SCWDS BRIEFS

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Southeastern Cooperative Wildlife Disease Study

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West Nile Virus

An outbreak of human illness due to infection with West Nile virus in the New York City area has caused 7 deaths, and more than 50 individuals have been hospitalized with viral meningitis. West Nile virus is a mosquito-borne virus that has not previously been diagnosed in the Western Hemisphere. The outbreak was detected in mid-August, and the last human case was diagnosed on September 16th. Public health officials in the affected area encouraged people to take precautions to reduce exposure to mosquitoes, and an intensive mosquito spraying program was implemented.

West Nile virus is one of a large group of viral agents that are spread by biting arthropods, hence the classification arthropod-borne virus or "arbovirus." Originally discovered over 60 years ago in Uganda, West Nile virus has been found in countries throughout Africa, the Middle East, southern Europe, the Mediterranean, and Eurasia. Human illness usually consists of flu-like symptoms such as fever, headache, muscle soreness, sore throat, and rash. Severe cases involve inflammation of the brain and meninges (meningeo-encephalitis) and heart (myocarditis). Overall, mortality in people ranges from 3-15% and is skewed toward the elderly.

West Nile virus has been isolated from over 40 species of mosquitoes and some species of ticks. In the eastern hemisphere the virus cycles between apparently healthy birds and mosquitoes, and birds are considered the maintenance vertebrate hosts for the agent. The current problem in the New York area is unique from previous occurrences because wild birds actually are dying from infection. Hardest ht have been American crows, but infection has been confirmed in 18 species of native birds in Connecticut, Maryland, New Jersey, and New York. One well-publicized avian mortality event occurred at the Bronx Zoo where multiple bird species died, including a bald eagle, Chilean flamingos, exotic pheasants, and an exotic cormorant. In addition to the eagle, other native bird species that have succumbed to the virus at the zoo include American crow, fish crow, bluejay, laughing gull, American robin, rock dove, mallard, sandhill crane, and black-crowned night heron. In addition to the human and avian cases, three horses on Long Island, New York, were confirmed positive by virus isolation.

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Wildlife and public health authorities in the region consider crows to be an "indicator" species for viral activity because of their apparent susceptibility, and diagnostic investigations of bird mortality events are encouraged, particularly for corvids such as crows, ravens, and jays. The pattern for death losses in crows is an accumulation of individual bird mortalities over time in contrast to a sudden event such as pesticide poisoning. Therefore, even the deaths of a few crows may be significant. Necropsy findings are non-specific and include weight loss, heart muscle necrosis, enlarged spleen and liver. hemorrhage in the upper intestine and on the liver surface, and, occasionally, inflammatory lesions of the brain.

West Nile virus is not considered transmissible to human beings via handling infected birds, but persons are encouraged to avoid bare-handed contact with bird carcasses. Sick crows or crows dead less than 36 hours are considered suitable diagnostic specimens. Carcasses should be double-bagged and refrigerated immediately for submittal to a diagnostic laboratory. If the bird cannot be shipped within 24 hours, it should be frozen, preferably on dry ice. At necropsy, the desired specimens to collect include brain, heart, spleen, kidney, lung, liver, and one ml of serum, all frozen on dry ice. The National Wildlife Health Center in Madison, Wisconsin. is requesting notification of any unusual bird mortality (Dr. Linda Glaser, 608-270-2446, or Dr. Kathryn Converse, 608-270-2445). If SCWDS can be of assistance, please contact us at 706-542-1741. (Prepared by Victor Nettles)

EHDV-1 in the Eastern U.S.

Hemorrhagic disease (HD) in white-tailed deer can be caused by several viruses in the epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) serogroups. These include EHDV serotypes 1 (New Jersey) and 2 (Alberta) and BTV serotypes 10, 11, 13, and 17. In recent years, most of the HD outbreaks in the Southeast and Midwest have been caused by EHDV-2. In fact, of the more than 70 virus isolations that SCWDS has made from whitetailed deer since 1989, all but 3 have been EHDV-2. Therefore, it was surprising when our first white-tailed deer isolate in 1999 was an EHDV-1.

