Viral Disease Surveillance in Select Wildlife Populations in Mississippi

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ABSTRACT Raccoons and opossums are quickly becoming a common sight in urban areas, providing a higher risk of human exposure to zoonotic pathogens. Feral swine are also becoming more common and problematic for many. We conducted serological surveys for avian influenza (AI), West Nile virus (WNV), rabies virus (RV), and canine distemper virus (CDV) in raccoons (n=119), opossums (n=126), and feral swine (n=105) in two regions of Mississippi. Raccoons had antibodies to all four viruses, while feral swine had antibody responses to WNV and opossums exhibited responses to WNV and RV. Active wildlife disease surveillance is a vital aspect of wildlife damage management and allows for the opportunity to detect possible disease threats and act accordingly. If potential disease threats can be caught early, the threat of interspecies transmission can be reduced.

KEY WORDS avian influenza, distemper, Mississippi, rabies, West Nile virus

Over half of infectious diseases known to be pathogenic to humans are zoonotic (Taylor et al. 2001). The ability of pathogens to infect multiple species is of extreme importance to public health, economic stability, and wildlife conservation (Cleaveland et al. 2001, Lafferty and Gerber 2002). To determine pathogenicity and range of hosts of infectious agents, continuing surveillance of known and potential animal reservoirs is needed (Zinsstag et al. 2007).

Agriculture production and human waste receptacles provide wildlife with convenient meals, increasing direct contact with humans, and providing a higher risk of human exposure to zoonotic pathogens. For purposes of this study, we focused on specific mammal species that are commonly seen and have direct contact with humans, providing a direct threat to human health. In both rural and urban areas of Mississippi, raccoons (*Procyon lotor*) and opossums (*Didelphis virginiana*) are common, widespread, and likely to have the most direct contact with humans (Junge et al. 2007, Suzan and Ceballos 2005). Feral swine (*Sus scrofa*) are becoming a nuisance to farmers and rural landowners. They are extremely adaptable and can coexist with raccoons and opossums, providing an opportunity for disease transmission between species. Behavioral characteristics and susceptibility to various parasites and pathogens makes feral swine a particular species of interest in disease surveillance. Insufficient data are available regarding the exposure of these animals to various pathogens and their actual risk to other species (Root et al. 2005, Junge et al. 2007). The primary objective of this study was to provide baseline data regarding exposure of raccoon, opossum, and feral hog populations in Mississippi to specific viral pathogens: avian influenza (AI), West Nile virus (WNV), rabies virus (RV), and canine distemper virus (CDV). This study also provided the opportunity to determine if land use patterns in Mississippi affected prevalence of viral pathogens.

STUDY AREA

This study was conducted in the Lower Mississippi Alluvial Valley (LMAV) and the Upper Coastal Plain (UCP) regions of Mississippi (Fig. 1). The LMAV was

historically a bottomland hardwood forest (King and Keeland 1999). Because of deforestation for agriculture and flood control, only about 28% of the original forest remains intact. Efforts have been taken to reforest the LMAV, but habitat loss and influences on coastal waters have made a lasting impact on fish and wildlife populations (King and Keeland 1999, Stanturf et al. 2000). About 70% of this major land resource area is devoted to agricultural crops and catfish production (USDA 2008). In the LMAV, samples were taken from private land in Issaquena County, Holmes County, and Sharkey County. Samples were also taken from national wildlife refuges (NWR): Morgan Brake NWR, Hillside NWR, and Panther Swamp NWR.

Figure 1. Mississippi land use areas: Upper Coastal Plain (right) and Lower Mississippi Alluvial Valley (left).

The UCP is a mix of hardwoods and pines (USDA 2008). The majority of the UCP is forested with timber being the largest economic resource. As resources in the LMAV are predominately agricultural, the UCP is more developed. In the UCP, private landowners in Noxubee and Oktibbeha counties participated in this survey. Samples were also collected from Noxubee NWR and Trim Cane Wildlife Management Area.

