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SCWDS BRIEFS

A Quarterly Newsletter from the
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Johne's Disease and Southeastern White-tailed Deer

Paratuberculosis or Johne's disease, caused by the bacterium *Mycobacterium avium* subspecies *paratuberculosis* (Mptb), occurs worldwide and is recognized as a significant health problem for domestic ruminants, wild ruminants in zoological collections, and farmed cervids. In the United States, a 1996 national survey found that 22% of dairy cattle herds had at least a 10% infection rate and 41% of herds had at least one seropositive cow. Infection with Mptb has been confirmed in multiple farmed red deer (*Cervus elaphus elaphus*) herds in the United States and in more than 300 red deer operations in New Zealand. Compared to traditional livestock or captive wild ruminants, reports of clinical or subclinical infection with Mptb among free-ranging wild ruminants are infrequent. Among free-ranging native wild ruminants in North America, clinical paratuberculosis has been reported in populations of Rocky Mountain bighorn sheep (*Ovis canadensis*), tule elk (*C. e. nannodes*), Rocky Mountain elk (*C. e. nelsoni*), and Key deer (*Odocoileus virginianus clavium*). Additionally, Mptb was isolated from 2 of 10 clinically normal wild white-tailed deer from a known-infected cattle farm. Both mule deer (*O. hemionus*) and white-tailed deer (*O. virginianus*) have been infected experimentally.

Worldwide, Mptb infection has been infrequently reported in non-ruminant species, including primates.

While it is clear that free-ranging wild ruminants are susceptible to infection, in North America the infection apparently is maintained within only a few wild populations. A better understanding of the prevalence, distribution, and ecology of paratuberculosis within wild ruminant populations is desirable from a wildlife health perspective. Furthermore, this epidemiologic information would be important in the development of a national paratuberculosis control program for livestock.

In collaboration with Dr. Becky Manning at the Johne's Disease Testing Center in the University of Wisconsin's School of Veterinary Medicine, SCWDS recently completed a culture and serologic survey for Mptb infection among white-tailed deer populations in the southeastern United States. From July 1998 through October 2002, radiometric culture (ileocecal lymph node, mesenteric lymph node, and feces) and serologic testing using an enzyme-linked immunosorbent assay (ELISA) were used to survey white-tailed deer for Mptb infection. Mptb was isolated from the ileocecal lymph node of 1 of 313 deer (0.3%) originating from 63 populations in Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, North

Carolina, South Carolina, Tennessee, and West Virginia. Six deer (2%), all from different populations, had ELISA results above a 0.25 sample-to-positive (S/P) cut-off value, but none of the ELISA reactors originated from the population from which the single Mptb isolation was made. These six deer were seronegative when tested by agar gel immunodiffusion (AGID). These data suggest that white-tailed deer currently do not constitute a broad regional reservoir for Mptb; however, further study is warranted to clarify the significance, if any, of infected deer to the epizootiology of paratuberculosis on a local scale. Because culture is expensive and labor intensive, adaptation and validation of a reliable serologic assay for use with deer and other wildlife would markedly enhance Mptb surveillance capabilities and be of great use in epidemiologic studies involving wild species. (Prepared by Randy Davidson)

New USDA/SCWDS Studies

Recent USDA funding to SCWDS is allowing expansion of several ongoing programs. SCWDS has been involved in exotic tick research and surveillance throughout the United States and the Caribbean region for over 25 years, and surveillance efforts now are being expanded in the southeastern United States and Puerto Rico. In recent years, efforts have been focused on the status of wildlife as hosts for exotic ticks at specific sites with known exotic tick infestations. The current expansion of this program will allow for development and implementation of a regional program for exotic tick surveillance in the Southeast.

Exotic ticks introduced into the United States may bring with them both human

and animal diseases. One current concern is the tropical bont tick, *Amblyomma variegatum*. This tick is native to Africa but also is established in the Caribbean. It is a vector of heartwater and African tick-bite fever and is associated with acute bovine dermatophilosis. Heartwater is a rickettsial disease of ruminants known to be present on three islands in the Caribbean, and in experimental trials it was fatal to white-tailed deer. Acute bovine dermatophilosis affects cattle in the tropics, and African tick-bite fever is a disease of humans. Both diseases also are present in the Caribbean.

