The Prevalence of Equine Leptospirosis in New York State

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*We carried out a cross-sectional study to determine the seroprevalence of seven different serovars of *Leptospira interrogans* in horses in New York State and examine the geographic distribution of the disease in the state. A random sample of 2,967 horses was selected from the equine population in New York State using the 1988 New York State Equine Census as a sampling frame. The samples were tested for the presence of antibodies against seven serovars: *L. pomona*, *L. hardjo*, *L. icterohaemorrhagiae*, *L. grippityphosa*, *L. canicola*, *L. autumnalis*, and *L. bratislava*. The microscopic agglutination test was used to determine seropositivity at a range of dilutions from 1:100 to 1:12800. A titer of ≥ 100 was considered positive. We also examined the association between seroconversion at higher (≥ 1600) titers among those serovars. Cluster analysis was used to examine the distribution of the disease for special pattern or clustering. The following prevalences were determined: *L. pomona* (7.6%), *L. hardjo* (1.0%), *L. icterohaemorrhagiae* (11.3%), *L. grippityphosa* (6.5%), *L. canicola* (16.2%), *L. autumnalis* (35.1%), and *L. bratislava* (40.7%). The majority of the titers for all serovars were relatively low. Most of the samples tested positive for *L. pomona* had titers ranging from 100 to 400. For all the other serovars, most samples tested positive had titers ranging from 100 to 200. We found significant associations of seroconversion between all pairs of serovars except *L. grippityphosa* and *L. pomona*, and *L. grippityphosa* and *L. autumnalis*.*

Key words: epidemiology, equine, leptospirosis, prevalence

J. Equine Sci.
Vol. 9, No. 4
pp. 119–124, 1998

Leptospirosis is a zoonotic disease affecting humans, domestic and wild animals [13, 15]. It is prevalent worldwide and is caused by a bacterium species *Leptospira interrogans*, which is subdivided into pathogenic serovars. Serologic evidence of exposure to *L. interrogans* is common in horses, and horses may be a maintenance host for *L. bratislava* [10, 17]. Leptospirosis can cause clinical disease in horses [12, 23]. Clinical signs may include fever, jaundice, anorexia, and lethargy [12, 23]. Leptospirosis is associated with equine uveitis [14, 19, 23], renal dysfunction [4] and liver dysfunction [1, 14]. Other important consequences of *Leptospira* infection in horses are abortion and stillbirth [2, 6–8, 10, 16]. It appears that the reported number of abortions and stillbirths due to *L. interrogans* is increasing [2, 3, 7]. The prevalence of leptospirosis in different countries and

regions is reported to vary widely [10, 13, 25, 27, 28]. Little has been done to examine the disease in horses of New York State. The objectives of our study were to: 1) estimate the prevalence and distribution of seven serovars of *L. interrogans* in the horses of New York State; and 2) examine the association between those serovars.

Methods and Materials

Study Population

The study population was selected from the 39,000 equine operations in the 1988 New York State Equine Census [21]. In order to recruit at least 1% of the target population, a total of 3,000 equine operations were randomly selected and solicited by mail to participate in this study. Of these, 864 operations agreed to participate. Five hundred and seventy-two operations consisting of 2,967 horses were included in this study. The remaining

This article was submitted March 11, 1999 and was accepted March 19, 1999.

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Fig. 1. The distribution of equine operations included in the prevalence study of *L. interrogans* serovars in New York State.

292 farms which we were unable to visit, in which 1,695 horses resided, were evenly distributed throughout the state. We had hoped to visit them in an attempt to have a larger representation of those counties with small equine populations but we were not able to visit the operations because the project was terminated in 8/93.

Data collection

Equine operations enrolled in the study were visited to collect blood samples and administer a questionnaire. The questionnaire was developed to collect geographic, demographic management, and health data on the study population. The questionnaire was administered by personal interview. The farms were visited from 5/91–8/93, and an effort was made to visit different regions of the state from week to week to prevent seasonal and geographical biases. Blood samples were collected into sterile vacutainers and stored in ice while transported to the New York State Diagnostic Laboratory, Cornell University. A total of 24 to 120 samples were collected and processed per week. Figure 1 shows the locations of the farms visited in New York State.

