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SCWDS BRIEFS

A Quarterly Newsletter from the
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First Culture of the *STARI* Agent

Lyme disease, caused by *Borrelia burgdorferi* sensu lato, is the most common tick-borne disease of humans worldwide and the most frequently reported vector-borne disease in the United States. The hallmark of acute Lyme disease is a distinctive "bull's-eye" rash, called erythema migrans, which is present in 60 to 90% of patients. Several other nonspecific multisystemic symptoms also occur, including fever, malaise, fatigue, headache, muscle aches, and joint aches. If left untreated, a chronic disease may develop involving the heart, musculoskeletal system, or neurological system. This bacterial agent is maintained in nature through a cycle involving rodent reservoir hosts and *Ixodes* sp. tick vectors. The disease is endemic in the Northeast, mid-Atlantic states, Midwest, and West Coast. However, in much of the South, where *Ixodes* sp. ticks are seldom found on humans, epidemiologic evidence and case reports suggest that classic Lyme disease is relatively rare, despite the presence of *B. burgdorferi* in wild rodent populations and ticks.

Since the mid-1980s, physicians have described a Lyme disease-like illness in patients from the southeastern and south-central United States in which an erythema migrans rash and mild flu-like symptoms develop following the bite of a lone star tick, *Amblyomma americanum*. This disease is alternatively referred to as southern tick-associated rash illness (STARI), Master's disease, or southern Lyme disease. *Amblyomma americanum* has been shown to be an incompetent vector for *B. burgdorferi*, and serologic testing of STARI patients does not support a diagnosis of classic Lyme disease, despite microscopic evidence of spirochetes in biopsy samples of affected skin. This has led researchers to speculate that another *Borrelia,* tentatively named *Borrelia lonestari*, may be responsible.

All previous attempts to culture spirochetes from patients with STARI and from ticks have failed. However, researchers at SCWDS recently cultured lone star ticks and were able to isolate *B. lonestari*, using an embryonic cell line derived from *Ixodes scapularis* ticks. The report of this first isolation of *B. lonestari* was published this spring in the *Journal of Clinical Microbiology* and provides a microscopic, molecular, and ultrastructural description of the organism in cell culture. Although culture isolation of *B. lonestari* from human cases of STARI is necessary to demonstrate a causative link to the illness, our results will help establish the critical foundation necessary for further understanding of this disease in humans. Cultured organisms are critical for the development of accurate diagnostic assays and not only will allow investigation of the clinical manifestations of infection in humans but also further our understanding of the epidemiology and natural history of *B. lonestari*, including host and vector competence, natural maintenance cycles, and geographical distribution. (Prepared by Page Luttrell)

Wyoming Elk Mortality

Unusual wild elk mortality that began in late January to early February 2004 in the Red Rim area southwest of Rawlings, Wyoming, has been preliminarily diagnosed as lichen (*Xanthoparmelia chlorochroa*) toxicosis. The first affected elk were found on February 8, 2004, and since then over 300 elk have died or have been euthanized due to this previously unrecognized cause of elk mortality. Numerous agencies cooperated in the disease investigation, which was led by the Wyoming Game and Fish Department and the Wyoming State Veterinary Laboratory.

The affected elk were afebrile, had progressive weakness, and usually were found down and unable to rise. The elk appeared to be in good body condition and were alert and reactive, but they became moribund as the disease progressed. All affected elk either died or were euthanized, and it appears that elk cannot recover after the onset of clinical signs. Postmortem examinations revealed a consistent lesion of degenerative myopathy; however, it was unknown whether these lesions represented a primary effect of the disease agent or were lesions of exertional myopathy secondary to struggling. Diagnostic testing ruled out infectious agents such as viruses, bacteria, fungi, parasites, and prions leading investigators to suspect toxins and noninfectious etiologies as the cause of the mortality. However, results of toxicologic tests on elk tissue and blood samples were consistently negative for multiple organic toxins, heavy metals, common plant toxins, trace minerals, and vitamin imbalances.

Investigators found large quantities of *Xanthoparmelia* lichen in areas where affected elk were found, as well as in the rumen contents of elk at necropsy. Literature from the 1950s suggests that this lichen could cause similar syndrome in cattle and sheep. In the absence of other significant necropsy findings, the lichen was suspected as a possible cause of the epidemic. *Xanthoparmelia* lichens contain usnic acid, an unique compound previously believed to have been associated with degenerative myopathy in cattle and sheep. It is not known if usnic acid is the toxic compound involved in the elk mortality. In order to test the theory that lichen toxicosis was the cause of the mortality, *Xanthoparmelia* lichen was fed to healthy elk captured from outside the Red Rim area. Clinical signs and muscle lesions similar to those seen in field cases developed in 2 of 3 experimental elk within 7 to 10 days.