Introduction of the tropical bont tick or other exotic ticks into the United States could occur by various means, including importation of domestic animals or captive wildlife, on animals in the pet trade, or on migratory birds. Studies conducted by SCWDS from 1988 to 1992 documented that cattle egrets on islands in the Caribbean were infested by the tropical bont tick and that these birds moved between islands in the region and to the United States mainland. The threat to the United States was highlighted when one of the cattle egrets marked in Guadeloupe as part of this study was observed on Long Key, Florida.

A second area that is gaining additional attention is research on Johne's disease, or paratuberculosis. Johne's disease is caused by the bacterium *Mycobacterium avium* subspecies *paratuberculosis* (Mptb). Johne's disease primarily is a disease of domestic ruminants, but reports of infection in wild ruminants in the United States and in a variety of wildlife species in Scotland have led to interest in determining any potential role

of wildlife in the epidemiology of this disease.

SCWDS studies currently are focused on infected wildlife associated with farms in the midwestern and southeastern United States. The long-term goal is to determine if wildlife play a role in the epidemiology of Johne's disease in livestock. These studies are being conducted in collaboration with Dr. Becky Manning at the Johne's Disease Testing Center in the University of Wisconsin's School of Veterinary Medicine.
(Prepared by Joe Corn)

Surveillance for Human Ehrlichioses Agents Using White-tailed Deer

For 3 years, SCWDS has been conducting research on the natural history of two emerging tick-borne human pathogens, *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum*, among white-tailed deer across a multistate region. This study is funded by a grant from the National Institutes of Health. *Ehrlichia chaffeensis* infects primarily monocytes, and the disease it causes is called human monocytotropic ehrlichiosis (HME). *Anaplasma phagocytophilum* primarily infects granulocytes, thus causes human granulocytotropic anaplasmosis (HGA). As a point of clarification, a recent taxonomic reclassification of these rickettsial agents synonymized the "human HGE agent," *E. equi*, and *E. phagocytophila* as a single species and transferred these organisms to *Anaplasma phagocytophilum*. HME has been confirmed in more 1,200 people in over 30 states (mainly southeastern, south-central, and mid-Atlantic states). HGA is found primarily in the

northeastern and north-central states, and over 1,000 cases have been reported. Both infections range from subclinical to fatal. When present, the most common symptoms are fever, headache, nausea, and malaise.

A major goal of this study was to implement and evaluate an extensive fine-scale (county) serologic surveillance system for these two emerging disease agents using white-tailed deer as natural sentinels. In the eastern United States, lone star ticks (*Amblyomma americanum*) are the principal vectors of HME, and blacklegged ticks (*Ixodes scapularis*) are the principal vectors of HGA. White-tailed deer are the preferred host for adult stages of both ticks. Thus, the force of transmission of both agents is focused on white-tailed deer, and this was the rationale for using deer as natural sentinels.

To accomplish this goal, serologic testing was performed on serum samples from 3,275 white-tailed deer from 18 states in the south-central and southeastern United States. Most samples came from the SCWDS serum bank, which contains frozen white-tailed deer serum samples obtained over many years during herd health evaluations, hemorrhagic disease sampling, and individual research projects. Additional samples were collected during the 2001 and 2002 white-tailed deer hunting seasons.

All states tested, except for West Virginia, had deer populations with antibodies reactive to *E. chaffeensis* and/or *A. phagocytophilum* (Figs. 1 and 2).

Figure 1. Serologic results from testing white-tailed deer for the HME agent (*Ehrlichia chaffeensis*)