Serology

Sera were separated and stored at -70°C until tested. Using the microscopic agglutination test (MAT) for seropositivity to the seven serovars of: *L. pomona*, *L. hardjo*, *L. icterohaemorrhagiae*, *L. grippotyphosa*, *L. canicola*, *L. autumnalis*, and *L. bratislava* [20]. All sera were initially screened at a dilution of 1:100. Those with a positive reaction were titrated in a serial two-fold dilution and

the end-point titer was recorded. All sera reacting to a dilution of $\geq 1:100$ were considered positive. The same observer determined all of the results of the MAT tests.

Statistical analysis

The prevalence of specific serovars of *L. interrogans* was computed as the proportion of samples that tested positive in the MAT for a specific serovar of all samples examined for that serovar. The county specific rate was computed as the proportion of seropositive samples among all samples from each county. These proportions were plotted, using a computerized graphics program [24].

The association between seroconversion in a horse at higher (≥ 1600) titers among those serovars, as an indication of multiple exposure, was evaluated using the chi-square or fisher-exact tests (depending on the number of observations) in BMDP [5].

Results

Figure 2 shows the prevalence of the seven serovars of *L. interrogans* in the study population. The prevalence of these serovars varied from 1% for *L. hardjo* to 41% for *L. bratislava*. One thousand six hundred and fifty nine horses tested (56%) were seropositive to at least one of the serovars.

The county specific prevalence for *L. pomona* is shown in Fig. 3. No samples were collected from Hamilton, Nassau, or Rockland counties. All other counties had at

Fig. 2. The prevalence of *L. interrogans* serovars in the equine population of New York State.

Fig. 3. The county-specific prevalence of *L. pomona* in the equine population in New York State.

least seven samples. Only one county had a prevalence above 20% (Livingston; prevalence = 21.3%). The majority of the counties had a prevalence above 6%; these counties are located primarily in central and western New York State. On the other hand, the distribution of *L.*

bratislava appeared to have no distinct pattern throughout the state (Fig. 4). Montgomery and Lewis counties had the highest prevalence for this serovar (67 and 63%, respectively).

Table 1 shows the distribution of the titers among the

Fig. 4. The county-specific prevalence of *L. bratislava* in the equine population in New York State.

samples that tested positive. The majority of the titers for all serovars were relatively low. Most of the samples tested positive for *L. pomona* had titers ranging from 100 to 400. For all the other serovars, most samples testing positive had titers ranging from 100 to 200. *L. pomona* and *L. autumnalis* were the only serovars which had samples whose end-point titers were at a dilution of 1:12,800.

Table 2 shows the p-values for the association between two serovars at higher (≥ 1600) titers, as an indication of multiple exposure. There were significant associations between all pairs of serovars except *L. grippityphosa* and *L. pomona*, and *L. grippityphosa* and *L. autumnalis*.

Discussion

The objectives of the study were to determine the prevalence of seven *L. interrogans* and determine their distribution throughout New York State. A dilution of $\geq 1:100$ was used as a cut-off point for seropositivity. Most laboratories using the MAT test as a diagnostic tool for serology in equine leptospirosis consider a dilution of 1:100 as positive [26]. There is a general belief that infections can be identified and non-specific reactions

are less likely to occur at this dilution [26].

Figure 1 shows the wide range of prevalence of seven serovars of *L. interrogans* in New York State. *L. hardjo* has the lowest prevalence of 1%. Of all horses tested, 40.7% tested positive to the serovar *L. bratislava*. This high prevalence suggests that horses might be a reservoir host for this serovar. However, there have been studies that have indicated an association between disease and infection of *L. bratislava*, but it is unclear what the significance is of this association [10]. Figure 2 shows that *L. bratislava* is widely distributed throughout New York State, with prevalences in Lewis and Montgomery counties greater than 60%. There is no distinct pattern to the distribution of this serovar throughout the state. A high number of horses also reacted to *L. autumnalis* (35.1%), but it is not clear what role this serovar plays in the disease, or if horses may serve as a maintenance host for this serovar.

