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## SEROSURVEY OF LEPTOSPIROSIS IN FERAL HOGS (*SUS SCROFA*) IN FLORIDA

Jenifer Chatfield, D.V.M., Michael Milleson, M.S., Robyn Stoddard, D.V.M., Ph.D., Duy M. Bui, B.S., and Renee Galloway, M.P.H.

**Abstract:** *Leptospira* is a global pathogen of emerging public health importance in both developing and industrialized nations and can infect almost all mammalian species, including humans. As suburbanization and the popularity of outdoor recreational activities increases, so do human–wildlife and companion animal–wildlife interfaces. Florida offers a tropical climate favorable for outdoor activities and a semirural landscape that sustains an abundant feral hog population. Because no survey of leptospirosis in feral hogs (*Sus scrofa*) in Florida has been published to our knowledge, we sought to establish preliminary seroprevalence of leptospirosis exposure in feral hogs in Florida. Blood samples were collected opportunistically from 158 male and 166 female feral hogs taken at managed hunts and by permitted trappers in the northern, central, and southern regions of Florida. Samples were then analyzed using the microscopic agglutination test (MAT) for antibody titers to 20 *Leptospira* serovars representing 17 serogroups. A titer of >1:100 was considered positive; 33% (107/324 total samples) were positive to at least one serovar, and 46% of those were positive to multiple serovars. Antibodies to *L. interrogans* serovar Bratislava strain Jez Bratislava (serogroup Australis) was the most common, with 18% (58/324) testing positive for antibodies. These initial data indicate that there is a significant possibility of feral hogs having a larger role in the complex etiology of leptospirosis in Florida than historically estimated and that further investigation is warranted.

**Key words:** feral hog, Florida, leptospirosis, *Sus scrofa*, zoonosis.

### INTRODUCTION

Leptospirosis is a pathogen of emerging public health importance in both developing and industrialized nations, with symptoms of infection ranging from subclinical to renal failure and, in some cases, death. *Leptospira* spp. can infect almost all mammalian species, including humans, and has caused severe illness and death in the United States in the last 10 years.<sup>15,17,22</sup> Many wildlife species have been implicated as reservoirs for the bacteria, including squirrels, raccoons, opossums, and feral hogs.<sup>6</sup> As suburbanization and the popularity of outdoor recreational activities increases, so do human and companion animal-wildlife interfaces. Certainly, wildlife were implicated as the source of environmental contamination that led to infection in some recent

human cases of leptospirosis in the United States.<sup>22</sup> However, no survey of evidence of exposure to leptospirosis in feral hogs (*Sus scrofa*) in Florida has been published to our knowledge, despite the fact that feral hogs can serve as maintenance hosts for *Leptospira* spp. and continue to shed the bacteria in urine after infection. Because Florida has one of the most abundant populations of feral hogs in the United States,<sup>9,13</sup> a tropical climate, and significant opportunity for human contact with contaminated water in semirural areas due to recreational activities, we sought to establish preliminary seroprevalence of leptospirosis exposure in feral hogs.

### MATERIALS AND METHODS

Blood samples were collected opportunistically from 158 male and 166 female (324 total) feral hogs killed at managed hunts and by permitted trappers throughout Florida. Age was estimated based on dentition; 118 adult male and 135 adult female and 40 subadult male and 31 subadult female hogs were sampled.<sup>16</sup> Samples were obtained <3 hr postmortem from the orbital sinus or via cardiac stick using a 16-gauge needle and 35-ml syringe (Monoject, Covidien, 15 Hampshire Street, Mansfield, Massachusetts 02048, USA). Samples were immediately placed in serum separator tubes (BD, 1 Becton Drive, Franklin Lakes, New Jersey 07417, USA), placed in a cooler,

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transported to the U.S. Department of Agriculture field office (where the samples were centrifuged within 12 hr and serum transferred into cryovials) (Nalgene,<sup>75</sup> Panorama Creek Drive, Rochester, New York 14625, USA), and then stored at  $-20^{\circ}\text{C}$ . Samples were then tested at the Centers for Disease Control and Prevention in Atlanta, Georgia, using the microscopic agglutination test (MAT) to detect antibody titers against 20 *Leptospira* serovars representing 17 serogroups.<sup>3</sup> Samples with  $\geq 50\%$  agglutination on the screen were serially diluted and titrated with each positive antigen. The titer reported was the reciprocal of the last well showing at least 50% agglutination using dark field microscopy.

## RESULTS

Thirty-three percent of hogs sampled (107/324) were positive to at least one serovar. Of those, 46% (50/107) were positive for antibodies to multiple serovars. *L. interrogans* serovar Bratislava strain Jez Bratislava (serogroup Australis) was the most common serovar for which antibodies were detected, with 18% (58/324) testing positive for antibodies to this serovar. See Table 1 for listing of serovars assayed and sample results. No correlation between positive titer, age, sex, or sample collection location was evident. Titers  $\geq 1:800$  were detected in nine animals, with four samples having titers of 1:1,600. Typically, antibody titers of  $\geq 1:800$  are considered evidence of recent or current infection.