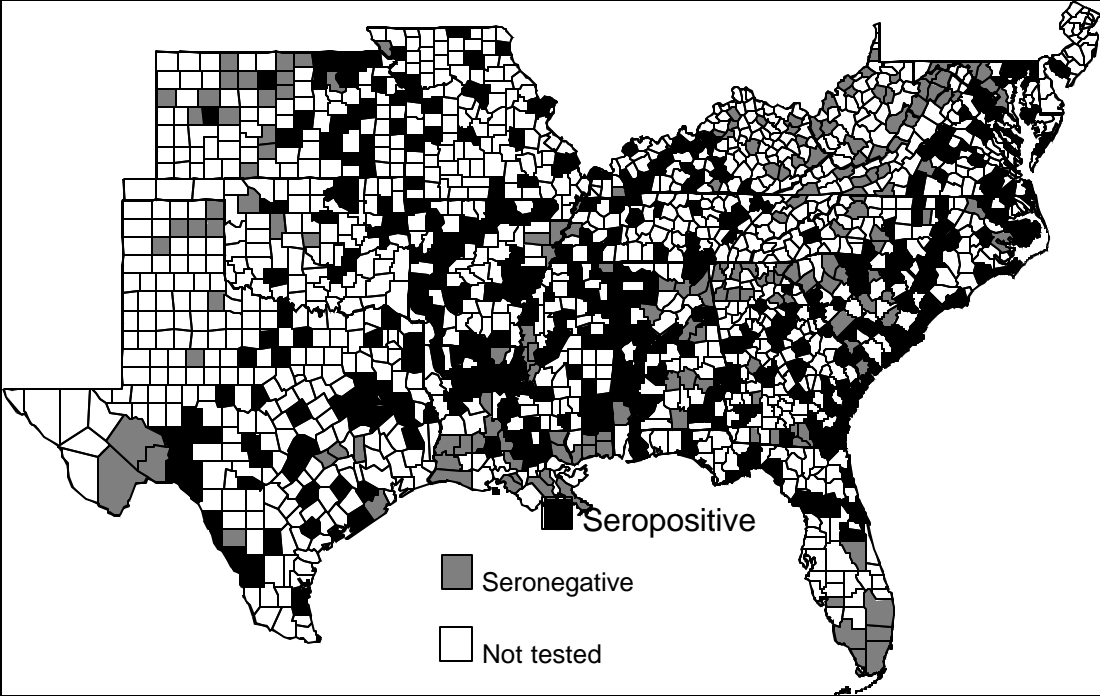
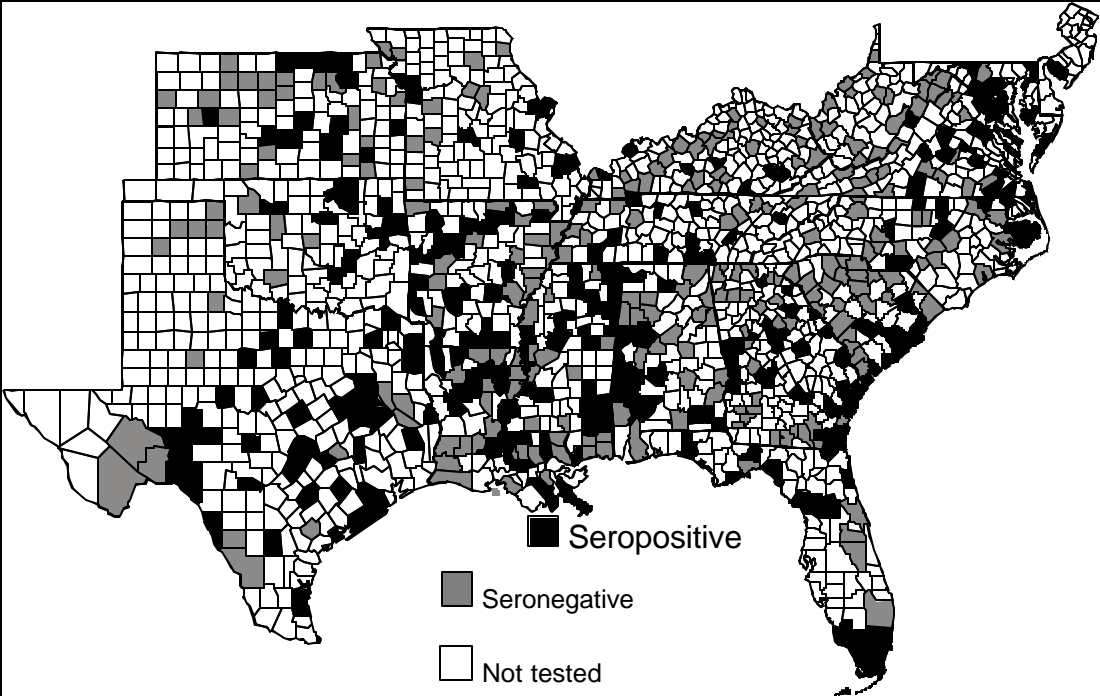