METHODS

Feral swine, raccoons, and opossums were captured from May 2007 through June 2008 through a variety of trapping and shooting methods. Immediately following the death of the animal, 5–15 ml of blood via cardiac puncture was collected. The blood samples were centrifuged at approximately 4000 rpms for 20 minutes. The serum was then separated using a pipette and frozen in 2 ml vials. Sera were frozen (-80°C) until transported to the appropriate testing facility. Each sample was identified with a unique label.

Testing of samples for AI and WNV was performed at the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Wildlife Services (WS), National Wildlife Research Center, Fort Collins, Colorado. Agar gel immunodiffusion assay (AGID), a common screening test for influenza A viruses, was used for AI testing (Vandalen et al. 2009). Fifteen milliliters of agar gel was placed in a 100 x 15 mm petri plate. Wells were cut into the agar gel and filled with 0.05 ml of sera, antigen, or control. After wells were filled appropriately, each plate was covered and incubated for 24 hrs at room temperature. A positive result was recorded if a straight line was observed separating the antigen well from the test well, indicating a reaction between the antigen and antibodies of the test sera. A negative reaction was indicated by anything other than a straight line between the wells.

Testing for WNV antibodies was performed with the blocking ELISA as described by Blitvich et al. (2003). A 96-well plate was coated with antigen and incubated overnight. Blocking buffer was added to each well and then incubated for 40 minutes. Diluted test serum (50µl) was added in duplicate, meaning there were two wells for each sample. Positive and negative controls also were added. Each test well was

labeled and the plate was incubated for 2 hrs. Diluted monoclonal antibody (MAb) 3.1112G was then added (50µl) to each well and incubated for an hour. Diluted IgG conjugate (50µl) was added to each well and incubated again for another hour. The well plate was washed four times between each described step. Finally, 75µl of ATBS substrate was added to each well. The plate was then inserted into the plate reader and optical density was measured at 415nm. A reaction was determined to be positive if the optical density was ≥ 30% inhibited.

Rabies testing was conducted at Kansas State University's Veterinary Diagnostic Laboratory. Rabies serum titers were obtained using the rapid fluorescent focus inhibition test (RFFIT) described in the World Organization for Animal Health's Terrestrial Manual (OIE 2008). The RFFIT is more sensitive than the previously used mouse neutralization test and can be completed within 24 hours (Lee et al. 1977). End titers were determined to be the serum dilution factor at which 50% of observed microscopic fields at 160–200x magnification showed one or more infected cells (OIE 2008).

Distemper testing was conducted at Mississippi State University using indirect fluorescent assays. Sera were diluted in buffered saline and incubated in wells of pre-prepared CDV infected slides provided by Gentaur Molecular Products (Brussels, Belgium). The slides were incubated for 30–45 minutes to allow the serum to react with the fixed antigens. Slides were then rinsed thoroughly three times with a prepared phosphate buffer solution (PBS). The fluorescent IgG conjugate was then added and the slides were incubated for another 30–45 minutes. The slides were incubated in the dark so as not to interfere with the fluorescence. The slides were rinsed again as before and viewed under a fluorescent microscope as soon as possible while the fluorescence was brightest. A positive and negative control well were added to each slide as reference wells. A positive reading was determined if fluorescent green inclusions were seen in ≥7–15% of each observed microscopic field at 400x magnification.

RESULTS
Samples were collected from 105 feral swine (LMAV = 91; UCP = 14), 119 raccoons (LMAV = 49; UCP = 70), and 126 opossums (LMAV = 49; UCP = 77). Of those samples, 347 were tested for AI (feral swine = 104; raccoons = 118; opossums = 125), 320 were tested for WNV (feral swine = 98; raccoons = 103; opossums = 119), 223 were tested for rabies (raccoons = 117; opossums = 106), and 225 were tested for CDV (raccoons = 119; opossums = 106).

Only eight raccoons were found to be positive for exposure to avian influenza, resulting in an exposure rate of 6.8% in raccoons and 0% in both opossums and feral swine. Of those AI-positive raccoon samples, four were collected from Morgan Brake NWR in Holmes County, three were collected from Panther Swamp NWR in Yazoo County, and one was collected from Noxubee NWR in Noxubee County. Statistical analysis demonstrated that exposure rates of AI were not different (p = 0.0728, Fisher's exact test) between the LMAV (3.8%) and the UCP (0.62%). Overall, 2.3% of samples tested were shown to have AI exposure.