We received our first report of suspected HD this year on August 19 in a penned deer in Walton County, Georgia. Later that same month, viruses were isolated from one additional penned deer from Walton County and two wild deer from Harris County, Georgia. All of these viruses were identified as EHDV-1. As of September, we had additional isolates of EHDV-1 from Maryland, New Jersey, North Carolina, and Virginia. Submissions from North Carolina, Virginia, and South Carolina have continued through October. At present, we have 16 isolates of EHDV-1 and

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one isolate of BTV-13. The latter virus was isolated from a deer in an enclosure where EHDV-1 also was confirmed. Significant deer mortality has been reported from these states, especially from New Jersey.

This year's outbreak is interesting because of its distribution as well geographic as the predominant serotype involved. This is the first time since 1975 HD has been confirmed in New Jersev. Prior to the 1975 outbreak, the only other reports of HD from New Jersey occurred in 1955, when the prototype EHDV-1 was isolated and described. From 1976 to present, EHDV-1 has been isolated only five times, viz., 1981 from deer in California, 1982 from deer in Georgia, 1983 from bighorn sheep in California, and 1991 and 1996 from deer in Tennessee. Serologic evidence suggests that EHDV-1 also was responsible for a widespread HD outbreak in Mississippi, Tennessee, Alabama, and Georgia during 1991.

Our antibody surveys on normal deer provided evidence that EHDV-1 is enzootic in the coastal plain of the southeastern United States and Texas. Thus, the scarcity of virus isolations from deer in these areas does not reflect the absence of this virus. Instead, the few virus isolations probably indicate that debilitating clinical disease and mortality from EHDV-1 in these areas are minimal, and it is presumed that most EHDV-1 infections in the enzootic area are mild or inapparent.

It is interesting to speculate as to where this virus originated. The timing of this outbreak suggests that it moved northward up the east coast from Georgia (See Figure). Our serologic results from Georgia during 1998, suggested an upswing in EHDV-1 activity in coastal plain deer populations. The number of virus isolations (16) that we currently have from this year's outbreak is exceptional, and it may afford a rare opportunity to determine the genetic relatedness of these isolates of EHDV-1 throughout this geographic range and to compare these viruses with previous EHDV-1 isolates from the Southeast. In addition, in cooperation with state wildlife agencies, we will

be conducting a serologic survey of hunterkilled white-tailed deer from Georgia to New Jersey. It is hoped that this will give us a better understanding of the extent of infection in these herds and a better delineation of the viruses that may have been involved. As always, we greatly appreciate all the assistance we have received from our cooperating wildlife agencies and state diagnostic laboratories, without whose support none of these samples and subsequent virus isolations would have been available. (Prepared

*A pictoral view of the southeastern distribution of EHDV/BTV isolations of white-tailed deer for 1999 is on page 7.

Insight From Recent EHDV Isolations

by David Stallknecht)

In the last issue of SCWDS BRIEFS (Vol. 15, No. 2) we reported on the sensitivity of two cell culture lines for the isolation of EHDV from white-tailed deer clinical samples, i.e., cattle pulmonary artery endothelial cells (CPAE) and baby hamster kidney cells (BHK₂₁). In our laboratory, CPAE cells worked best for EHDV-2 virus. Of 31 EHDV-2 isolations last year, CPAE cells accounted for successful isolation from 30 of 31 samples, while BHK₂₁ cells were successful in only 9 attempts. However, we stated "Had not the BHK₂₁ cell line been used ... we would have missed an EHDV-1 isolation from Tennessee." Based on this observation, we advised that BHK₂₁ cells continue to be used for EHDV isolation attempts.