Figure 2. Serologic results from testing white-tailed deer for the HGA agent (*Anaplasma phagocytophilum*)



Overall, 47% and 24% of deer were seropositive for *E. chaffeensis* and *A. phagocytophilum*, respectively. The higher seroprevalences for *E. chaffeensis* were noted in Arkansas (67%), Missouri (57%), North Carolina (60%), Oklahoma (60%), and Virginia (62%), which correspond with states with the higher incidences of HME. Similarly, *A. phagocytophilum* seroprevalence was higher in states (Maryland [80%] and New Jersey [80%]) where the disease incidence in humans and densities of *I. scapularis* are higher. Serologic data for selected populations were validated by PCR assays and/or by culture isolation.

Differences in antibody prevalence were not noted among age or gender categories, indicating that all white-tailed deer 6 months old or older are suitable for use as sentinel animals. For *E. chaffeensis*, in contrast to the high, stable prevalence of antibodies among age classes, PCR evidence of rickettsemia declined with age. This pattern conforms to proposed infection dynamics in naturally infected white-tailed deer, whereby animals less than 1.5 years old are more likely to be rickettsemic than older adults. In the present study, the prevalence of PCR positivity among deer younger than 9 months old (53%) was dramatically higher than deer older than 1.5 years (8%). The majority of deer tested from some seropositive populations were more than 1.5 years old, and only deer older than 1.5 years old from other populations were available for PCR testing, which may explain why not all seropositive populations were confirmed by PCR. Because the probability of a white-tailed deer being rickettsemic declines with age, serology represents a better tool for surveillance than PCR, and under natural conditions

of re-exposure to ticks, titers do not appear to decline over time. Similar analyses for *A. phagocytophilum* infected white-tailed deer are currently underway.

This ongoing research has confirmed that white-tailed deer serve as excellent natural sentinels for both *E. chaffeensis* and *A. phagocytophilum*. Furthermore, data from this prototypic surveillance system provide the basis of an investigation on the landscape epidemiology of these two pathogens. Based on current serologic data, spatial analyses are being used to predict the infection status of deer in unsampled counties. Also, a suite of available environmental, climatic, and land use variables are being analyzed as potential predictor variables for the distribution of these two human pathogens. (Prepared by Michael Yabsley, Vivien Dugan, and Randy Davidson).

Characterization of Ehrlichia chaffeensis from Deer

As part of ongoing studies at SCWDS to investigate the role that wildlife serve as reservoirs for zoonotic ehrlichiae, we investigated the genetic variation of *Ehrlichia chaffeensis* from naturally infected white-tailed deer. *Ehrlichia chaffeensis* causes human monocytotropic ehrlichiosis (HME) and is an important emerging tick-borne pathogen in the southeastern and south-central United States. *Ehrlichia chaffeensis* is maintained in an epidemiologic cycle involving white-tailed deer and the lone star tick.

Because isolation attempts for *E. chaffeensis* are not routinely conducted in hospitals, few human isolates have been available for genetic studies. In regard to

non-human vertebrate hosts, *E. chaffeensis* DNA has been detected in domestic dogs, white-tailed deer, lemurs, coyotes, and domestic goats, but the organism only had been isolated in culture from seven white-tailed deer, one domestic goat, and one lemur. Since 2001, SCWDS has successfully isolated *E. chaffeensis* from nine additional white-tailed deer and two ring-tailed lemurs. Furthermore, we have amplified DNA from over 100 white-tailed deer from 12 states using PCR.

Two gene targets believed to be useful in distinguishing strains have been used to characterize *E. chaffeensis* isolates. These genes encode for two surface expressed antigens, the variable length PCR target (VLPT) antigen and the 120-kDa antigen. To date, molecular characterization of *E. chaffeensis* has been restricted to only a few human isolates and positive tick samples, primarily because of the lack of availability of isolates from nonhuman vertebrates. Genetic characterization of *E. chaffeensis* DNA from these few human and tick samples suggested that genetic variants (strains) might be limited to certain geographic regions.

