SCWDS BRIEFS

A Quarterly Newsletter from the

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HPAI in Wild Birds in the USA

On December 2, 2014, an H5N2 highly pathogenic avian influenza (HPAI) virus was confirmed in a chicken and a turkey operation in British Columbia, Canada. Genetic analyses of the virus revealed that the H5 is derived from a HPAI Eurasian H5 strain (H5N8) and the N2 is of North American lineage. This was the first detection of any lineage of HPAI H5 Eurasian viruses in North America.

Following the detection of H5N2 in western Canada, increased surveillance for HPAI in the United States (U.S.) was initiated by the U.S. Department of Agriculture-Animal and Plant Inspection Service (USDA-APHIS), U.S. Fish and Wildlife Service (USFWS), and the U.S. Geological Survey (USGS) in cooperation with state wildlife and animal agricultural agencies. On December 10, 2014, H5 HPAI infections were confirmed in northern pintails (*Anas acuta*) and a captive gyrfalcon (*Falco rusticolus*) in Whatcom County, Washington. The pintails were infected with the same H5N2 found in the Canadian birds; however, the gyrfalcon was infected with HPAI H5N8. This H5N8 virus was of Eurasian lineage with 99% genetic similarity to the H5 of one Korean isolate and 99% similarity to the N8 of another Korean HPAI isolate. Since then, HPAI H5N2 and H5N8 viruses have been confirmed in domestic and/or wild birds in Washington, Oregon, California, Utah, Idaho, Nevada, Minnesota, and Missouri. Domestic species have included chickens, turkeys, ducks, geese, guinea fowl and pigeons. Wild birds (free-living and captive) have included northern pintail, gyrfalcon, peregrine falcon (*Falco peregrinus*), red-tailed hawk (*Buteo jamaicensis*), Cooper's hawk (*Accipiter cooperii*), mallard (*Anas platyrhynchos*), American widgeon (*Anas americana*), gadwall (*Anas strepera*), and American green-winged teal (*Anas carolinensis*). The HPAI viruses were detected in captive and free-ranging wildlife, backyard poultry flocks, and single commercial turkey operations in Stanislaus County, California; Pope County, Minnesota; and Jasper and Moniteau counties in Missouri. Affected domestic bird facilities are quarantined, depopulated, cleaned, and disinfected, following confirmation of HPAI.

In addition to the H5N2 and H5N8 viruses, HPAI H5N1 was found in a green-winged teal in Whatcom County, Washington. This virus, like the HPAI H5N2, includes a Eurasian H5 and a North American N1, and it should not be confused with the Eurasian H5N1 that has been associated with human disease.

As a result of the many new reassortant viruses emerging in North America, a new classification has been proposed: "intercontinental group A (icA)" now is used to reference H5 viruses of mixed Eurasian and North American lineage. Fortunately, none of the icA viruses found recently in North America have been associated with human health problems; however, the U.S. Centers for Disease Control and Prevention recommend that persons with flu-like illness and recent contact with sick or dead birds be tested for novel influenza A viruses and treated with antiviral medication.

Unfortunately, many details are unavailable regarding clinical signs and presentation of wild birds infected with the HPAI viruses. The pintail with HPAI H5N2 in Whatcom County was found during the investigation of a waterfowl mortality event, but it is unknown if HPAI killed the birds. Other waterfowl isolates have come from hunter-killed birds that appeared healthy. Mortality was reported in the gyrfalcon with HPAI H5N8 infection and in three peregrine falcons from Idaho infected with HPAI H5N2. The falcon mortalities were associated with ingestion of meat from wild ducks.

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Prior to 2002, detection of HPAI virus in wild birds was uncommon, with only a single documented incident in 1961 in South Africa in common terns (*Sterna hirundo*) infected with HPAI H5N3. However, this changed in 2002 when HPAI H5N1 caused morbidity and mortality in wild birds (in addition to domestic poultry and captive exotic birds) in Asia. Over the next several years, HPAI H5N1 affected wild birds (and domestic poultry) sporadically across Asia and eventually in Europe and Africa. In North America, where surveillance activities date back to the 1970s, there is no historic record of any HPAI viruses in wild birds. More recently, surveillance of more than 400,000 wild birds in the United States from 2006-2011 failed to detect HPAI H5N1 or any other HPAI virus in wild birds.