The additional EHDV-1 isolations made this year have allowed us to learn more about the sensitivity of these two cell lines. EHDV-1 will grow in either CPAE or BHK₂₁ cells. Of the EHDV-1 isolates identified during 1999, there were 15 occasions where the suspect samples were inoculated in both CPAE and BHK₂₁ cells. Thirteen of these 15 isolations were made on both cell lines; one virus recovery was restricted to either CPAE or BHK₂₁ cells. Nevertheless, we have additional evidence that isolation of EHDV-2 is enhanced by CPAE cells. Samples were submitted from three clinicallyaffected cows from Iowa in conjunction with an investigation by Emergency Programs, Veterinary Services, USDA. Unlike deer, cattle have a very low level of virus in their bloodstream and virus isolations are more difficult. Three EHDV-2 viruses were isolated in CPAE cells, and none were isolated in $BHK_{21}s$. These sensitivity differences, while unexplained, are consistent with the fact that EHDV-1 and EHDV-2 are related but distinct viruses, and perhaps generalizations regarding diagnostics, and even epidemiology, should be approached with caution. (Prepared by David Stallknecht)

CWD in Penned Elk in CO and MT

Chronic wasting disease (CWD) of cervids recently was diagnosed for the first time in captive commercial elk in Colorado and Montana. CWD is transmissible spongiform а encephalopathy that is similar to, but distinct from, scrapie of sheep, bovine spongiform encephalopathy of cattle, and Creutzfeld-Jakob disease of humans. CWD, for which there is no live-animal test, had been recognized for many vears in free-ranging elk and deer in north-central Colorado and southeastern Wyoming as well as in some research cervid herds in those areas. However, since 1997 CWD has been detected in commercial elk operations Nebraska. in Oklahoma, South Dakota and Saskatchewan.

In September, CWD was diagnosed in a single elk from a small alternative livestock farm in Colorado. The herd was placed under quarantine and arrangements are being made for the state to depopulate purchase and the animals. Approximately 18 months prior to the CWD diagnosis, the affected elk was purchased from a larger commercial herd in Colorado, and this larger herd remains under quarantine until the completion of an epidemiological investigation. Colorado initiated mandatory CWD surveillance of privately-owned cervids in 1998.

In Montana, CWD has not been detected in wild deer and elk, but it was found in an elk that died (a in a commercial facility in October. This herd in had been under quarantine since 1998 when an elk with CWD in a captive facility in Oklahoma de was traced to the Montana herd. The Montana herd of approximately 80 elk will be depopulated. CWD has not been detected in 40 commercial facility from other Montana operations that have been tested since mandatory surveillance began, and results are pending on 200 additional animals.

In free-ranging deer and elk, surveillance last year did not reveal CWD in over 400 animals tested. Surveillance of wild cervids for CWD will occur over a broader geographic area in Montana this autumn. (Prepared by John Fischer)

Typhus and Flying Squirrels

Recently, SCWDS received an inquiry from a wildlife biologist involved in a project to protect the red-cockaded woodpecker, an endangered species that nests and roosts in holes in older (60+ years) southern yellow pines. During the examination of many tree holes, the biologist and his co-workers are in contact with southern flying squirrels (*Glaucomys volans*) and their nesting material. SCWDS was requested to provide information regarding the role flying squirrels may play in the epidemiology of louse-borne typhus fever of humans.

Also known as epidemic typhus, louse-borne typhus fever is caused by the bacterium *Rickettsia* The disease is well-known in less prowazekii. developed nations where transmission among people occurs via the human louse. Epidemic typhus rarely occurs in people in the United States because there is better personal sanitation. Here, the natural cycle primarily is limited to the established reservoirs, flying squirrels and their ectoparasites, and people only occasionally become infected. The strain of R. prowazekii in flving squirrels appears to be genetically distinguishable from the human louse strain.

Infection spreads among the squirrels primarily in the winter months when they congregate in their nests and there is a marked increase of ectoparasites. The flving squirrel louse (Neohaematopinus scuiropteri) is considered an important vector. The rickettsiae grow rapidly in the gut epithelium of the louse and are released in louse feces. When an infected louse defecates while taking a blood meal, the bacteria can be inoculated into the skin by subsequent scratching. Flying squirrel lice are host-specific and do not feed on humans. The squirrel flea (Orchopeas howardi) and fleas of other mammals also are highly susceptible and might be vectors. It is possible that fleas may transmit by biting, or if crushed, rickettsiae could be rubbed into the abraded skin. Inhalation of infected louse feces in dust may account for some infections.