The circumstances leading to the elk mortality at Red Rim are not completely understood. Possible explanations include the growth of more lichen than in other years, production of higher amounts of usnic acid or other toxic compounds by the lichen, or greater consumption of the lichen by elk, perhaps due to environmental factors. Wyoming and many other parts of the West have been suffering a severe drought for several years. Wyoming Game and Fish Department personnel have been compiling data on the mortality event, including GPS coordinates of the affected elk, date found, age, and sex of the elk, in order to create a more complete epidemiological picture.

Although lichen toxicosis previously has been seen in sheep and cattle, caribou and reindeer that often consume large quantities of lichen apparently are not affected. It is hypothesized that these animals have specialized rumen microorganisms that neutralize the toxic compound. In the Red Rim area, morbidity and mortality were not observed in other species, including horses, cattle, deer, and pronghorns. It is unknown whether these animals consumed the lichen or had some type of resistance to it. One theory is that animals in the Red Rim area were somewhat resistant to the effects of the toxic compound because of repeated exposure to it and elk that were affected may have been migrating through from another area. Investigators will continue to piece together various aspects of the epidemic in an effort to understand all the factors contributing to the outbreak. (Prepared by Rick Gerhold)

EHDV and BTV Isolations During 2003

During 2003, hemorrhagic disease (HD) was documented in seven states through virus isolation at SCWDS. Most of our isolations were associated with an outbreak in western Idaho and adjoining eastern Washington. Both epizootic hemorrhagic disease virus (EHDV) and bluetongue disease virus (BTV) were found. In Idaho, EHDV-2 was isolated from 17 white-tailed deer and one mule deer during a significant mortality event. Also, BTV-10 was isolated from a pronghorn antelope and a bighorn sheep, and

BTV-13 was isolated from an additional bighorn sheep. In adjoining counties in Washington, EHDV-2 was isolated from two white-tailed deer, and BTV-17 was isolated from one deer. An interesting aspect of this outbreak was the variation observed in viruses isolated from deer as opposed to sympatric herds of domestic sheep and cattle. In Idaho, BTV-17 was isolated from blood samples collected from 16 cattle in 2 herds that were located within the same area where white-tailed deer were dying. Of 6 viruses isolated from 21 blood samples collected from domestic sheep, 5 were BTV-17 and 1 was EHDV-2.

In the Southeast, things were relatively quiet on the Atlantic side, with HD reported from relatively few counties. EHDV-2 was isolated from two deer each in Georgia and South Carolina, and from three deer from Tennessee. It appears from preliminary data, however, that significant HD events occurred in Alabama, Arkansas, Louisiana, and Mississippi, but confirmatory virus isolations are not available. A significant outbreak also occurred from northern Missouri through northeastern Kansas, Nebraska, South Dakota, and southwestern North Dakota. EHDV-2 was isolated from a penned deer in Missouri and isolations from Kansas included EHDV-2 from four white-tailed deer and BTV-17 from one deer.

Although numerous, most virus isolations during 2003 were related to a single outbreak in Idaho and Washington or were associated with relatively sporadic activity in the Southeast. In order to confirm an HD diagnosis, identify the actual viral serotypes involved in these outbreaks, and to provide biological material to better understand the epidemiology and molecular biology of these viruses, we encourage submissions of diagnostic samples for virus isolation during future outbreaks. (Prepared by David Stallknecht)

Your Viruses at Work

Each year we receive samples to be tested for epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) from throughout the United States. From 1978 to the present, we have amassed a respectable collection of viruses representing field isolates. This work is important because it provides confirmation of hemorrhagic disease (HD) in wildlife populations and provides long-term epidemiologic data relating to specific serotypes involved in these outbreaks. Recently, we decided to take a much closer look at these isolates, specifically their genetic sequences. Molecular techniques, such as phylogenetic analysis of gene sequences, are routinely incorporated into epidemiologic studies and provide insight into such things as a pathogen's potential for genetic change, virulence factors, and potential geographic associations (topotypes). All of these may be important to our understanding of HD.

Recently, in collaboration with Dr. Jim MacLachlan's research group at the University of California, Davis, we sequenced a large number of our EHDV-2 and EHDV-1 isolates for phylogenetic analysis. This work was done by Dr. Molly Murphy, a recent SCWDS PhD graduate. The EHDV serotypes were selected because they represented the majority of our isolates and because less is known about their genetic variability in relation to BTV. Two genes from each of over 100 viruses were sequenced. The genes included S10, which encodes for NS3 (a potentially conserved non-structural protein) and L2, which encodes for VP-2 (a potentially variable protein associated with attachment to mammalian cells and the neutralizing antibody response). Findings from this study are helping us to better understand EHD and are summarized as follows.