At present, the risk of HPAI H5N8 and related icA viruses to wild avian populations is unknown. It also is unknown whether these viruses or their Eurasian genes will persist in North American wild bird populations. Research on Eurasian HPAI H5N1 viruses in free-ranging populations has failed to provide evidence that they are maintained in wild bird populations. However, there is good evidence in two cases that foreign influenza viruses, or specific HA genes can successfully invade North America. This occurred with the Eurasian H6 gene that replaced the North American H6 genes and with the recent discovery and subsequent spread of H14 viruses in North America (SCWDS BRIEFS Vol. 30, No. 1). However, only the HA gene has persisted in both of these situations. The mechanisms behind such successful introductions are not currently understood, and we have no scientific basis for predicting the fate of viruses, such as the Eurasian H5N8.

Based on our current knowledge, we have evidence that Eurasian H5N8 and icA viruses: 1) may be widespread in wild birds wintering in the Pacific flyway; 2) can infect and be transported by migratory waterfowl; 3) are reassorting genetically with North American influenza viruses; 4) can be transmitted to other birds through ingestion of infected birds and appear to be transmitted to backyard poultry flocks; 5) are a threat to the commercial poultry industry due to high mortality and trade restrictions; 6) do not pose a documented health risk to humans; and 7) can cause a varied clinical response in wild birds probably ranging from subclinical to fatal infection, depending on the host species. At present we cannot: 1) predict the spread, potential for establishment in wild bird populations, or the potential impacts of these viruses on wild bird populations and their management; 2) predict how they will interact or assimilate in the North American avian influenza virus gene pool; 3) recommend how to best track these viruses in North America; 4) determine how they entered North America; or 5) completely rule out the possibility of human infection.

It will take a combined surveillance and research effort to answer such questions, and unfortunately, they cannot be answered in the short-term. SCWDS will provide updated information as projects are conducted to find the answers. In the meantime, eliminating potential contact between wild birds, backyard poultry, and commercial poultry, and implementing stringent biosecurity standards are the only recourses to prevent the potential impacts associated with these HPAI H5 viruses. (Prepared by Rebecca Mattucks, University of Missouri College of Veterinary Medicine and Dave Stallknecht)

GI Bacteria in Pet Geckos

The United States imported nearly 1.5 billion live animals between 2000 and 2006. Approximately 92% of the imports were designated for commercial purposes (the majority for the pet trade), of which the third most abundant taxonomic group was reptiles. Most of the reptiles were wild-caught, not required to undergo pathogen testing, and originated from Southeast Asia, a region known for emerging infectious diseases. Of particular significance is the potential role these animals may play in the ongoing, multi-state, multi-national, human salmonellosis outbreaks linked to reptiles in the United States.

Tokay geckos (*Gekko gecko*) are small, relatively long-lived (up to 20 years in captivity), nocturnal, arboreal lizards that are caught in the wild in Indonesia for export in the pet trade. In the wild, they are solitary animals that display aggressive behavior to other geckos if they come within 30 meters of each other. The number of Tokay geckos imported into the U.S. annually is unknown but is estimated to be in the millions.

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The gastrointestinal (GI) bacterial flora of reptiles has not been studied comprehensively. Describing the flora shed by reptiles in the pet trade is important to public health because reptiles are known to carry numerous bacteria, including *Salmonella*, which can cause opportunistic infections in humans, particularly in children. It also is important to identify the GI bacteria of imported pet reptiles, because the animals experience stress when captured and transported, and this can cause shifts in the composition of the flora, including pathogen colonization and/or overgrowth of resident, nonpathogenic bacteria. Transport conditions, such as overcrowding, thermal extremes, poor diets, and poor ventilation, have been shown to be stressful to livestock and may be more stressful to captured wild animals. In domestic animals, stress has been associated with colonization and shedding of human pathogens such as *Salmonella* spp.