Human disease has been reported in Georgia, Massachusetts, North Carolina, Pennsylvania, Tennessee, Virginia, and West Virginia. Some patients had a history of close or frequent contact with flying squirrels, their nests, or ectoparasites, but the mode of transmission to humans was not always clear. Occurrence of the illness is not necessarily seasonal, although the majority of reported human cases have occurred in the winter months. Person-to-person transmission has not been documented. Symptoms in people are characterized by acute onset of fever, headache, nausea, and muscle aches, with some patients developing a rash. Most cases are mild, but infection can be life-threatening. Prompt treatment of this disease with antibiotics prevents complications and results in early resolution of symptoms.

It was interesting to find that typhus cases had not been reported in wildlife biologists working directly with flying squirrels. Nevertheless, biologists should be aware of this special disease risk. If someone experiences the rather generic symptoms described above, they should inform their physician accordingly in order to get early diagnostic action and antibiotic therapy. Because studies have shown that *R. prowazekii* is present in populations of flying squirrels throughout a wide area of the eastern United States, the following precautions are recommended for wildlife workers exposed to flying squirrels and their nests: (1) wear gloves; (2) inspect yourself for the presence of lice and fleas; (3) use Permanone® on field clothing; (4) handle nesting materials or flying squirrels with care; and (5) seek timely medical attention for potential symptoms. (Prepared by Jane Huffman and Victor Nettles)

Wildlife Seed Treatment

Recently, SCWDS responded to questions about the safety of chemical treatments on seeds offered to members of the National Wild Turkey Federation (NWTF) for use in wildlife food plots. This inquiry was assigned to Ms. Cynthia Tate, a senior veterinary student from the Virginia-Maryland Regional College of Veterinary Medicine, who was working on a SCWDS veterinary externship. She gathered information on potential toxicological problems associated with the chemicals of concern.

The seeds and seed treatment products were as follows: (1) Corn: Maxim® (fludioxonil) and Apron® (metalaxyl); (2) Wheat: Dividend XL® (difenoconazole and mefenoxam); (3) Sorghum: Captan®; and (4) Sunflower: Captan®. All of the aforementioned chemicals are registered fungicides and, when used as seed dressings, they must contain a dye that imparts an unnatural color to the seed. In addition to one or more active compounds, these seed dressings contain various other ingredients such as surfactants, solvents, and fillers.

The Environmental Protection Agency (EPA) requires that all chemical crop protectants be evaluated for ecological toxicity. Accordingly. these products have been tested to determine toxic doses in small mammals, birds, fish, insects, and other invertebrates such as earthworms. In general, toxicologic data for birds usually are acquired using mallard ducks and bobwhite quail. Results are reported as either LD_{50} or LC_{50} . LD_{50} is the lethal dose, in milligrams of chemical per kilogram of body weight, at which 50% of the birds die. LC_{50} is the lethal concentration, in parts per million of chemical to dry food, at which 50% of the birds die. In addition. reproductive data are acquired via 12-20 week

feeding trials. A "no effect" level, in parts per million, is based on parameters such as eggshell thinning; fertility, viability, and hatchability of eggs; 14-day survival of hatchlings; and body weight of both parents and young.

Each of the fungicides listed above is labeled with a statement prohibiting the use of treated seed for feed animals, and this recommendation also applies to wildlife. All treated seed should be covered with soil after sowing in order to prevent large-scale consumption by wildlife; however, consumption by wildlife is inevitable because some of the seed will be "top sown" and there also may be some spillage. Fortunately, the LD/LC_{50} doses for all of the products listed above are sufficiently high for the EPA to classify them as "practically nontoxic" to birds. To put the term "practically nontoxic" in perspective, consider the product Apron 50W®, as labeled for seed corn. If the highest labeled application rate was used to treat the corn, a three-pound mallard duck would have to eat over twice its weight at one feeding to reach the LD_{50} . Field experience corroborates the non-toxic nature of these seed treatments: no case reports of wild bird toxicosis associated with the use of any of the listed products were found in the literature.

