National Wildlife Disease Program

The Carrier

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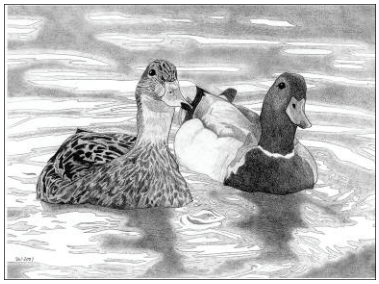

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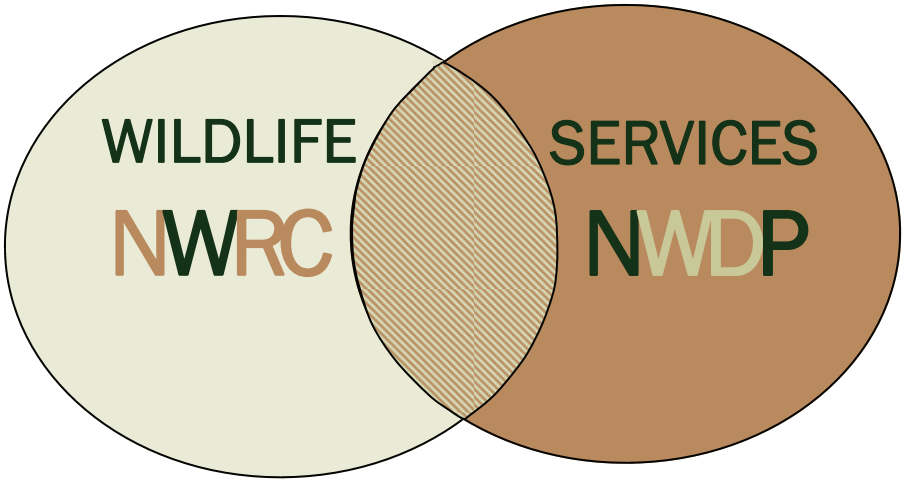
Program Commentary

By Dr. Thomas DeLiberto

It's hard to believe that we are fast approaching the end of 2012. It's been quite a year—the Olympics, U.S. Presidential Election, Superstorm Sandy, discovery of the Higgs boson ("God Particle"), Curiosity's landing on Mars, and Queen Elizabeth II celebrating her Diamond Jubilee. In the midst of all these and the other notable events of the year, I hope you and your family have benefited from good health and fortune. I also hope that your programs have had a productive year.

The NWDP has been fortunate to have experienced another successful year of collaborations with our partners in contributing to the health of wildlife, agriculture, and ecosystems. We have also greatly benefited from our merger with Wildlife Services' National Wildlife Research Center, headquartered in Fort Collins, CO. Since the inception of the NWDP in 2003, we have been co-located with the Research Center. However, in February of this year the NWDP officially became part of the National Wildlife Research Center. Hopefully this merger went pretty much unnoticed by those of you outside Wildlife Services; if so, then we were probably successful in delivering our services without much interference and confusion often associated with institutional reorganizations.

From my perspective, this reorganization has not only been successful administratively, but also has positioned Wildlife Services to provide our customers with a more efficient, coordinated, and relevant team of wildlife health scientists. We have already taken the opportunity to enhance the effectiveness of the services our program delivers by combining research and management teams to address bovine tuberculosis, heartland disease virus, and influenza viruses in birds and feral swine. We have also been working diligently here in Fort Collins to combine our research and diagnostic laboratories into a centralized biological laboratory unit that will enhance our capacity to conduct research on diseases, as well as adding capacity to provide diagnostic support for specific diseases of concern.



In the following pages, we have not only included updates on our surveillance activities, but also provided overviews of some of our research projects on wildlife health. We will continue to include highlights on our surveillance and research efforts in future issues of The Carrier, particularly as they relate to collaborations with our partners.

