Leptospira-Specific Antibody ELISA in Human Sera

Sample	ELISA OD		
	LigA (Lk90)	Sph2 (Lk73.5)	Pomona Lysate
Farm Workers	0.31[a] (0.22-0.63)[b] 0.11[c]	0.21 (0.15-0.48) 0.09	0.34 (0.21-0.43) 0.07
Equine Veterinarians	0.39* (0.24-0.87) 0.18	0.24** (0.15-0.55) 0.11	0.34 (0.21-0.66) 0.14
Positive Control	1.36 (0.52-1.62) 0.67	0.49 (0.22-0.99) 0.27	0.47 (0.29-0.64) 0.16

[a]Mean, [b]range, [c]standard deviation, *p=0.05, **p=0.03

The majority of the veterinarians had little or no elevation in serum antibody (OD) level; however 5 had an increase in OD to two or all three of the antigens, which showed a strong correlation to each other. The correlations between the ELISA results of the three antigens are as follows: Pomona lysate to LigA r=0.88; Pomona lysate to Sph2 r=0.91; and LigA to Sph2 r=0.93. Results are in Figures 1-3. Veterinarians were classified according to their ELISA responses, as belonging to either a low or high reactivity group. Those veterinarians in the high group had significantly higher OD levels for all three antigens. Results are listed in Table 4.

Table 4 Comparison of ELISA OD* Levels for Grouping of Veterinarian Sera Showing High and Low Reactivity

Sample	ELISA Optical Density		
	Lig A	Sph2	Pomona Lysate
Veterinarians with low reactivity (n=18)	0.35[a] 0.09[b]	0.24 0.04	0.31 0.06
Veterinarians with high reactivity (n=5)	0.69* 0.16	0.47** 0.07	0.63*** 0.08

*Z=0.002; **Z=0.001; ***Z=0.0009;
[a]Mean,[b]standard deviation

Figure 1 Regression Correlation LigA to Sph2

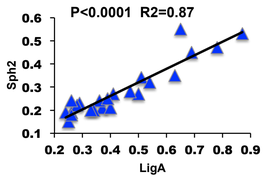


Figure 2 Regression Correlation Pomona Lysate to Sph2

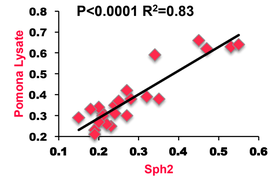
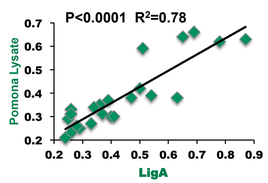


Figure 3 Regression Correlation Pomona Lysate to LigA



No horses from UF were positive for leptospirosis compared to 3/12 (25%) from AF (positive is considered a titer level of >1600 for any of the serovars). Low levels of exposure (titer levels 1:100 to 1:1600) were seen in both groups; horses with high titers can also show a cross reaction with other serovars; the serovar with the highest titer is considered the primary serovar.

Conclusions

ELISA showed an increased risk of seroconversion to *Leptospira* antigens in veterinarians compared to farm workers. This study suggests LigA and Sph2 are expressed during sub-clinical infection and are useful as markers in individuals who have been exposed to and sub-clinically infected with *Leptospira*. LigA is surface-expressed, Sph2 is secreted, and both are implicated in the pathogenesis of leptospirosis (10,11,12).

Antibodies to both Sph2 and LigA have been shown to increase in horses and humans with leptospirosis (12,13). LigA antibody has also been shown to increase in horses and humans with leptospirosis (12). It is possible that some or all of the veterinarians that had increased ODs could have experienced a mild case of leptospirosis; however none of the veterinarians indicated they had recently been ill. There were no differences between the low and high groups in age, length of time practicing equine medicine or time since last exposure. To date there are no studies known to us that demonstrated these antibodies in people who have been exposed, but not diagnosed with leptospirosis. This is also the first study to show seroconversion in veterinarians in strictly equine practice.

The sample size in this study was small. To more accurately determine the influence of exposure level, a larger sample size would have to be employed with collection of several sera over a period of time. Although there was no evidence of clinical disease, exposures significant enough to cause seroconversion are of possible concern. ELISA appears to be more sensitive then MAT and IHA in detecting past exposure to *Leptospira*. Further investigation is needed to validate the findings. Additionally, research is also required on the potential role of LigA and Sph2 in vaccines for both humans and animals.

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