In regard to the toxicology of these products, two other points deserve mention. First. these compounds, with the exception of metalaxyl and mefenoxam, are stated to be highly toxic to fish and other aquatic organisms. Second, Captan® has been classified by the EPA as a possible human carcinogen. We do not foresee these statements as relevant in this case as long as treated seeds are used in the manner for which they are intended. Based on the toxicologic data available, as well as the lack of case reports of wild bird toxicoses associated with the use of these products, there does not appear to be a significant threat to wildlife health when the seeds are properly planted. (Prepared by Cynthia Tate and Victor Nettles)

New Deer Parasite Research

Dr. Murray Lankester and co-workers at the Department of Biology, Lakehead University, Ontario, Canada, currently are testing a technique for the detection and differentiation of some important nematodes by examining larvae in the feces of cervids. They are using a polymerase chain reaction (PCR) assay developed by Dr. Alvin Gajadhar at the Centre for Animal Parasitology in Saskatoon that requires relatively fresh feces. This test will enable them to determine the host and geographic distribution of such parasites as meningeal worms (Parelaphostrongylus tenuis), muscle worms (P. andersoni), western species of muscle worms (P. odocoilei), tissue worms (Elaphostrongylus rangiferi), lungworms (Dictyocaulus sp.), and others. Although the project initially is directed at nematodes whose adults or larvae infect whitetailed deer, mule deer, and black-tailed deer, they also are interested in testing samples from other ungulates such as caribou, muskox, elk, and wild sheep.

During herd health evaluations conducted in August and September, SCWDS assisted their research effort by collecting fecal samples for PCR testing. Samples were obtained from whitetailed deer in Alabama, Arkansas, Louisiana, Mississippi, and West Virginia and elk from Arkansas. These samples were shipped to Dr. Lankester's laboratory for analysis.

Another Canadian researcher, Michael Duffy, a graduate student from the University of New Brunswick. is developing immunodiagnostic procedures to detect P. tenuis infections in cervids. He has isolated a protein specific for P. tenuis that has proved to be diagnostic for this parasite. He has demonstrated that antibodies in sera from experimentally infected deer react with this protein on a Western Blot procedure. In September. accompanied Mike **SCWDS** personnel on deer herd health evaluation trips to Louisiana and Mississippi to collect serum

samples and adult *P. tenuis* and *P. andersoni* from white-tailed deer to be used as part of a field evaluation of his test. (Prepared by Page Luttrell)

PA Professor on Sabbatical at SCWDS

Dr. Jane E. Huffman, professor of microbiology at East Stroudsburg University (ESU) in Pennsylvania, is spending 7 weeks on sabbatical at SCWDS as a visiting scientist. During her stay, Dr. Huffman is interacting with the staff and faculty at SCWDS and gaining information on how the concepts and technology of wildlife disease investigation can be used in the undergraduate research program and graduate curriculum at ESU. By being involved in many of the day-to-day duties performed by various SCWDS personnel, she is seeing firsthand the synergistic effort used to resolve questions in wildlife disease studies.

Dr. Huffman is participating in a Warnell School of Forest Resources' Wildlife Graduate Seminar (FORS 8300) on current wildlife health issues conducted by Drs. Randy Davidson, John Fischer, and David Stallknecht. Seminar discussions relate to appropriate actions to enhance diagnosis and management of wildlife disease.

Dr. Huffman's husband, Dr. Doug Roscoe, is a research scientist with the New Jersey Division of Fish, Game, and Wildlife. He and Jane not only share many mutual interests in wildlife diseases but are fortunate in that they have the opportunity to work together occasionally. Jane's sabbatical at SCWDS, which ends at Thanksgiving, has been mutually beneficial.

While she expresses her appreciation for exposure to the talent, expertise, and generosity of the staff at SCWDS, she has brought some fresh ideas onto the scene, and we have thoroughly enjoyed her being here and having the opportunity to work with her. We wish her and her family well. (Prepared by Gary Doster)





Recent back issues of SCWDS BRIEFS can be accessed on the Internet at SCWDS.org.