SCWDS conducted a study to: 1) describe the enteric commensal flora in the feces of wild Tokay geckos that were captured in Indonesia and immediately shipped to Athens, Georgia; and 2) determine if overcrowding (as a proxy for stress) has an effect on the diversity and prevalence of GI bacterial species.

To mimic typical importation conditions, free-ranging geckos were captured on the island of Jakarta and individually placed in clean plastic containers from the moment of capture until they arrived at The University of Georgia College of Veterinary Medicine. Once there, they were transferred to sterile containers and housed individually for approximately 10 days to allow them to acclimate. During the first 48 hours of this 10-day period, we cultured fecal samples to identify their "baseline" commensal flora, although we recognized that they had already undergone the stress of captivity and shipment. After the acclimation period, geckos were moved to mesh cages in groups at low, medium, or high densities. Approximately six months later, geckos were individually placed in sterile containers until a fecal sample was collected for culture.

From the 110 geckos we studied, we recovered 189 isolates of bacteria in the family *Enterobacteriaceae*, which is a large family of gram-negative bacteria of the intestinal tract that includes pathogenic and harmless, commensal organisms. The genera *Citrobacter*, *Klebsiella*, *Enterobacter*, and *Kluyvera* were found most frequently, and seven isolates each of *Salmonella enterica* subspecies *arizonae* and *Escherichia coli* were cultured. The commensal enteric bacteria we cultured were comprised of genera previously described from reptiles.

There was no difference in the diversity of genera recovered between individually-housed geckos and geckos housed in groups, nor in comparisons of the groups of differing densities, even when taking into account the number of animals or number of isolates. However, there were some important shifts in the prevalence of genera from individually-housed geckos compared to the combined geckos. For example, the prevalence of *Citrobacter* spp. increased from 55% in individually-housed geckos to 75% in geckos housed in groups, and *Klebsiella*, decreased from 20-4%. The prevalence of the other two most commonly isolated genera (*Enterobacter* and *Kluyvera*) from the combined animals decreased, and there was a complete loss of two bacteria: *Serratia* sp. and *E. coli*. The prevalence of *Salmonella enterica* subsp. *arizonae* was 12% in animals living in groups, compared to 1% or less in individually-housed geckos. These shifts illustrate that the GI flora of geckos is affected by the stress of the shipping and holding conditions typical of the pet trade.

Experimental overcrowding of geckos resulted in a decrease in the diversity of lactose-fermenting GI flora and a higher prevalence of *Salmonella* shedding, very similar to what has been documented in livestock. Regulations are in place to diminish stress and prevent pathogen shedding in production animals, but the pet trade is not subject to such regulations. Promoting humane conditions for the importation of pet reptiles is vital for welfare concerns, but may also help to maintain diverse, commensal microbial communities that protect individual animals from pathogen colonization and prevent pathogen dissemination. The increased prevalence of *S. enterica* subsp. *arizonae* is important because the popularity of reptiles as pets continues to increase, suggesting that pet reptile-associated salmonellosis will continue to be a significant public health concern. (Prepared by Sonia Hernandez and Chrissy Casey)

Snake Fungal Disease in Georgia

A free-ranging, sub-adult, female mud snake (*Farancia abacura***)** with facial swelling, poor body condition, and improperly shed skin was found in mid-May 2014 in Bulloch County, Georgia. The snake was submitted to The Orianne Society, a local reptile conservation organization, where it was held for several days with no improvement. Georgia Department of Natural Resources personnel were contacted and facilitated delivery of the snake to SCWDS for euthanasia and necropsy.