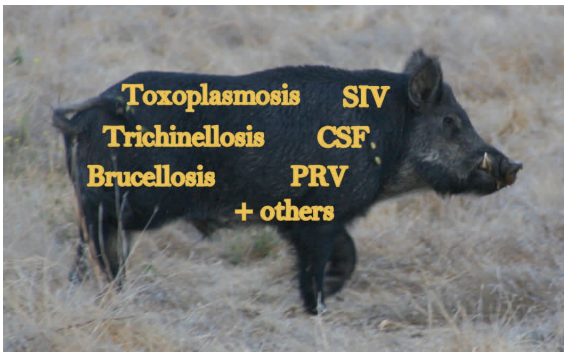
NWDP

The wildlife drawings on this page are original artworks created by the National Wildlife Disease Program's former Administrative Support Assistant Erika Kampe (prairie dog) and Wildlife Technician Sarah Goff (ducks)

Leveraging the Value of Feral Swine Samples

By John Baroch

The cost of obtaining serum and tissue samples from feral swine (*Sus scrofa*) is the same regardless of how many diseases are subsequently monitored with the samples; however, the value of the samples increases dramatically when they are used to monitor multiple diseases. The NWDP is constantly exploring new collaborative opportunities to get the most value from samples collected in the field.



Since feral swine sampling began in 2007, the NWDP has partnered with Veterinary Services to identify and monitor a core group of diseases in feral swine. These diseases initially included classical swine fever, swine brucellosis, and pseudorabies. Later, toxoplasmosis, trichinellosis, swine influenza, Hepatitis E, and leptospirosis were included in the list of monitored diseases. More specifically, the NWDP collaborates with several other federal agencies and universities in a surveillance effort that seeks to increase our understanding of the dynamics of type A influenza viruses in feral swine populations. Hepatitis E is monitored using an ELISA serology based test by the National Institutes of Health in Maryland. Leptospirosis testing is conducted in collaboration with Colorado State University.

A number of other diseases have been the subject of short-term monitoring or research studies. A study on lesions caused by experimental infection with foot-and-mouth disease in feral swine was completed in 2010, in collaboration with the Foreign Animal Disease Diagnostic Laboratory at Plum Island, New York. Results were presented in a pocket field guide illustrating foot and mouth lesions in feral swine. Two diagnostic negative cohort studies, on foot-and-mouth disease and African Swine Fever in feral swine, were conducted in partnership with several National Animal

(Continued on page 4)

Ukraine Workshop on Wildlife Disease Surveillance

By John Baroch

NWDP is collaborating with USDA, Foreign Agricultural Service to improve capacity within Ukraine to conduct disease surveillance in wild-life. Specific objectives for this project include strengthening Ukraine's wildlife management and disease surveillance programs, promoting effective and efficient use of samples collected from the field to broaden surveillance of animal diseases, and working on existing response systems for emerging wildlife disease outbreaks.

A Ukrainian delegation representing government laboratories and Academy of Sciences visited the United States from August 19th to 26th. Subject matter experts from Wildlife Services and Veterinary Services provided information on surveillance and control of wildlife diseases, on how to evaluate the economic impacts of outbreaks, on the role of epidemiology/risk assessments, and on the importance of diagnostics. The group also met with scientists at Colorado State University to discuss diagnostics and tour laboratory facilities. The delegation traveled to Texas for the second half of the week. WS Texas State Director provided an overview of wildlife damage management, with specifics on both managing feral swine and disease surveillance in feral swine. The WS Texas program demonstrated devices for capturing feral swine and aerial gunning to reduce populations. A historical perspective of the rabies program in Texas was also presented. How the program operates along the border with Mexico was of particular interest, because it related to the Ukrainian's effort to address the threat of European wild boars carrying African swine fever across their borders with other countries.

The Ukrainian delegation also worked with Wildlife Services to develop a wildlife disease workshop for a larger group in Kiev, Ukraine this fall. Agreed upon topics will cover overviews of wildlife damage management and wildlife disease surveillance. This will include developing surveillance plans, capture techniques, sampling techniques, and laboratory diagnostics. The diseases of primary interest include African swine fever, classical swine fever, brucellosis, pseudorabies and influenza in wild swine. There also will be sessions on conducting wild bird surveillance to detect avian influenza and Newcastle disease, along with red fox management, and surveillance and control of rabies.

