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Orbiviruses New & Old - What Do We Need to Know?

Bluetongue viruses (BTV) and epizootic hemorrhagic disease viruses (EHDV) recently have expanded their geographic and host ranges. Disease outbreaks have occurred with BTV-8 in Europe, EHDV-7 in Israel, EHDV-9 in Algeria and Morocco, and EHDV-6 and BTV-1, -3, -5, -6, -14, -19, and -22 recently have been detected in the United States. Needless to say, these events have many experts scratching their heads, and, although tempting, it is premature and overly simplistic to attribute all of this to global warming. Although the causes of these events may or may not be connected, it is clear that we need to answer (and perhaps re-examine) some very fundamental questions to begin to understand why they occurred.

In 2006 and 2007, BTV-8 caused an extensive outbreak in northern Europe. During 2006, sheep and cattle were affected on over 2,000 farms in the Netherlands, Belgium, France, and Germany, and during 2007 the virus “re-emerged” to infect livestock in this same region and expanded its range to Denmark, Switzerland, and the United Kingdom. This outbreak is significant not only because of its scale but also because of its spatial distribution. Historically, bluetongue outbreaks have not been observed in Northern Europe, and this is not an area where Culicoides imicola, an important vector for bluetongue in southern Europe and northern Africa, is known to exist. This ongoing example of range expansion does not stand alone. Since 1999, BTV-2, -4, -9, and -16 have been observed in Bulgaria, Croatia, Kosovo, Macedonia, and Yugoslavia, all of which are located north and west of historic bluetongue range. These events challenge our existing knowledge of bluetongue epidemiology, especially as related to the identification of vector-competent Culicoides species and our understanding of how climatic conditions can affect vector range and movement, as well as viral replication in competent vectors. The fact that BTV-8 occurred in two consecutive years in Northern Europe also highlights our failure to understand how these viruses overwinter. In other words, there is a growing list of researchable questions that need to be addressed to understand the current situation.

Concurrent with the BTV-8 outbreak in Europe in 2006, EHDV-7 caused disease in cattle in Israel, and a similar outbreak of EHDV-9 was reported in northern Africa (Algeria and Morocco). These are the first confirmed reports of EHDV-related disease in cattle since 1959. The 1959 event in Japan involved Ibaraki virus, which now is recognized as a strain of EHDV-2. It is well established that EHDV can infect cattle, but these viruses generally are not associated with disease in this or other domestic animal species. These recent events test our understanding of EHDV pathogenesis, the potential impacts of this disease, and the distribution of these viruses worldwide. With regard to the latter, the minimal connection between EHDV and livestock disease has led to a situation where reference viruses, representative field isolates, and sequence data for most of the recognized EHDV subtypes are either not available, or are very limited.

We should not be complacent about EHDV in the United States. Both EHDV-1 and EHDV-2 have been responsible for numerous and large-scale mortality events in wild ungulates, especially white-tailed deer, and during 2002 and 2007 we have witnessed major outbreaks in the eastern United States. The unprecedented 2007 EHDV-2 outbreak exhibited two characteristics that should spark some concerns when viewed in relation to the outbreaks of BTV-8 in Europe and EHDV-7 in Israel. In 2007, EHDV-2 occurred in many northern states where deer normally are never or rarely infected. There also is evidence that many cattle herds were affected during this outbreak. This is not the first time that EHDV-2 (concurrent with outbreaks in white-tailed deer) has been suggested as a cause of cattle disease in the United States and not the first continued…
time EHDV-2 was isolated from affected cattle herds. However, due to limited research, clinical disease in cattle never has been produced experimentally with EHDV-2 or any other EHDV subtype other than the Ibaraki strain, and no challenge studies have been done for most of the EHDV subtypes.

The detection of EHDV-6 in white-tailed deer in Illinois and Indiana during 2006 as reported in the SCWDS BRIEFS (Vol. 23, No. 2) and again in Missouri in 2007 highlights additional problems in dealing with these viruses. The first relates to diagnostics, and the second relates to surveillance. In the case of diagnostics, polymerase chain reaction assay (PCR) protocols routinely are used for detecting both BTV and EHDV in clinical samples. It is important, however, to understand that these techniques should supplement rather than replace traditional virus isolation and identification protocols. PCR-based diagnostics would have represented a rapid and reliable method for identifying EHDV as the cause of death in these EHDV-6 cases. However, without additional follow-up, the presence of this exotic serotype in the United States would have gone undetected, even with the cases in hand. A second issue relates to surveillance sensitivity, which at present is unknown. To put this potential problem into perspective, the single isolation of EHDV-6 in Missouri in 2007, which was needed to fully understand if this new serotype is established in the United States, was only possible to achieve with close to 300 successful orbivirus isolations from white-tailed deer. Generally, in a single year there are fewer than 100 combined BTV and EHDV isolates from both domestic and wild animals throughout the entire United States.