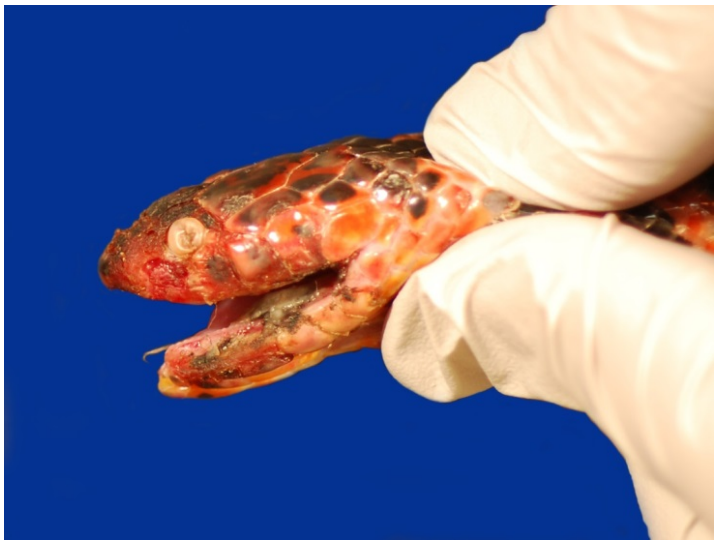

Figure 1.

The snake was moribund and in poor nutritional condition with obvious muscle wasting. Gross lesions included a cloudy eye, ulcerative dermatitis on the face and body, improperly shed skin, and multifocally roughened and thickened scales on the dorsum and ventrum (Figure 1). Microscopic examination revealed inflammation and ulceration of the affected areas of skin, as well as numerous fungal hyphae and arthroconidia consistent with *Ophidiomyces* (formerly *Chrysosporium*) *ophiodiicola*, the proposed fungal agent associated with Snake Fungal Disease (SFD). The presence of this fungus was confirmed at SCWDS by fungal culture and subsequent polymerase chain reaction and genetic sequencing.

The species susceptibility range and the geographic distribution of SFD remain poorly characterized. There is growing interest in this disease, as well as other emerging fungal skin infections, such as chytridiomycosis caused by *Batrachochytrium dendrobatidis* in amphibians, *Batrachochytrium salamandrivorans* in salamanders, and White Nose Syndrome of bats.

Snake Fungal Disease is thought to cause dermatitis and facial disfiguration in a wide variety of free-ranging and captive snakes. In 2009 near Sparta, Georgia, a novel fungus was isolated from a captive black rat snake (*Elaphe obsoleta obsolete*) with dermatitis and facial disfiguration. This fungus is now being referred to as *Ophidiomyces ophiodiicola* and has been associated with SFD, although a causative relationship has not yet been demonstrated.

Increasing numbers of fungal skin infections in a number of species of free-ranging snakes have been reported within the United States since 2006, although it is not currently known if the fungus was introduced recently or previously occurred in the environment. Biologists may have observed animals with cloudy eyes, retained shed skin, crusty skin lesions, and facial disfiguration, but samples were not analyzed by diagnosticians. Cases in wild snakes have been reported in Florida, Illinois, Massachusetts, Minnesota, New Jersey, New York, Ohio, Tennessee, and Wisconsin. It is suspected that a number of other states also have had snakes with SFD, although information is lacking in the published literature. Species in which the disease has been reported include the northern water snake (*Nerodia sipedon),* eastern racer (*Coluber constrictor*), rat snake (*Pantherophis obsoletus* species complex), timber rattlesnake (*Crotalus horridus*), massasauga rattlesnake (*Sistrurus catenatus*), pygmy rattlesnake (*Sistrurus miliarius*), and the milk snake (*Lampropeltis triangulum*). In some rattlesnakes, local, population-level declines have been associated with the detection of the fungus and its characteristic lesions.

Mud snakes are non-venomous, primarily aquatic snakes found throughout wetlands in the southeastern United States, and as such may be vulnerable to wetland degradation. Mud snakes are not currently considered a protected species. To our knowledge, SFD has not been confirmed previously in this species, or in any free-ranging snake from Georgia.