## DISCUSSION

Leptospirosis is a widespread bacterial zoonosis that is transmitted directly between hosts or indirectly through environmental contamination. These bacteria thrive in warm, moist environments, and hosts become infected most commonly through ingestion of contaminated food or water. The bacteria can also enter a host through mucus membranes or abraded skin. It is possible for the organism to penetrate intact skin if the skin has been immersed in water for a long period. Additionally, the bacteria can be acquired after aerosolization of contaminated urine or water. In tropical climates, such as Florida, many people pursue outdoor activities involving water directly, such as swimming, boating, and fishing, and indirectly, such as adventure racing, hunting, and farming. Thus, Florida's human population has substantial contact with the environment and wildlife. Florida also has one of the highest feral hog populations nationwide, estimated  $>500,000$

individuals.<sup>9</sup> Although many recent serologic surveys of leptospirosis in free-ranging wildlife have been performed,<sup>1,2,10-12,14,18,20,21,23,24</sup> no data are readily available on seroprevalence of leptospirosis in the feral hog population of Florida, despite the state's popularity as a tourist destination and escalating number of organized outdoor activities. Traditionally, raccoons, squirrels, rats, and other wildlife species are credited as the reservoirs for leptospirosis, with rodents being most often implicated.<sup>6</sup> However, as the feral hog population continues to expand across the United States and to increase in Florida, it is important to determine if a concurrent increase in environmental contamination with pathogenic leptospires is possible. These data indicate that feral hogs in Florida could serve as a significant source of environmental contamination by *Leptospira*, because 33% of those sampled were positive for at least one serogroup. These positive results do not necessarily indicate that the hog was actively shedding the bacteria at the time of sample collection, but they certainly indicate previous exposure and potential infection with possible bacterial shedding prior to death. These data indicate higher prevalence in Florida's feral hog population when compared with populations recently surveyed in Japan,<sup>24</sup> Kansas,<sup>8</sup> Northern Spain,<sup>5</sup> and Italy.<sup>4</sup> This could be a result of multiple environmental factors present in Florida, such as warm climate, greater annual rainfall, and more wetlands, conducive to increased survival time for the bacteria. Further investigation of environmental factors and a greater sample size would be necessary to determine the definitive cause for the apparent higher seroprevalence in Florida's hog population. Additionally, urine culture would clarify if serology correlated with active *Leptospira* spp. shedding. A recent serosurvey of feral hogs in the Great Smoky Mountains National Park, USA, had 44% samples positive for one of five tested serovars and 89% of positive samples were positive to 5/5 serovars.<sup>19</sup> However, the sample size in that survey ( $n = 108$ ) was considerably smaller than in this investigation ( $n = 324$ ), so the higher prevalence may be artifactual.

Outdoor activities such as adventure races, water sports, hunting, and others are very common in Florida. A recent leptospirosis outbreak in race participants may have been a result of swamp waters contaminated with infected hog urine.<sup>22</sup> Although a direct causal relationship was not established in these adventure racers, environmental evidence of routine hog use, such as rooting areas, were noted and many racers

**Table 1.** Total samples positive for antibodies to listed *Leptospira* spp. serovars.

<i>Leptospira</i> spp. serovar	No. samples positive
Australis	25
Bratislava	58
Autumnalis	37
Canicola	2
Celledoni	3
Cynopteri	6
Djasiman	4
Grippotyphosa	15
Icterohaemorrhagiae	37
Mankarso	3
Georgia	4
Pomona	4
Alexi	4
Pyrogenes	3
Ballum	0
Bataviae	0
Borincana	0
Javanica	0
Wolffi	0
Tarassovi	0

reported being chased by hogs during the event. At the time of this outbreak, no leptospirosis surveillance was done in local wildlife. However, current data indicate positive titers in the hogs in the affected county. It seems plausible that feral hogs contributed to the leptospirosis exposures in these racers.

Many of the samples included in this study were collected postharvest at population management hunts in environmentally sensitive areas. Thus, the hunters certainly were at risk for exposure to *Leptospira* spp., as well as other zoonotic pathogens, such as *Brucella* spp., when butchering the affected hogs. Educational material regarding potential zoonoses and feral hogs were made available to hunters, who also received verbal encouragement to use appropriate personal protective equipment when processing the hogs. However, as the popularity of recreational hog hunting increases so does the potential for increased exposure to swine-related zoonoses. Thus, it is important to establish seroprevalence of zoonoses, such as leptospirosis, in feral hog populations.<sup>7</sup>

These initial data indicate a significant possibility of feral hogs having a larger role in the transmission of leptospirosis in Florida than historically estimated. Certainly, wild hogs as a source of disease for humans and other animals is hardly novel. However, the role of the feral hog in

the complex etiology of leptospirosis in Florida is worthy of further investigation.

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