The detection of exotic orbiviruses in the United States is not limited to EHDV. In 2007, experts at USDA’s National Veterinary Services Laboratories (NVSL) announced that BTV-3, -5, -6, -14, -19, and -22 were isolated and identified from Florida. During the last three years, SCWDS, with the assistance of NVSL, reported the isolation of BTV-1 and BTV-3 from white-tailed deer in Louisiana and Mississippi, respectively (see SCWDS BRIEFS Vol. 22, No. 3). The detection of these exotic BTVs in the United States underscores all of the questions, problems, and limitations discussed above. With these recently reported events, we may be dealing with the introduction of new viruses, genetic changes in existing viruses, the introduction of new vectors, range expansion by existing vectors (Culicoides insignis), transmission by established but currently unrecognized vectors, and changes in all of the climatic and land use factors that can influence vector/host relationships. Our understanding of the current situation also is hindered by limited surveillance for these viruses as described for EHDV-6 and, ironically, by the fact that all of these exotic BTVs are regulated as Select Agents. Although this designation was made to protect livestock and wildlife health, it also has served to restrict research and limit diagnostic and surveillance capabilities.

In viewing these problems, one is tempted to ask if they could have been predicted. This obvious question has no answer. However, it is possible that our ability to understand and possibly prevent these “new” events has been impacted by our generalized concept of the diseases caused by these viruses. For example, bluetongue has long been clearly recognized as a significant disease of sheep, but one that could be controlled through vaccination. In cattle, bluetongue was viewed primarily as a trade barrier rather than an important animal health issue. Epizootic hemorrhagic disease viruses were associated only with problems in wild ungulates in North America, not cattle. It also was widely accepted that the primary vectors for BTV were clearly identified and that they predictably regulated the distribution of these viruses. Although these generalized concepts are supported by the scientific literature, their complete acceptance has limited research opportunities and the surveillance needed to detect and fully understand the potential for change. In light of the evolving BTV and EHDV situation, perhaps the most basic question that we need to answer about these viruses and the diseases they cause is “Do we know as much as we think we know?” (Prepared by David Stallknecht)

Orbivirus Vector Surveys

SCWDS has conducted research and surveillance on foreign animal disease vectors in the United States and the Caribbean region since the 1960s. Under a Cooperative Agreement with USDA-APHIS-Veterinary Services that began in 2003, SCWDS conducts surveillance for exotic ticks and other livestock arthropods in the southeastern United States and Puerto Rico. This program has been centered on detecting exotic ticks and other ectoparasites on wildlife in Florida, with limited surveys conducted in Alabama, Georgia, Mississippi continued…
and Puerto Rico (see SCWDS BRIEFS Vol. 21, No. 4). Due to the recent expansion of bluetongue viruses in Europe and the detection of several non-endemic bluetongue and epizootic hemorrhagic disease viruses in the United States, the program is being expanded to include surveys for the biting fly vector, *Culicoides* spp., in Florida and the coastal plain regions of Alabama, Georgia, Mississippi, and Louisiana. Results of these surveys may be helpful in determining whether exotic species of *Culicoides* have facilitated introduction of exotic orbiviruses into the United States.

Exotic arthropods can enter the United States through various pathways, including migratory birds, winds and tropical storms, imported plants and animals or their products, and on persons returning to the United States from international travel. In some cases, introduced arthropods may become established and can cause significant economic or environmental damage, as well as harm to domestic animal, wildlife, and/or human health. Early detection is critical to the success of eradication efforts.