Although ribonucleic acid (RNA) viruses such as EHDV are expected to undergo a high rate of mutation and genetic variability, results from this study suggest that on the contrary there is a high level of genetic conservation with both the EHDV-1 and EHDV-2 isolates. This unexpected finding was consistent with both genes and may relate to the fact that these viruses must replicate in both insects (*Culicoides*) and mammalian hosts. This

requirement for replication in multiple taxons may leave little tolerance for genetic change.

Genetic variation, although minimal, was sufficient to detect relationships between individual viruses. In general, viruses did not group by location or year of isolation but did group in relation to specific outbreaks. An example of this was seen with three EHDV-2 isolates from a localized outbreak in two counties in West Virginia during 1993. Sequences for both genes analyzed in these viruses segregated in distinct groups when compared to all other EHDV-2 viruses. Likewise, the EHDV-1 isolates from a 1999 epizootic in the eastern United States formed a separate group. These results imply that new viruses move in and out of areas where HD epizootics occur. An interesting aspect of this work relates to genetic diversity within enzootic areas, i.e., areas where these viruses are maintained and transmitted annually. Most of the viruses used in this study were isolated from deer found dead during HD outbreaks, and consequently we have few representative viruses from enzootic areas. The exception to this involved four EHDV-1 viruses that were isolated from clinically normal white-tailed deer from a single pasture in Texas on the same day. More genetic variation was detected in these four viruses than in all other field EHDV-1 isolates combined. This is an interesting area for future research as it suggests that HD epizootics may represent “virus escapes” from enzootic areas such as Texas.

In relation to regional genetic variation (eastern versus western United States), differences were detected with EHDV-1 but not with EHDV-2. This is an interesting finding and is not fully understood. However, the lack of regional topotypes with EHDV-2 suggests a potential for long distance movement as has been suggested for BTV. The existence of a single genotype of EHDV-1 representing isolates from eastern Georgia to New Jersey in 1999 also is consistent with long-range movement of these viruses during a single epizootic.

The limited genetic variation observed in the viruses sequenced in this study conforms with recent work at the Arthropod-borne Animal Diseases Research Laboratory, ARS, USDA, at Laramie, Wyoming. In that study, which was conducted by Drs. James Mecham and Bill Wilson using some of these same EHDV-1 and EHDV-2 isolates, a high level of genetic conservation in the S7 gene was reported. This gene codes for VP7 (a viral protein associated with allowing these viruses to replicate in the *Culicoides* vector).

Although genetic variability was not extreme and gave no indication of rapidly evolving viral populations, this type of analysis has great potential for future epidemiologic studies. In addition, this work has built an important genetic data base. For example, we now have a means to link individual cases to better define HD outbreaks geographically. Such definition is critical to identifying risk factors associated with these events. We may also have a means to identify regional movement and local persistence of these viruses. Both of these are poorly understood and are critical to our understanding of outbreak dynamics.

We thank our collaborators for all their assistance and all of those field biologists who supplied us with the biological material to do these studies. Please do not hesitate to send tissue from suspected HD cases in the future; we will put your viruses to good use. (Prepared by Molly Murphy and David Stallknecht)

Hair Loss in West Virginia White-tailed Deer

Since 1998, personnel with the West Virginia Wildlife Resources Division have received sporadic reports from the public regarding white-tailed deer with extensive hair loss (Figure 1). Case histories indicated that variable amounts of supplemental deer feeding with shelled corn occurred at most of the locations where affected deer had been reported. In April 2003, six affected deer, 9 months to 5 years of age, from four counties were examined in an attempt to ascertain the cause of this condition.

Figure 1. Hair loss in white-tailed deer in Grant Co., West Virginia.

Necropsies, microscopic examinations, and detailed dermatologic evaluations were performed on the deer. All deer were in fair body condition, with adequate stores of adipose tissue. Gross abnormalities consisted of areas of bilateral, patchy hair loss on the trunk, with sparing of the head, limbs, and tail. The hair in affected areas was easily detached and appeared broken off in many areas, leaving hairs that were lighter and shorter than normal. There was excessive dryness, scaling, and hyperpigmentation of the underlying skin in affected areas, some of which contained areas of hair regrowth. Variable amounts of hair and supplemental corn were found in the rumen contents but significant pathologic changes other than hair loss were not apparent in any of the deer.