In our study of *E. chaffeensis* in white-tailed deer, these two genes were amplified and characterized from a much larger number of samples. DNA sequences of both genes from infected deer were similar to those reported from humans and ticks, although a new variant of the 120-kDa gene was detected. Additionally, coinfections with multiple VLTP and 120kDa genetic types were detected in individual deer and in multiple deer populations. Sequence analysis of both genes revealed more nucleotide variation than previously reported for *E.*

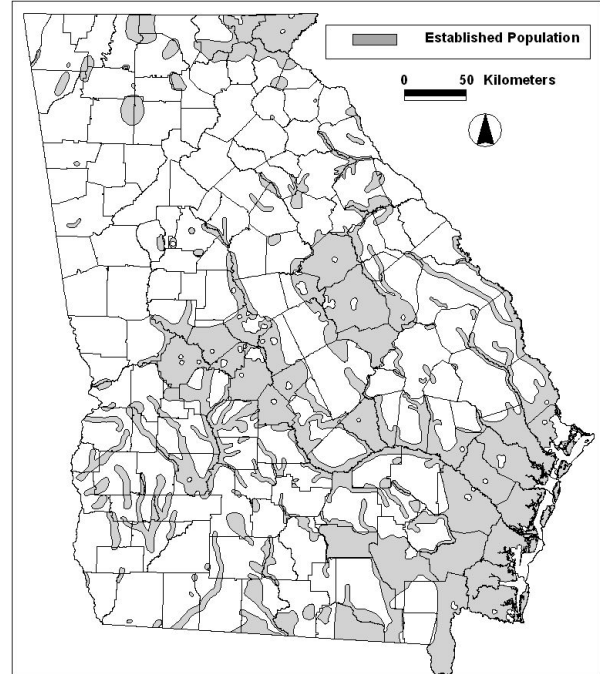
chaffeensis from infected humans or ticks. Additionally, analysis of the larger number of samples from white-tailed deer disclosed that strains present did not conform to any geographic pattern, contradicting the concept that *E. chaffeensis* strains are spatially restricted. This is the most extensive study of *E. chaffeensis* genetic variation to date and is the first to examine strain variation in *E. chaffeensis* from a nonhuman vertebrate host. Results of this study are being published in the November 2003 issue of the Journal of Clinical Microbiology. (Prepared by Michael Yabsley and Randy Davidson)

GIS-Based Pseudorabies Surveillance

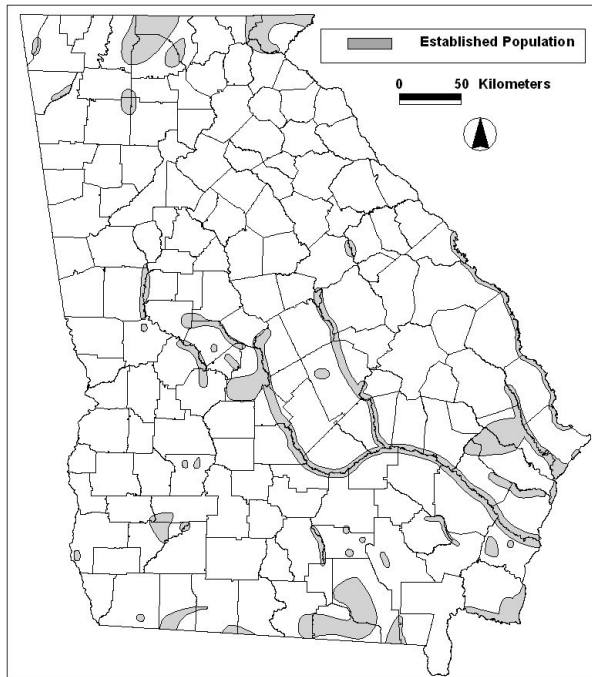
In the 14 years since the U.S. Department of Agriculture (USDA) launched the National Pseudorabies Eradication Program, pseudorabies virus (PRV) has been nearly eliminated from the nation's domestic swine herd. However, feral swine in 10 states have tested seropositive for PRV, and SCWDS studies have shown that once infected, feral swine populations remain infected indefinitely. Furthermore, distribution and abundance of feral swine appear to be increasing in many areas. In order to better understand the potential risks that feral swine pose for re-infection of domestic herds, SCWDS used Georgia as a model to develop a targeted strategy for conducting PRV surveillance among feral swine populations. With funding from a Cooperative Agreement with USDA's Animal and Plant Health Inspection Service, maps of feral swine distribution, domestic hog production, and previous feral swine PRV surveillance were developed and processed with geographic information systems (GIS) software in order to

identify high priority areas where PRV surveillance should be conducted.