Our initial surveys have been designed to identify bluetongue virus vectors, including exotic *Culicoides* spp., at selected sites in the Gulf Coast region and will include areas where non-endemic orbiviruses recently have been detected. Surveys will include insect trapping at sites throughout the region, with processing and identification of *Culicoides* spp. at SCWDS. Dr. William Grogan, an entomologist at Salisbury University who is a Cooperating Scientist with the USDA-ARS Systematics Entomology Laboratory, is providing support with systematics. (Prepared by Joe Corn)

**Studies on H5N1 HPAI Virus in Swans and Geese**

An important research project on H5N1 highly pathogenic avian influenza virus (HPAI) recently was completed, and the results were disseminated in the monthly scientific journal published by the Centers for Disease Control and Prevention (Brown, Stallknecht, and Swayne. 2008. Experimental infection of swans and geese with highly pathogenic avian influenza virus (H5N1) of Asian lineage. *Emerging Infectious Diseases* 14(1): 138-142). The study was a collaborative effort between scientists at SCWDS and USDA’s Southeast Poultry Research Laboratory (SEPRRL) and was funded by grants from the USDA-Agricultural Research Service, the United States Poultry and Egg Association, and Morris Animal Foundation.

Since 2002, H5N1 highly pathogenic avian influenza (HPAI) virus has caused morbidity and mortality in wild birds in Southeast Asia, where the virus is endemic in domestic poultry (including domestic ducks). During the winter of 2005-2006, H5N1 HPAI virus spread to Central Asia, followed by multiple introductions into Europe. During this 2005-2006 Eurasian epidemic, H5N1 HPAI virus was reported in 13 European Union member states and some surrounding countries and caused mortality in both domestic and wild birds. The majority of wild bird mortalities (and H5N1 HPAI virus isolations) occurred in a select number of species in the Order Anseriformes (ducks, geese, and swans), some of which included mute swans, whooper swans, tufted ducks, bar-headed geese, and Canada geese. Though unproven, a growing body of genetic and epidemiologic data suggests that some of these clinically affected species of migratory waterfowl may have had a role in the geographic spread of H5N1 HPAI viruses during these Eurasian outbreaks. However, H5N1 HPAI viruses rarely have been isolated from clinically healthy wild birds in Europe that were not associated with ongoing mortality events, despite intense surveillance efforts, and there is no clear evidence to suggest the virus can be maintained or geographically spread by infected asymptomatic wild birds. A reliance on dead bird surveillance has left several gaps in our understanding of the role that wild birds played in the 2005-2006 H5N1 HPAI outbreaks in Eurasia. The question is whether the wild birds that died from H5N1 HPAI infection were reservoirs for the virus or simply dead-end hosts.

The objective of the SCWDS/SEPRL study was to evaluate the susceptibility and viral shedding patterns in four species of swans and two species of geese experimentally infected with an H5N1 HPAI virus and to potentially extrapolate on each species’ ability to serve as a reservoir and/or a geographic disseminator of the virus. The species included in this study were whooper swans, black swans, trumpeter swans, mute swans, bar-headed geese, and cackling geese.

The results of this study indicated the following: (1) all four species of swans were highly susceptible to H5N1 HPAI virus, as evidenced by 100% mortality, though the swan species varied in their onset of clinical signs, duration of morbidity, and mean death times. The H5N1 HPAI virus also was virulent for...
both species of geese but less than for the swans, as indicated by the lower mortality rates; (2) clinical signs in the birds that died in each species were neurologic, and the most severe microscopic lesions were in the brain; (3) birds in all six species shed virus in respiratory secretions and the feces prior to the onset of detectable clinical signs, but species-related variability was evident. Mute swans, cackling geese, and bar-headed geese had the longest duration of asymptomatic viral shedding and excreted the highest concentrations of virus prior to the onset of clinical signs. Based on these data, infected birds in these migratory species would have the greatest potential to geographically spread H5N1 HPAI virus.

While the results of this study suggest that swans and geese are highly susceptible to infection with H5N1 HPAI viruses, this is not the case for all anseriform species (see SCWDS Briefs No. 22, Vol. 1). In a previous experimental infection trial conducted by SCWDS and SEPRL, only wood ducks exhibited high mortality after exposure to H5N1 HPAI viruses, while the remaining anseriform species (mallards, blue-winged teal, redheads, and Northern pintails) were resistant to infection and did not die or exhibit signs of disease. Taken together, these experimental infection studies indicate that susceptibility to H5N1 HPAI virus is species-dependent within the Order Anseriformes. Furthermore, these studies suggest that relatively few avian species, rather than all waterfowl, may have contributed to the geographic spread of H5N1 HPAI virus in Eurasia in 2005-2006.