Inflammation secondary to bacterial, fungal, parasitic, or viral infection can cause hair loss, but microscopic examination revealed no inflammatory processes and no bacterial, parasitic, or fungal organisms were apparent. In fact, the gross and microscopic findings indicated that the dermatologic lesions in affected deer were non-inflammatory and were characterized by excessive numbers of involuting and resting hair follicles, while developing follicles were under represented. Differential diagnoses for non-inflammatory alopecia include nutritional deficiency or stress, endocrine disruption via ingestion of toxins, excessive grooming/self trauma, hypothyroidism, hyperadrenocorticism, mycotoxin ingestion, and genetic defects.

The case histories did not suggest a genetic cause, and excessive itching or scratching was not reported. Nutritional stress, such as protein starvation, may cause alopecia in domestic ruminants; however, protein starvation has not been described as a primary cause of alopecia in deer, and these animals did not have gross or serologic evidence of such malnutrition. Consistent mineral imbalances involving copper, molybdenum, and selenium were not detected through mineral analyses of liver and kidney, although three deer had low copper levels. Vitamin E values were within normal ranges for all deer.

Based on histological and gross features, relatively normal nutritional values, and the absence of infectious agents, hair loss in these white-tailed deer could be related to an endocrine disorder. Endocrine disorders include anomalies associated with one or more of the endocrine organs (pituitary, thyroid, parathyroid, endocrine pancreas, adrenal glands, and reproductive organs) or their products. The affected deer had evidence of hair cycle growth arrest, a

condition that sometimes is associated with endocrine-related alopecia. However, identifying sources of endocrine-related disruption in wildlife populations can be very difficult.

Nearly all of the animals in this small study came from sites where supplemental feed, primarily corn, was available to wild deer. Corn can support fungi, such as *Fusarium moniliforme*, that are known to produce estrogenic mycotoxins. However, establishment of a relationship to feeding corn was not possible, particularly because a significant amount of time had elapsed between the onset of the alopecia problem in the deer and our examination of the animals. The underlying cause of the non-inflammatory alopecia in these deer remains unknown. (Prepared by Karen Wolf, Nicole Gottdenker, and Randy Davidson)

Pseudorabies and Brucellosis Problems in Feral Swine

The distribution and density of feral swine populations are increasing rapidly throughout many parts of the United States. Surveys conducted by SCWDS in 1988 indicated that feral swine populations were established in portions of 18 states, primarily in the Southeast, California, and Hawaii. Since then, feral swine have become more widespread in the southeastern states, and new populations have been reported in Illinois, Indiana, Kansas, Missouri, Ohio, and Oregon. Although local expansion of feral swine populations can occur naturally, anecdotal evidence suggests that expansion in the Southeast and into the Midwest may have been augmented by translocation of feral swine by hunters. The expansion of feral swine populations presents numerous epidemiological challenges because the pigs carry a variety of diseases that can infect wildlife, livestock, and humans. Pseudorabies and swine brucellosis are two diseases of feral swine that are of particular interest to livestock producers. In the case of swine brucellosis, humans also are at risk. The role that feral swine can play in pseudorabies and swine brucellosis epidemiology provides a glimpse into one of the numerous problems posed by expanding feral swine populations.

Pseudorabies

Pseudorabies (PRV) is an infectious disease of swine caused by porcine herpesvirus-1. Also known as Aujeszky's disease or "mad itch," PRV rarely causes morbidity or mortality in adult swine but frequently causes abortion in pregnant sows and death of neonatal piglets, especially in naive domestic herds. Infection of domestic swine usually occurs by oronasal or aerosol transmission, but feral swine most often are infected via venereal transmission. Once infected, swine are carriers for life, sporadically shedding virus in the saliva and/or reproductive mucosa. PRV does not appear to limit the growth of feral populations and, once infected, populations remain infected indefinitely. In secondary hosts, such as cats, cattle, dogs, goats, and sheep, PRV produces an acute, fatal infection of the central nervous system marked by pruritus, convulsions, excessive salivation, and other rabies-like symptoms (hence the name pseudorabies). PRV does not affect humans, but it has been implicated as an infrequent cause of mortality among numerous wildlife species including coyotes, black bears, brown bears, mink, raccoons, and the endangered Florida panther. Transmission to secondary hosts probably occurs from ingestion of contaminated feed or carcasses or possibly from bite wounds obtained during aggressive encounters. Despite the severity of disease in secondary hosts, reports of infection in wildlife and domestic animals other than swine are rare. Potential effects of PRV on wildlife populations are unknown.