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Further investigations are needed to better understand the potential ecological impacts of SFD. The fungus appears to have temperature-dependent growth and has been isolated only from snakes and not from other orders of reptiles or amphibians. No infections have been reported in humans or domestic animals. For more information about Snake Fungal Disease, please refer to SCWDS BRIEFS, Vol. 29, No. 2. For more information regarding SCWDS diagnostic activities associated with SFD and other diseases of amphibians and reptiles, please contact Dr. Heather Fenton at hfenton@uga.edu or (706)-542-1741. (Prepared by Heather Fenton, Lisa Last, and Jessica Gonynor-McGuire from Georgia Department of Natural Resources)

Elk Hoof Disease

Prior to 2008, sporadic cases of lame elk (*Cervus elaphus*) with deformed, overgrown, broken, or sloughed hooves were reported in the Cowlitz River Basin in southwestern Washington. However, dramatic increases in numbers of limping elk and size of the affected area since 2008 have some scientists believing a new disease may have entered the elk population around that time.

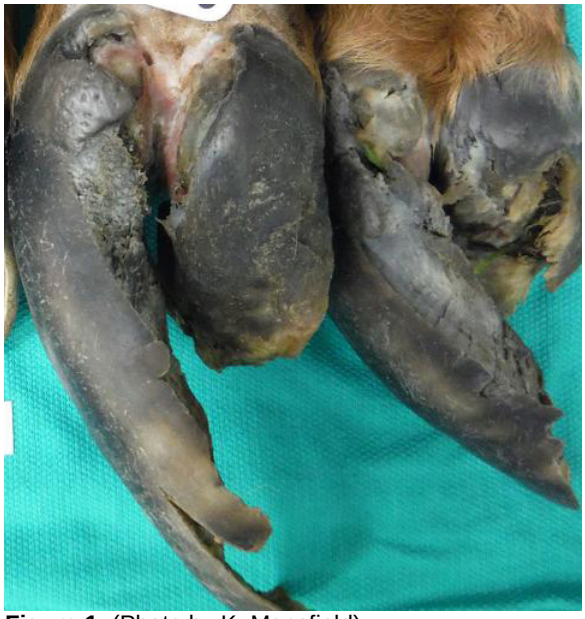

Figure 1. (Photo by K. Mansfield)

In 2009, the Washington Department of Fish and Wildlife (WDFW), Washington State University, Colorado State University (CSU) and others initiated preliminary investigations to determine the cause of severe hoof disease in elk and identify potential management actions to minimize its impact on affected populations. Initial results showed that affected animals had extensive asymmetrically elongated hoof horns, laminar necrosis, hoof horn sloughing, sole ulcers, and/or mild to severe laminitis (Figures 1 and 2). Disease in affected animals appeared to be isolated to the distal limbs with no evidence of systemic spread. Copper and selenium levels were low in affected elk, but this finding also was observed in unaffected animals. An ongoing, expanded study that began in 2013 has involved veterinary personnel and diagnostic or research laboratories in several states, the U.S. Department of Agriculture, and the University of Liverpool. Findings have been similar to those of the 2009 study; however, detailed analyses indicated involvement of spirochete bacteria in the genus *Treponema*. These treponemes and other anaerobic organisms cultured from the hooves were similar to those found in digital dermatitis, a hoof disease of domestic livestock.

Treponeme bacteria have been linked to hoof disease in domestic cattle and sheep. Bovine digital dermatitis, first reported in 1974 in Italy,

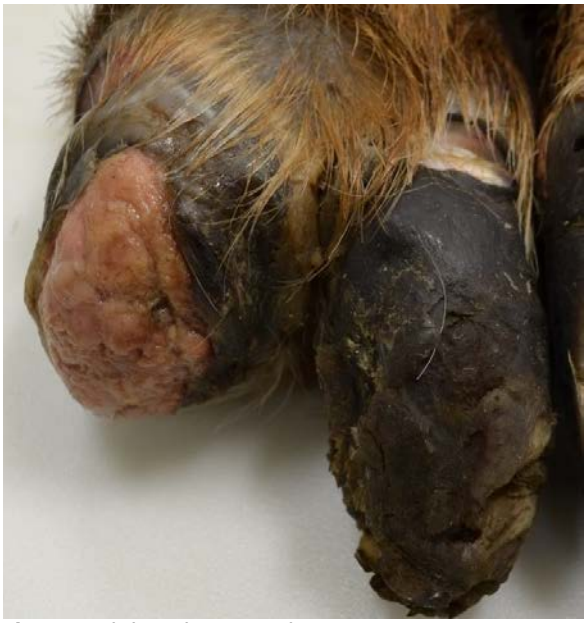

Figure 2. (Photo by S. Han)