This study shows that some waterfowl species, based on concentration and duration of asymptomatic viral shedding, do have the ability to spread H5N1 HPAI virus over limited geographic distances. However, laboratory studies and existing field data have failed to identify a wild avian species that possesses characteristics consistent with a reservoir host, such as prolonged duration of asymptomatic viral shedding or frequent isolations of virus from clinically healthy birds. Rather, the data suggest that H5N1 HPAI virus infections in wild birds likely represent short-term spill-over events from domestic poultry and that an asymptomatic or “silent” wild avian reservoir for H5N1 HPAI virus may not exist. The entire manuscript may be accessed on the CDC website at www.cdc.gov/eid/content/14/1/136.htm. (Prepared by Justin Brown)

**Dr. Justin Brown Awards**

Dr. Justin Brown, SCWDS Post-doctoral Research Associate, attended the 31st Annual Meeting of The Waterbird Society held in Barcelona, Spain, in November 2007 and received The Best Student Paper Award for his presentation entitled “Experimental H5N1 HPAI Infections in Waterbirds: Clinical Response and Viral Shedding Patterns.”

Justin also is to be honored by the American Association of Avian Pathologists (AAAP) at its annual meeting in New Orleans in July 2008. Justin will receive The Reed Rumsey Student Award for Advancement of Avian Medicine for his research on H5N1 highly pathogenic avian influenza virus in wild birds. This award was established by former AAAP president Dr. Reed Rumsey shortly before his death in 1980. Dr. Rumsey was “highly respected by his peers in the industry and academia, he also had great faith in young people and the future of the poultry industry.”

To qualify for the award, an applicant must have already received his or her DVM degree or be pursuing a DVM degree and be engaged in research in avian medicine or have a case report to submit. Preferential consideration is given to nominees who express a commitment to devoting their career to avian medicine. The award consists of $1,000, plus travel expenses to the American Veterinary Medical Association’s annual meeting. Justin was nominated for the award by Dr. David E. Swayne, Laboratory Director of USDA’s Southeast Poultry Research Laboratory in Athens, Georgia, who was a member of Justin’s Doctoral Advisory Committee.

We are extremely proud of Justin for these and his many other achievements at SCWDS and offer our congratulations and thanks for his excellent work. (Prepared by Gary Doster)

**Impacted Turkey Gizzard**

On November 12, 2007, a landowner in Louisiana found an adult male wild turkey that could not walk or fly. The bird was easily captured and was delivered to the Louisiana Department of Wildlife and Fisheries, where it was euthanized. The wildlife biologist who submitted the case was aware that large amounts of corn were provided for wildlife at the site from which the turkey originated, and he was concerned about the possibility of aflatoxicosis.
The carcass was shipped to SCWDS for postmortem examination.

The turkey was severely emaciated and weighed only 7.5 pounds. The pectoral muscles were severely atrophied, and there was no visible fat. The liver was only about half its expected size, and the kidneys filled only 70% of the renal fossae, the cavities adjacent to the backbone that they normally fill completely.

The most significant lesion, however, was a hard 7-x 7-x 4-cm mass that distended more than half of the gizzard (Figure 1). This mass consisted of tightly packed fibrous plant material, grit, a small amount of acorns, and wheat (Figure 2). Such an indigestible mass of plant material in the gastrointestinal tract is termed a phytobezoar. The portion of the gizzard distended by this mass was extremely thin and necrotic. The opening to the small intestine was patent, but its diameter was reduced because the lining was thickened.

We are not aware of any previous reports of gizzard impactions due to phytobezoars in wild turkeys, and this condition is not likely to be of any significance to turkey populations. At some point this bird may have consumed an excessive amount of roughage that tipped the scales towards formation of the phytobezoar.

The concerns of potential aflatoxicosis expressed by the submitter were realistic in light of the history of supplemental corn being applied in the vicinity. Aflatoxicosis in domestic poultry has been associated with many of the signs and lesions described here, including inappetence, reduced activity, unsteady gait, and variable atrophy of the liver. Clinical signs and history are invaluable in disease investigations, but in some cases a definitive diagnosis is only possible with a thorough postmortem examination. (Prepared by Kevin Keel)

**Regional Disease Workshops in 2008-09**

In 2007, SCWDS received a 3-year Multi-State Conservation Grant (MSCG) to provide wildlife disease workshops for biologists with state fish and wildlife management agencies across the country. The MSCG Program is funded by the U.S. Department of the Interior and is intended to address regional or national-level priorities of state fish and wildlife agencies. The program is cooperatively administered by the U.S. Fish and Wildlife Service (FWS) and the Association of Fish and Wildlife Agencies (AFWA). The AFWA solicits, selects, and recommends to the FWS a priority list of projects to be funded. Such projects must address a National Conservation Need identified by the state wildlife agency directors to be high priority and must benefit at least 26 states, a majority of states in a FWS region, or a regional fish and wildlife agency association.