L. pomona is the serovar often associated with clinical and pathological disease in the horse [2, 3, 10, 16, 23], and it is also widely distributed throughout the state (Fig. 3), although the overall prevalence (7.6%) was lower than that of *L. bratislava*. Most counties had a prevalence of *L. pomona* greater than 6%; these are located primarily in Central and Western New York. We do not believe

Table 1. The distribution of the positive titers of the seven serovars of *L. interrogans* in the horses in New York State

Serovar	Titer								Total number
	100	200	400	800	1600	3200	6400	12800	
<i>L. pomona</i>	23.5	20.8	19.5	16.8	12.8	3.5	1.3	1.8	226
<i>L. hardjo</i>	83.9	9.7	3.2	3.2	0	0	0	0	31
<i>L. ictero.</i>	67.5	25.4	8.4	3.0	0.9	0.9	0	0	335
<i>L. grippo.</i>	53.1	28.4	8.8	7.2	2.1	0.52	0	0	194
<i>L. canicola</i>	51.3	31.9	12.5	2.5	1.0	0.42	0.42	0	480
<i>L. autumnalis</i>	53.3	27.1	10.0	5.6	2.0	0.38	0.38	0.19	1041
<i>L. bratislava</i>	50.0	30.5	12.2	4.4	2.0	0.5	0.5	0	1208

Table 2. The significance of association between two serovars of *L. interrogans* from samples with positive titers ≥ 1600

Titer	<i>L. pomona</i>	<i>L. ictero.</i>	<i>L. grippo.</i>	<i>L. canicola</i>	<i>L. autum.</i>	<i>L. bratis.</i>
<i>L. ictero.</i>	0.0862	—	0.0117	0.0000	0.0031	0.0000
<i>L. grippo.</i>	0.9277	0.0117	—	0.0175	0.9277	0.0649
<i>L. canicola</i>	0.0073	0.0000	0.0175	—	0.0002	0.0000
<i>L. autum.</i>	0.0000**	0.0031	0.9277	0.0002	—	0.0000*
≥ 1600 <i>L. bratis.</i>	0.0000**	0.0000	0.0649	0.0000	0.0000*	—

**p-value calculated using chi-square test.

that this pattern is due to direct transmission among horses; however, if we were to speculate, the pattern may be due to geographical variability in soil type, climate, or wildlife distribution [2, 3]. Livingston, Tompkins, and St. Lawrence counties are those counties with the highest prevalences of *L. pomona*, with Livingston county having over 20% of the horses testing positive.

Our prevalence findings agree with previous studies conducted [10, 18, 28]. However, it is important to keep in mind that the prevalence of serovars can vary among regions (due to climate and geographical variations). Also, sampling biases may occur. This study was conducted with a true random sample of horses from New York State to minimize sampling bias.

In this study we found that 56% of the horses tested positive to at least one of the seven serovars. Many horses tested positive to multiple serovars. It is possible that there was multiple exposure to more than one serovar, but it also possible that some cross-reactivity occurred between the serovars. This could also help explain the abundance of low titers, as shown in Table 1. Because *L. hardjo* has a low prevalence of 1%, and the majority of positive samples had a titer of 100, this could indicate cross-reactivity, and not true infection. Since it is believed that cross-reactivity occurs predominately in lower titers we based our evaluation of the associations between serovars on samples which reacted at a dilution of

$\geq 1:1600$. We expect that any significant associations are based on multiple exposure to serovars and not cross-reactivity. We found that multiple exposure is both common and probable. Previous studies have also found multiple infection to be common [14, 28]. All pairs of serovars were found to be significantly associated with one another except for the pairs of *L. grippotyphosa* and *L. pomona* (p-value = 0.9277), and *L. grippotyphosa* and *L. autumnalis* (p-value = 0.9277).

The MAT test is a subjective test. To carry out this diagnostic procedure, the observer must determine if at least 50% of the antigen has agglutinated with the antibodies in the serum. In order to reduce observer variability in our study, only one observer (the senior author), was used to determine the results of all of the tests.

Studies should be continued to further understand the epidemiology of this disease. A better understanding will lead to the development of recommendations for horse owners on how to avoid equine *Leptospira* infection and improve preventive measures. This may not only help prevent equine infection, but infection of other mammals as well, including humans.

Acknowledgment

This research was supported by funds from the Unrestricted Alumni Funds, College of Veterinary Medicine, Cornell University.

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