Emerging Viral and Bacterial Diseases in Wildlife Research

By Drs., Alan Franklin, Jeff Root, Susan Shriner, and Toni Piaggio

The Ecology of Emerging Viral and Bacterial Diseases project at the National Wildlife Research Center conducts research on viral and bacterial pathogens in wildlife that affect agricultural and human health, as well as wildlife conservation. Staff scientists have expertise in microbiology, quantitative ecology, statistics, genetics, and wildlife disease ecology. Scientists evaluate and identify risks associated with diseases in wildlife disease in an effort to foster the development and implementation of mitigation strategies for agricultural and human health. The project focuses on: 1) accurate and quantifiable detection of the presence of and exposure to pathogens; 2) surveillance and monitoring different pathogens, which is critical for understanding the spatial and temporal distribution of the pathogens, hosts, reservoirs and their movement across landscapes; 3) implementation of key research studies to support the development of disease risk assessment and management strategies; and 4) development of quantitative risk assessment models for transmission of pathogens among wildlife populations, agricultural operations, and humans, based on data collected from surveillance, monitoring, and research studies. This approach incorporates feedback loops among risk assessment, monitoring, and research for further refinement and provides additional

NWRC geneticist Dr. Toni Piaggio

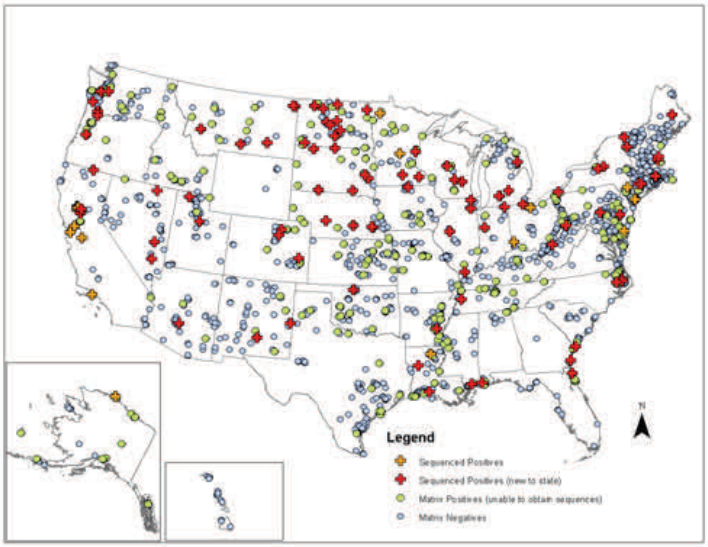
(Continued on page 5)

Phylogenetic Analysis of Avian Influenza

By Dr. Toni Piaggio

In 2006, a United States interagency avian influenza surveillance plan was initiated for early detection of highly pathogenic avian influenza viruses in wild birds. The plan included a variety of wild bird sampling strategies including the testing of fecal samples from aquatic areas throughout the United States from April 2006 through March 2011. Although highly pathogenic avian influenza was not detected through this surveillance effort, thousands of samples were determined to be positive for avian influenza viruses. Researchers at the National Wildlife Research Center have been analyzing these samples in an effort to increase knowledge of influenza viruses in nature.

The National Wildlife Research Center's Wildlife Genetics Laboratory recently completed a study on low pathogenic avian influenza in collaboration with the NWDP and the American Museum of Natural History. This study involved the collection of avian fecal samples and the application of DNA sequencing. During the national surveillance effort, 75,000 fecal samples were collected and type A avian influenza was detected in 759 of them. From those positive samples, 136 DNA sequences were obtained. These genetic sequences, along with DNA sequences from a public influenza database, were used in a phylogenetic assessment of hemagglutinin diversity in the United States. Scientists analyzed sequences from all hemagglutinin subtypes except H5, H7, H14, and H15 to examine genetic variation, exchange between Eurasia and North America, and geographic distribution of LPAIV in wild birds in the United States. This study confirms recent and ongoing intercontinental exchange of some hemagglutinin subtypes (including a newly documented H9 exchange event), as well as identifies subtypes that do not regularly experience intercontinental gene flow but have been circulating and evolving in North America for at least the past 20 years. Most hemagglutinin subtypes have high levels of genetic diversity with many lineages co-circulating within the wild birds of North America. The surveillance effort that provided these samples demonstrates that such efforts, albeit labor-intensive, provide important information about the ecology of LPAIV circulating in North America.