PRV is of considerable interest to swine producers worldwide because of the economic losses associated with reduced productivity and piglet fatalities. The U.S. Department of Agriculture initiated a nationwide PRV eradication program in 1989, and the disease has been virtually eliminated from U.S. domestic swine herds; however, PRV has been reported in feral swine from at least 10 states. The persistence of infection in feral populations, coupled with their expanding geographical

distribution, has created the potential for reintroduction of virus to domestic herds. As such, spatiotemporal separation of feral and domestic swine is necessary to guarantee that feral swine will not reinfect PRV-free domestic herds.

Swine Brucellosis

Swine brucellosis is an infectious disease of pigs caused by the bacterium *Brucella suis*, one of at least six closely related species of *Brucella* that cause disease in a variety of wild and domestic mammals worldwide, including humans. *Brucella suis* should not be confused with *B. abortus,* which causes Bang's disease, a serious disease of domestic cattle. Of the five *B. suis* biovars identified to date, biovars 1, 2, and 3 can infect swine. Transmission occurs sexually, through ingestion of water or feed contaminated with reproductive fluids, or by suckling of piglets on infected sows. Acute infection of swine with *B. suis* results in systemic infection and/or localized infection of the reproductive organs and skeletal joints, often leading to abortion in pregnant sows. As with PRV, morbidity and mortality due to swine brucellosis are rare in adults, and most acutely infected swine appear perfectly healthy; however, some swine develop chronic infections, characterized by infertility, posterior paralysis, and/or swollen genitalia and joints. Such animals may shed the bacteria intermittently throughout life and repeatedly infect other swine within a herd. Swine brucellosis does not appear to limit the growth of feral swine populations. Domestic cattle, dogs, fowl, and horses are potential secondary hosts for *B. suis*, but morbidity and mortality among these animals are rare. Nonetheless, infected secondary hosts can transmit the disease to healthy swine herds or humans.

In addition to domestic animals, *B. suis* is infectious to a variety of other species. Non-porcine wildlife species serve as reservoirs for three of the five *B. suis* biovars. European wild hares are infected with biovar 2 in areas where their range overlaps with European wild boars; caribou and domestic reindeer are infected with biovar 4 throughout arctic Alaska, Canada, and Russia; and small rodents in the Caucasus region of Asia are infected with biovar 5. Biovars 1, 3, and 4 are pathogenic to humans with symptoms including malaise, loss of appetite, myalgia, depression, and intermittent fever. Traditionally, brucellosis was an occupational hazard for abattoir workers, farmers, and veterinarians. Hunters can reduce their risk of contracting swine brucellosis by wearing rubber gloves while field dressing feral hogs and by cooking the meat thoroughly.

Human health concerns and financial losses to livestock producers led to a cooperative state and federal brucellosis eradication program that began in 1934. Since its inception, human infections with all *Brucella* species in the United States have dropped from a high of more than 6,000 in 1947 to approximately 100-200 cases annually. Bovine and swine brucellosis have been almost eradicated from domestic livestock in the United States; however, bison and elk in the Greater Yellowstone Area are infected with *B. abortus* and feral swine are the primary reservoir for *B. suis*, particularly in the southern portion of their range.

Infected feral swine populations, which have been expanding via natural dispersion and human-assisted movements, are potential reservoirs for transmission of PRV and *B. suis* to domestic pigs. Although federal law prohibits interstate translocation of feral swine unless they have tested negative for PRV and brucellosis, the law is difficult to enforce and anecdotal evidence suggests that it frequently is violated. In areas where feral swine exist, appropriate biosecurity measures are the best way to prevent transmission of PRV and *B. suis* from feral to domestic swine. In areas where feral swine do not yet exist, wildlife agencies, livestock interests, and sportsmen should do their best to prevent them from being established. Not only do feral swine pose disease risks, they also compete with native wildlife for resources and cause damage to agricultural crops and natural ecosystems. (Prepared by Clay George)

Stallknecht Wins Pfizer Award

Dr. David Stallknecht recently received the Pfizer Award for Research Excellence at the University of Georgia's College of Veterinary Medicine. The award was established in 1985 and is given annually to a faculty member at each of the nation's veterinary schools and colleges or teaching/research hospitals. The winner must be the principal investigator in recent research that has attained national recognition. The purpose of the award is to foster innovative research by recognizing outstanding research effort and productivity. The award consists of an engraved plaque, $1,000 cash, and national recognition.

David did not win the award for any single research effort but more as a "lifetime achievement" award for his many years of dedication and hard work. The award was presented to David on April 13, 2004, at a meeting of The Society of Phi Zeta, the honor society of veterinary medicine. David has fulfilled the requirements for this award many times over and is most deserving of this recognition. (Prepared by Gary Doster)

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