emerged as a significant disease of dairy cattle in the U.S. in the mid-1990s. This contagious and painful condition leads to sizable production losses in dairy and beef cattle, and currently is

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the leading cause of lameness in the American dairy industry. It is present in 70% of dairies nationwide and is responsible for 50% of lameness in dairy cattle. Digital dermatitis (DD) of cattle now occurs worldwide and is known by many names, including papillomatous digital dermatitis, foot warts, hairy heel warts, strawberry heel warts, and Mortellaro's disease. The disease appears to occur most often in cattle kept in wet conditions for prolonged periods of time. The precise role of *Treponema* spp. in the development of DD and other hoof diseases is currently unknown because a complex of organisms is found within the lesions. However, histology and immunohistochemistry of affected elk hooves have demonstrated treponemes (associated with inflammation and necrosis) deeply invading viable intact tissue at the interface of the inflammation. In human periodontal disease, a similar complex synergy is seen between *T. denticola* and other anaerobic bacteria, with the treponemes invading deeply into the tissue. It is unknown if lesions are initiated by compromise or damage of the skin, or by colonization with a bacterial species.

Contagious ovine digital dermatitis (CODD) was reported in domestic sheep in the United Kingdom and Ireland not long after the emergence of bovine digital dermatitis. Quite similar to what is seen in lame elk in Washington, ulcerative lesions in sheep develop at the coronary band and are followed by extensive tissue destruction beneath the keratinized hoof horn, with potential sloughing of the horn. As with bovine digital dermatitis, treponeme spirochetes and other bacteria have been found in studies of affected sheep.

Treponeme-associated hoof disease (TAHD) in free-ranging elk has spread from the Cowlitz River basin to Pacific, Grays Harbor, Lewis, Clark, and Wahkiakum counties and currently involves approximately 4,000 square miles in Washington. In addition, reports of lame elk and hunter-harvested elk with suspicious hoof lesions have been received from Multnomah and Washington counties in adjacent Oregon. Hoof abnormalities have been documented in male and female elk as young as three months of age, with lesion severity progressing with the age of the animal. Although transmission mechanisms have not been elucidated, it is speculated that infected animals carry treponemes on their hooves and the bacteria persist in moist soil where other animals are exposed to them.

A multi-agency Elk Hoof Disease Technical Advisory Group (EHDTAG) was formed in June 2014 with a mission to assist WDFW with the assessment of diagnostic test results and to advise WDFW on additional diagnostic approaches and possible management options to pursue. The EHDTAG also recommends that key points of future research should include the following: how the disease is maintained, transmitted, and progresses; whether elk develop immunity to the disease; its effects on local and population level reproduction and survival; and the ability to sample and identify the bacterium in the environment.

The WDFW currently is considering several management options to minimize the impacts of TAHD on elk populations including treatment, containment, population density reduction, continued sampling and pathogen isolation, prevalence and survival monitoring, and passive management approaches. The department implemented a new rule stating that hooves must be removed from any elk harvested in southwest Washington and left on site. A website to keep the public updated on the disease has been created by the WDFW (http://wdfw.wa.gov/conservation/health/hoof_disease/). The website also has an interactive link where members of the public can directly report lame or dead elk with hoof abnormalities. This cooperation between the public and wildlife officials is important to monitor and control this disease. (Prepared by Kevin Niedringhaus, SCWDS, and Jeff Curtiss, University of Florida College of Veterinary Medicine, with assistance from Kristin Mansfield, WDFW; Sushan Han, CSU; and Jennifer Wilson-Welder, USDA)