Location of fecal samples collected from wild birds.

Deer-Borne Cameras for Studying Disease Transmission

By Madeleine Thomas, Mike Lavelle, and Dr. Kurt VerCauteren

As management concerns regarding intra- and interspecies transmission of pathogens have increased, so has the need for quality behavioral data and more precise estimates of contact rates amongst species. Although researchers typically resort to direct observation to obtain such data, it is labor intensive and can produce insufficient results should unforeseen hindrances—like poor visibility—arise during data collection.

Understanding a need for gathering more precise data, researchers from the National Wildlife Research Center, University of Nebraska, Caesar Kleberg Wildlife Research Institute, and Missouri Department of Conservation decided to test the effectiveness of modified video camera collars on white-tailed deer as a means of obtaining unbiased behavioral data, directly from the animal's point of view.

In November 2010, the team traveled to private property located near Zapata, Texas with an enclosed population of white-tailed deer managed primarily for recreational purposes. After aerially immobilizing 26 adult male white-tailed deer, the team fitted each buck with deer-borne camera systems constructed from Telonics® SOB GPS collars and commercially available motion activated trail cameras. The cameras were also outfitted with proximity loggers, VHF beacons, and programmable collar-release mechanisms outfitted to automatically release after 14 days. The cameras were programmed to capture a 30-second video clip when motion was detected in the field.

Photos taken with a deer-borne camera system documenting interactions with other deer.

Once the cameras dropped off the deer, the team retrieved them using VHF telemetry receivers and hand-held antennae. Of the 26 systems deployed, 17 were found with retrievable data, four had moisture damage and inoperable cards, and five were found with missing cards. Over 21,000 digital video clips recorded over an average of 6.7 days were obtained. Video capture, as opposed to single still images, provides researchers an opportunity to improve understanding of animal behaviors and interactions. The team was able to document interactions with potential for direct transmission of pathogens including nose-to-nose contact, sparring, and breeding, as well as behaviors conducive to indirect disease transmission like using lick branches, and creating scrapes and rubs. Current disease transmission models primarily rely on estimates of contact rates among individuals, and camera systems such as these are a tool that could facilitate establishment of more reliable contact rates with potential for transmission of pathogens.

(Leveraging the Value of Feral Swine Samples—Continued from page 2)

Health Laboratory Network laboratories during 2010 and 2011. In addition, a retrospective study on two swine respiratory diseases, porcine reproductive and respiratory syndrome virus and porcine circovirus type 2, was conducted in collaboration with the Rollins Diagnostic Laboratory in North Carolina, National Veterinary Science Laboratory, and others, in 2011. Feral swine lymph nodes were used to culture *Brucella* at the National Veterinary Science Laboratory during 2011 and 2012 in an attempt to distinguish between *Brucella abortus* and *Brucella suis*. The Agricultural Research Service in Beltsville, Maryland, is extracting *Trichinella* larvae from feral swine tongues, and *Toxoplasma* tissue cysts from feral swine hearts, for genotyping analyses.

More recently, the NWDP began a collaborative pilot project with the University of Montreal to screen feral swine serum for several diseases of concern to the pork industry. These diseases include *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae,* and *Salmonella* species. Feral swine tonsils have also been submitted to test for the presence of a zoonotic disease, *Streptococcus suis.* NWDP expects the list of wildlife diseases monitored to continue to change, and likely grow, in the future as we learn more about the role of feral swine in animal and human health.