A map of current feral swine distribution was developed in cooperation with the Wildlife Resources Division of the Georgia Department of Natural Resources (DNR). Surveys were distributed to each of seven DNR regions, where biologists, technicians, and law enforcement officers indicated distribution and status of feral swine within the region. The resulting map indicated that feral swine populations are established in portions of 137 of Georgia's 159 counties. A comparison of the current distribution map with a similar map created in 1988 indicates an increase of approximately 350% in feral swine distribution over the 15 year period (see the figures below).



Feral Swine Distribution in Georgia in 2003



Feral Swine Distribution in Georgia in 1988

A map of domestic hog production was created in cooperation with the Georgia Agricultural Statistics Service using domestic hog data from the 1997 USDA Census of Agriculture. Hog production was calculated for each county as a function of domestic hog inventory and number of hog farms. Counties were then ranked by their hog production value and categorized as high-production, moderate-production, or low-production counties. The majority of high-production counties were located in the southern half of Georgia, although six high-production counties were located in the northern half of the state. A map of previous PRV surveillance was created using data from the SCWDS feral swine PRV database. PRV seropositive feral swine had been previously documented in 10 Georgia counties. Since PRV infection persists in feral swine populations indefinitely, populations in those counties were presumed to be currently infected. All

high and moderate hog production counties with established feral swine populations and no previous PRV detections were classified as high-priority and moderate-priority counties for PRV field surveillance.

The targeted surveillance map was used to conduct additional field surveillance in 12 high-priority and 2 moderate-priority counties from May to August 2003. Feral swine were collected in 10 of 14 counties visited, and PRV was detected in 2 high-priority counties where it previously had been undetected. GIS techniques appeared to provide a logical and efficient means with which to prioritize field surveillance of a disease such as PRV.

SCWDS plans to develop similar maps for other southeastern states. In addition to their utility with respect to PRV surveillance, such maps would be highly useful in the event of a foreign animal disease outbreak such as foot and mouth disease or classical swine fever.
(Prepared by Clay George)

Exotic Newcastle Disease Quarantine Lifted

On September 16, 2003, U.S. Secretary of Agriculture Ann Veneman announced the removal of the last remaining quarantines due to the recent outbreak of exotic Newcastle disease (END) in the United States. The presence of END in this outbreak was first confirmed in California on October 1, 2002. END is a contagious and fatal viral disease that affects the respiratory, nervous, and digestive systems of birds and is one of the most infectious poultry diseases in the world.

During the outbreak, which involved commercial and backyard poultry flocks, infected birds were found in Arizona, California, Nevada, and Texas. State and federal quarantines were established in these states and in New Mexico, adjacent to quarantined areas in Texas. During the course of the outbreak, 19,146 premises were quarantined: 18,435 in California, 490 in Texas, 155 in Nevada, and 66 in Arizona. A total of 3.21 million birds were depopulated on 2,206 premises. In addition, exports of poultry and poultry products from the affected counties, states, or, in some cases, the entire United States, were no longer accepted by many countries throughout the world. The outbreak cost more than \$160 million to fight.

Eradication of the disease was conducted through the agricultural agencies of the affected states and the Animal and Plant Health Inspection Service, USDA. END continues to be a threat to the United States because the disease is present in birds throughout much of the world. Highlighting the threat is the fact that the recent outbreak in the United States was the result of two separate END introductions, one into California and one into Texas. Much of the transmission of the disease during this outbreak was due to human-assisted movement of backyard poultry and game fowl. Agricultural agencies are providing educational materials to the public as to what biosecurity measures should be implemented by poultry producers, pet-bird enthusiasts, and backyard-poultry owners to help prevent further introductions of END. (Prepared by Joe Corn)

Pentobarbital Poisoning of Eagles

The United States Fish and Wildlife Service (FWS) has issued a press release regarding accidental poisoning of eagles by pentobarbital, a commonly used euthanasia drug. Furthermore, the Food and Drug Administration's Center for Veterinary Medicine is adding an environmental warning to the labels of euthanasia solutions that contain pentobarbital to help prevent future poisonings of wildlife.