The ELISA detected WNV antibodies present in nine raccoons (8.7%), 13 opossums (10.9%), and 15 feral swine (15.3%). Statistical analysis demonstrated that exposure rates of WNV were not different (p = 0.1483, Chi-square test) between the LMAV (9.2%) and the UCP (14.4%). Overall exposure to WNV was 11.6%.

Although specific validation for wildlife sera has not been performed, an end titer above 1:25 is believed to be positive (S. Moore, Kansas State University Veterinary Diagnostic Laboratory, personal communication). As a worse-case scenario, all samples with ≥1:12 end titers were considered positive and included in the data analysis. Of the 223 samples tested for rabies, 44 sera samples were positive (19.7%). Seven samples had titers greater than 1:25. There was no significant difference (p = 0.1871) between raccoons (23.08%) and opossums (16.04%). There were 131 samples from the LMAV and 92 samples from the UCP submitted for rabies testing. There was a difference (p = 0.0073) in exposure rates between the LMAV (28.26%) and the UCP (13.74%).

A titer of >1:25 is considered to be positive for CDV based on previous research of wild canids and raccoons (Arjo et al. 2003, Hoff et al. 1974). Ten raccoon samples (7.6%) from the UCP and four raccoon samples (4.3%) from the LMAV were positive for CDV exposure. There was no difference between the LMAV and UCP (p = 0.4051). No opossums showed positive antibody responses.

DISCUSSION

The results of this study provide evidence of wildlife exposure to avian influenza in Mississippi. Because wild birds are the natural host of AI and share habitat with both raccoons and opossums, it seems likely that contact with wild birds or contaminated water (or both) is the reason for the positive AI results. A definitive cause for the positive test results can only be speculated as further tests were negative.

West Nile virus has become established in the United States, but little is still known about which species are affected. In 2005, the United States Geological Survey, National Wildlife Health Center, reported

that WNV had not previously been found in raccoons, opossums, or feral swine. This study provided evidence that these species are exposed to WNV and mount an immune response to the pathogen. We have found no evidence to the contrary that mammals are dead-end hosts; therefore, there is no greater risk of transmission in having found WNV antibodies in all three species.

The last reported case of RV in Mississippi was 40 years ago (MSDH 2008). The recent absence of RV reports in Mississippi has always been curious given that all surrounding states continue to report cases of the disease. These results suggest that Mississippi may in fact have isolated cases of RV that go unreported. Alternatively, our results may indicate a recent introduction of RV into Mississippi that has not moved into the domestic animal population and has gone unreported. If such an introduction is occurring it could be through natural movement of the virus by wildlife, or through illegal importation of animals.

Another potential hypothesis could be that these animals were exposed to a RV vaccine. Vigorous vaccination programs have been established by WS along the eastern coast to prevent spread in surrounding states. Future research should be conducted to determine if the RV titers found in this study were a result of exposure to field or vaccine strains of the virus.

Exposure to RV was higher in the LMAV than in the UCP. These findings were unexpected because raccoons are generally the most common in the UCP. Potential differences in exposure rates to field and vaccine strain RV in the LMAV and UCP may have influenced these results.

Unlike RV, exposure to CDV was expected to be higher in the LMAV due to the greater likelihood of free-ranging canines in more rural areas. Results of this study show very little antibody response in

raccoons and opossums and no difference between land use areas. Such low levels of exposure suggested that CDV activity in the area was very low. The results from this study indicate that opossums were less likely than raccoons to transmit the virus.

MANAGEMENT IMPLICATIONS

Free-ranging wildlife play an important role in the ecology of many zoonotic pathogens. Disease surveillance in wildlife is important due to increased interaction between wildlife and humans. As human populations continue to increase and expand into new areas, the chance for transmission of diseases among humans and vulnerable wildlife species will increase. Active surveillance can provide the opportunity to foresee potential disease threats. This survey has led to increased awareness among wildlife experts in Mississippi, allowing for preparation if a pathogen that could severely affect human and wildlife health was introduced.