NWDP Leptospirosis Project

By Kerri Pedersen

Leptospirosis is a bacterial infection caused by one of several *Leptospira* serovars. Most mammals, including humans, can become infected. Humans typically become infected after exposure to water contaminated with animal urine. Infection in swine can lead to abortions or stillbirths.

The NWDP has been collaborating with Colorado State University to test samples from feral swine, raccoons, and coyotes for leptospirosis with the objective of determining the apparent prevalence in these species. Since sample collection for the leptospirosis surveillance project began in May 2012, 500 samples have been collected from 238 feral swine, 178 raccoons, and 84 coyotes. Serum samples from coyotes and raccoons have been tested using the microagglutination test to determine antibody titers to 5 serovars – *Hardjo*, *icterohaemorrhagiae*, *canicola*, *grippotyphosa*, *Pomona*, and *Bratislava*. These serovars are most commonly found in humans, livestock, domestic dogs, or rodents.

Sampling feral swine

As an extension of the original project, feral swine kidney samples are being collected from counties where positive swine serum samples have been identified previously; a paired serum sample will be tested as well. The kidneys will be analyzed using an rRT-PCR assay to determine whether active shedding of *Leptospira* is occurring; the assay will not distinguish between serovars of leptospirosis. NWDP is also increasing the number of counties being evaluated for presence of leptospirosis. Initially, only samples collected from counties where at least 10 serum samples were available through the archive were tested to reduce diagnostic costs. The project is now being expanded to include samples collected from any county where antibodies to leptospirosis have not been previously detected in feral swine serum.

Diagnostics have been performed on 164 of the 500 samples collected thus far. The samples consist of 25 coyotes, 72 feral swine, and 67 raccoons. The table below depicts the number of samples tested by species and result. Definitive conclusions cannot be drawn due to small number of samples tested thus far. However, initial results indicate that leptospirosis exposure may be fairly common in feral swine, coyotes and raccoons (42%, 28% and 30% respectively).

Species	Samples Collected	# of Positives	# of Negatives	# Pending Results
Feral Swine	238	30	42	166
Coyotes	84	7	18	74
Raccoons	178	20	47	118

(Emerging Viral and Bacterial Diseases in Wildlife Research —Continued from page 3)

questions for investigation that, in turn, require further research and monitoring.

Research on avian influenza virus focuses on the ecology of these viruses in the natural environment and how they can be transmitted to agricultural operations and humans where they become pathogenic. This work runs the gamut from participating in large-scale surveillance programs to conducting field research to laboratory experiments and assay development. For example, project scientists conducted an experimental infection studies to test hypotheses about the fecal-oral transmission of avian influenza viruses among waterfowl through water and the degree that infected waterfowl contaminate water with these viruses. Scientists also explored the role of wild peridomestic mammals, such as raccoons, skunks, rabbits and rodents, in the transmission of avian influenza viruses from aquatic habitats to agricultural operations. In addition to understanding the ecology of avian influenza viruses, these studies have direct applications for improving surveillance strategies for pathogenic strains, such as the highly pathogenic H5N1 virus.

More recently, scientists initiated research on the role of wildlife in the maintenance and transmission of pathogenic bacteria to humans and antimicrobial-resistant bacteria to agricultural facilities and, ultimately, into the consumer food chain. In 2008, children playing on a soccer field in Evergreen, Colorado were sickened with Shiga-toxin producing Escherichia coli O157:H7, which was genetically linked to feces from wild elk that had used the soccer field for foraging. Researchers are examining the relationship among elk and their use of developed areas in the maintenance and transmission of these strains of pathogenic E. coli.

In another effort, scientists are investigating the role of wildlife in disseminating antimicrobial-resistant bacteria to livestock facilities as part of a large-scale study in Canada and the U.S., which involves a number of agencies and universities. This study began this year and will assess whether peridomestic wild mammals maintain and move antimicrobial-resistant bacteria in livestock facilities, and determine the extent to which wildlife contribute to the colonization of livestock with antimicrobial-resistant bacteria.