Pseudorabies Kills 10 Dogs

The recent death of ten hog-hunting dogs from pseudorabies virus (PRV) infection highlights the risk PRV-infected feral swine pose to domestic animals other than hogs. In the incident, which occurred in December 2014 in Sevier County, Arkansas, the dogs showed clinical signs of pseudorabies within days of catching a feral hog or consuming its hide, viscera, head, and bones. Affected dogs displayed intense scratching and self-trauma, a common clinical sign responsible

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for the common name of “mad itch” for PRV infection in species other than swine. The animals were examined at the Arkansas Livestock and Poultry Commission (ALPC) Veterinary Diagnostic Laboratory, and PRV was confirmed at Iowa State University (ISU).

In the United States, feral swine serve as the source of PRV infection for domestic pigs and other animals including companion animals and wildlife. Humans are not susceptible to PRV infection; however, feral swine also may carry *Brucella suis*, which is a zoonotic pathogen. Pseudorabies is caused by a herpesvirus and infection often is subclinical in hogs, which are the primary reservoir of PRV. However, young pigs may die due to infection and pregnant sows may abort. As occurs with other herpesviruses, swine can become life-long, latent carriers of PRV after initial infection and develop clinical signs when stressed. In feral swine, PRV can be transmitted through the respiratory route, sexual contact, or cannibalism of animal carcasses.

In addition to swine and dogs, PRV has been reported in domestic mammals including cats, cattle, goats, and sheep. Infection and inflammation of the brain of non-suids is responsible for the severe clinical disease, and death generally occurs within 48 hours of onset of the first signs. Transmission to dogs may occur via biting infected hogs or consuming tissue from them. Clinical signs in dogs and other aberrant hosts include anorexia, depression, lethargy, incoordination, hypersalivation, diarrhea and vomiting, nervous excitability, shaking, convulsions, extreme pruritus with self-mutilation (often unilaterally on the head), paralysis, and coma.

Pseudorabies also has been reported in several native North American wildlife species including black bear (*Ursus americanus*), Florida panther (*Felis concolor coryi)*, raccoon (*Procyon lotor*), red fox (*Vulpes vulpes*), captive brown bear (*Ursus arctos*), captive coyote (*Canis latrans*), and farmed mink (*Mustela vison*). Experimental infections have been reported in numerous other species of mammals, as well as some birds, but not in cold-blooded animals.

The U.S. Department of Agriculture, National Pork Producers Council, and state animal health agencies began a PRV eradication program in 1989, and by 2004, all 50 states had been granted Stage V (Free) status in commercial swine operations. Unfortunately, PRV persists in feral swine populations, and the expanding distribution of feral swine in the United States affords increased opportunities for transmission of PRV from feral to domestic swine and other animals. SCWDS continues to maintain the National Feral Swine Mapping System (http://swine.vet.uga.edu/nfsms/), and 36 states currently report established feral swine populations.

Antibodies against PRV have been detected in feral swine populations in at least 26 states, and SCWDS studies have demonstrated that PRV persists in feral swine populations for decades. Factors affecting the occurrence of PRV in feral swine populations include age of the population, disease status of the source animals in the initial introduction, disease status of animals in subsequent introductions, and the potential for previous contact with infected domestic swine.

In addition to the ALPC and ISU, the recent canine mortality in Arkansas was investigated by the Arkansas Game and Fish Commission, Arkansas Health Department, and the U.S. Department of Agriculture-Animal and Plant Health Inspection Service-Wildlife Services. Arkansas takes feral swine issues seriously and implemented new regulations in 2013 prohibiting possession, release, and transport of feral hogs unless they carry an official tag provided by ALPC and are destined for a terminal facility.

Fatal PRV infections in dogs occasionally are reported in areas of the U.S. with feral swine populations, and it is anticipated that PRV will continue to occur in dogs used to hunt feral swine and in animals fed tissues from infected hogs. There is no PRV vaccine for dogs, so the primary preventive measures are to minimize exposure to feral swine and to avoid feeding their tissues to dogs. (Prepared by Joe Corn)

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