ACKNOWLEDGMENTS

LITERATURE CITED

Arjo, W. M., E. M. Gese, C. Bromley, A. Kozlowski, and E. S. Williams. 2003. Serological survey for diseases in free-ranging coyotes (Canis latrans) from two ecologically distinct areas of Utah. Journal of Wildlife Diseases 39:449–455.

Blitvich, B. J., R. A. Bowen, N. L. Marlenee, R. A. Hall, M. L. Bunning, and B. J. Beaty. 2003. Epitope-blocking enzyme linked immunosorbent assays for detection of West Nile virus antibodies in domestic mammals. Journal of Clinical Microbiology 41:2676–2679.

Cleaveland S., M. K. Laurenson, and L. H. Taylor. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range, and the risk of emergence. Philosophical Transactions: Biological Sciences 356:991–999.

Hoff, G. L., W. J. Bigler, S. J. Proctor, and L. P. Stallings. 1974. Epizootic of canine distemper virus infection among urban raccoons and gray foxes. Journal of Wildlife Diseases 10:423–428.

Junge, R. E., K. Bauman, M. King, and M. E. Gompper. 2007. Serological assessment of exposure to viral pathogens and Leptospira in an urban raccoon (Procyon lotor) population inhabiting a large zoological park. Journal of Zoo and Wildlife Medicine 38:18–26.

King, S. L., and B. D. Keeland. 1999. Evaluation of reforestation in the Lower Mississippi River Alluvial Valley. Restoration Ecology 7:348–359.

Lafferty, K. D., and L. R. Gerber. 2002. Good medicine for conservation biology: the intersection of epidemiology and conservation theory. Conservation Biology 16:593–604.

Lee, T. K., H. D. Hutchinson, and D. W. Ziegler. 1977. Comparison of rabies humoral antibody titers in rabbits and humans by indirect radioimmunoassay, rapid fluorescent focus inhibition technique, and indirect fluorescent antibody assay. Journal of Clinical Microbiology 5:320–325.

Mississippi State Department of Health [MSDH]. 2008. Rabies. Mississippi Morbidity Report 24:1–4.OIE [World Organization for Animal Health]. 2008. Rabies. OIE Terrestrial Manual 2008. <http://www.oie.int/eng/normes/mmanual/2008/pdf/2.01.13_RABIES.pdf>. Accessed 12 February 2009.

Root, J., J. S. Hall, R. G. McLean, N. L. Marlenee, B. J. Beaty, J. Gansowski, and L. Clark. 2005. Serological evidence of exposure of wild mammals to flaviviruses in the central and eastern United States. American Journal of Tropical Medicine and Hygiene 72:622–630.

Stanturf, J. A., E. S. Gardiner, P. B. Hamel, M. S. Devall, T. D. Leiningera, and M. E. Warren, Jr. 2000. Restoring bottomland hardwood ecosystems in the Lower Mississippi Alluvial Valley. Journal of Forestry 98:10–16.

Suzan, G., and G. Ceballos. 2005. The role of feral mammals on wildlife infectious disease prevalence in two nature reserves within Mexico

City limits. Journal of Zoo and Wildlife Medicine 36:479–484.

Taylor, L. H., S. M. Latham, and M. J. Woolhouse. 2001. Risk factors for human disease emergence. Philosophical Transactions: Biological Sciences 356:983–989.

United States Department of Agriculture [USDA]. 2008. Natural Resources Conservation Service. <http://www.mo15.nrcs.usda.gov/technical/mlra_ms.html>. Accessed 24 April 2008.

United States Geological Survey. 2005. National Wildlife Health Center. <http://www.nwhc.usgs.gov/disease_information/west_nile_virus/AffectedSpeciesList2005.doc>. Accessed 13 January 2009.

Vandalen, K. K., S. A. Shriner, H. J. Sullivan, J. J. Root, and A. B. Franklin. 2009. Monitoring exposure to avian influenza viruses in wild mammals. Mammal Review 39:167–177.

Zinsstag, J., E. Schelling, F. Roth, B. Bonfoh, D. de Savigny, and M. Tanner. 2007. Human benefits of animal interventions for zoonosis control. Emerging Infectious Diseases 13: 527–531.