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State Highlights

Western Region

Montana

Epizootic hemorrhagic disease is an orbivirus related to bluetongue virus, which can dramatically reduce local deer populations. Beginning in June, the NWDP initiated trapping to collect insects from two locations in an outbreak area to monitor for the presence of *Culicoides* species vectors and also the viruses it can carry.

Wildlife disease biologist Jerry Wiscomb collected samples weekly throughout the summer and submitted the samples to NWDP for analysis and testing. The objectives of the monitoring project are to identify the distribution and diversity of *Culicoides* species in the outbreak area, test *Culicoides* species for bluetongue and epizootic hemorrhagic disease viruses, and identify which species are vectors for the virus.

Oklahoma

A project is being conducted to isolate and identify *Trichinella* species infecting feral swine, by extracting larvae from tongue tissue. Genotyping of *Trichinella* in seropositive feral swine, combined with geolocation data will provide information on their role as a reservoir of *T. spiralis* and the potential risk to domestic swine. Wildlife disease biologist Patrick Whitley collected samples from feral swine trapped by Wildlife Services personnel in Love and Bryan Counties, which were causing damage to farms and ranches. In addition to testing for *Trichinella*, the feral swine samples are being tested for classical swine fever, pseudorabies, brucella, hepatitis E virus, and influenza.

Wyoming

A state-wide surveillance project has been established to evaluate the role of coyotes in the maintenance and transmission of *Echinococcus granulosus* (hydatid disease) in

Wyoming. During the month of July, the Wyoming State Veterinary Laboratory (WSVL) examined intestinal tracts from 9 coyotes for the presence of *E. granulosus*. All were negative for adult *E. granulosus* tapeworms. Wildlife disease biologist Mike Pipas also submitted the gastrointestinal tract from 6 grey wolves during the month (results pending). Results of this surveillance project will provide insight into various aspects of the ecology of this parasite, such as whether the sylvatic (wolf/wild ungulate) or pastoral (domestic dog/sheep) biotype occurs in Wyoming and apparent prevalence rates of *E. granulosus* in various definitive and intermediate hosts.

Eastern Region

Louisiana

In May, wildlife disease biologist Scott Woodruff was contacted by the Palmetto Island State Park Manager regarding a need to remove feral swine that were increasing in numbers. Beginning early June, three corral traps were set in the park and monitored by game cameras. In addition to these traps, three automatic feeders were placed in strategic locations in preparation for a three-day removal. Twenty-three feral swine were removed from the park, 11 of which were sampled for classical swine fever, swine influenza, and other diseases.

Illinois

Wildlife disease biologist Tim White assisted Illinois Wildlife Services with feral swine baiting, trapping, and landowner technical assistance to support the Illinois Comprehensive

Feral Swine Management Program. Tim assisted at a small-scale outdoor swine raising operation located in Fulton County. The animals are contained in a shady woodlot by a standard 4 foot hog panel, skirted by a single strand of electric fence 12 inches from the ground on the inside of the pen. Tim provided the landowner with information on diseases commonly found in feral swine, potential risks, and information on risk mitigation. Research conducted by the NWRC on the effectiveness of fences on feral swine was discussed, as well as the nature of feral swine and their desire to comingle with domestic herds.

New Hampshire

Wildlife Services hosted a Northeast Feral Swine Workshop for federal and state biologists in the New England area in July. The purpose of the workshop was to give biologists and land managers an opportunity to recognize signs that may alert them to the presence of this exotic invasive species, and to aid states in the prevention of population establishment. The workshop

had participants from NY, MA, RI, CT, ME, VT, and NH, representing 11 agencies and programs. Topics included hog biology and ecology, damage problems, distribution, disease issues, and trapping and removal equipment. Trap demonstrations were given by Wildlife Services New Hampshire State Director Parker Hall.

National Wildlife Disease Program

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PHOTOS OF THE QUARTER

Thurgood Marshal Intern Gabriel White collecting blood from a feral pig removed at Palmetto Island State Park, Louisiana

Juvenile feral pig in picnic area at Palmetto Island State Park, Louisiana