Unintentional pentobarbital poisoning of eagles has been diagnosed by the FWS National Wildlife Forensics Laboratory, the National Wildlife Health Center, SCWDS, and other laboratories (SCWDS BRIEFS, Vol. 16, No. 4). These poisonings are preventable, and veterinarians, animal owners, operators of solid waste facilities, and others are subject to prosecution when improper disposal of euthanized animal carcasses results in harm to wildlife.

Residue from sodium pentobarbital remains in the tissues of animals long after they have been euthanized, and fatal poisoning of more than 130 eagles in 16 states and British Columbia is known to have occurred due to consumption of contaminated carcasses. Birds may die immediately after consuming tissues containing pentobarbital, or they may fly several miles and die due to vehicle collision, electrocution, predation, drowning, falling, or hypothermia while they are sedated by the drug. Species confirmed with accidental pentobarbital poisoning include bald eagles, golden eagles, and other scavenging birds, such as ravens and magpies. Many other species of avian and mammalian scavengers,

including pet dogs and cats, may become intoxicated or die after ingestion of carcasses.

Proper disposal of the carcasses of euthanized animals is essential, and education of veterinarians, clients, staff, and operators of solid waste disposal facilities can help prevent this problem. Incineration is the preferred method of disposal; however, proper burial or disposal in a landfill also is effective. Burial must comply with local and state requirements. Proper disposal at a landfill may involve immediate burial of the contaminated carcass; however, when immediate burial is not possible sequestration in secure containers is acceptable.

Improper disposal of euthanized animals may lead to prosecution under state and federal laws, including the Golden and Bald Eagle Protection Act, Endangered Species Act, and the Migratory Bird Treaty Act. These laws are enforced by the FWS and carry penalties of fines and imprisonment for criminal or civil offenses resulting in harm to wildlife. FWS Special Agents conduct investigations of all reported incidents, including the circumstances of the poisoning and the source of the contaminated carcass. Penalties vary on a case-by-case basis with fines for criminal violations as high as \$250,000 per individual or \$500,000 per organization, forfeiture of vehicles and equipment, and up to 2 years in jail. Civil penalties range from \$500 to \$25,000.

Veterinarians and livestock owners can be held liable for accidental poisoning. Most cases pursued by FWS agents involve large animal carcasses left exposed to scavengers, oftentimes

because the need for rapid disposal of carcasses containing pentobarbital was not communicated to the animal owner by the veterinarian. Thus, misunderstanding, rather than malice, is the most frequent cause of pentobarbital poisoning. However, even persons unintentionally causing harm to protected animals may be held liable under criminal or civil law, and everyone involved with euthanasia and disposal of animals should take measures to prevent accidental poisoning of wildlife. Additional information can be found in a fact sheet titled Secondary Pentobarbital Poisoning of Wildlife at the FWS website: <http://mountain-prairie.fws.gov/poison.pdf>. (Prepared by John Fischer)

Receive the SCWDS BRIEFS Electronically

There is a great amount of information available on our web site about our facilities, staff, and services. Plus, current issues and recent back issues of the SCWDS BRIEFS can be easily accessed there. There also is an index to topics of previous BRIEFS articles to help the reader in locating information.

Because of the convenience of reading the BRIEFS online and the wish to reduce the amount of mail that comes into one's office, fewer and fewer of our newer readers are requesting to be added to our mailing list. Also, we are receiving requests from some of our earlier readers to be removed from our mailing list. We intend to continue to mail copies of the BRIEFS to all readers who want to remain on our mailing list, but for those who want the BRIEFS electronically we are establishing an email mailing list to inform you each quarter when the latest issue is available online. Send us an email if you wish, and we will add your email address to the list (gdoster@vet.uga.edu). One valuable advantage to this system is that you can read the BRIEFS online about a week earlier than you receive your mailed copy. Check out the website at www.scwds.org and let us hear from you. (Prepared by Gary Doster)

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Information on SCWDS and recent back issues of SCWDS BRIEFS can be accessed on the internet at www.scwds.org