The project awarded to SCWDS will benefit all 50 states by providing wildlife disease workshops for personnel with every state fish and wildlife agency. The four workshops will be organized and held within the four regional agency associations, namely the Southeastern, Northeastern, Midwestern, and Western Associations of Fish and Wildlife Agencies. Two regional workshops will be held in 2008, with the remaining two in 2009. The goal of the workshops is to train as many agency personnel as possible, and the bulk of the funding for this grant will be provided to state agencies to cover the costs...
of employee travel and attendance at the workshops. Delivery of these funds to the states will be administered by AFWA, and agency directors will receive letters with details on funding and other logistical information.

Course content and speakers for the workshops will vary with each region in order to highlight the significant diseases of the key wildlife species found there. General information to be provided in all regions will include protocols for wildlife mortality investigations; necropsy of wild birds and mammals; sample collection, processing, and shipping for diagnostic testing; foreign animal diseases; biosafety and biosecurity; zoonotic diseases of concern to biologists; reducing risk factors for the introduction and establishment of diseases in wildlife; and other topics. We look forward to seeing you at these workshops soon. (Prepared by John Fischer)

Dove Disease Research at SCWDS

Trichomonosis is the most significant infectious disease of mourning doves. With funding from the Dove Sportsman Society of Quail Unlimited, SCWDS recently conducted research to better understand the disease. Trichomonosis in doves often is referred to as crop canker by hunters and bird watchers. The disease appears as large yellowish-white cankers in the oral cavity and esophagus of affected birds and kills birds by interfering with feeding and swallowing. The cause of trichomonosis is a microscopic protozoan parasite, *Trichomonas gallinae*. The disease predominantly is observed in members of the Family Columbidae (doves and pigeons), due to their unique courtship rituals and methods of feeding nestlings. Doves and other birds also may acquire the parasite at contaminated bird feeders and birdbaths.

Trichomonosis has been reported throughout the world, and in the United States several large outbreaks have been documented in mourning doves. Recent outbreaks have occurred in mourning doves in Arizona and Nevada and in band-tailed pigeons in California. Generally, outbreaks occur focally and affect low numbers of birds. These focal outbreaks are reported by most states on a yearly basis and often are associated with contaminated bird feeders and/or birdbaths. *Trichomonas* also affects raptors that ingest infected birds and it can have significant local impacts on raptor populations. In raptors, trichomonosis causes lesions similar to those seen in Columbids and is called frounce.

It has been recognized that some strains of *T. gallinae* exist in apparently healthy birds, while other strains cause severe disease and death. Virulence factors contributing to the ability of different *T. gallinae* strains to cause disease are not fully known. Recent studies of a closely related *Trichomonas* parasite in humans have demonstrated that some of the human trichomonads are infected with viruses. Furthermore, the virus-infected protozoans may cause more severe disease than virus-free trichomonads.

SCWDS conducted a study to determine whether *T. gallinae* samples from mourning doves and other birds contain viruses. Twelve virulent and avirulent *T. gallinae* isolates were examined for viruses using molecular techniques and electron microscopy; however, viruses were not detected in any of the isolates. Consequently, in the isolates we examined we observed no association between the virulence of certain *T. gallinae* organisms and the presence of viruses.

Additionally, we genetically analyzed 42 isolates of *T. gallinae* collected from several avian species and found that although all of the parasites were morphologically similar, their genetic composition varied considerably. This genetic variability was significant enough to strongly suggest that the parasites may belong to at least three species. Interestingly, isolates from rock pigeons were distinct from isolates originating from white-winged doves, which also were distinct from those originating from common ground-doves, suggesting that rock pigeons, white-winged doves, and common ground-doves may have individual host-associated species of *Trichomonas*. We found that *Trichomonas* organisms infecting mourning doves and raptors belong to the genetic groups associated with rock pigeons or with white-winged doves. This information, combined with apparently higher infection prevalence in rock pigeons and white-
winged doves than in mourning doves, as well as higher prevalence among mourning dove populations that are sympatric with these two species, suggests that mourning doves and raptors may become infected via direct and/or indirect contact (sharing feed or water sources) with rock pigeons and white-winged doves.

As is the case with many research projects, it appears that our work has resulted in more new questions than answers to the old ones: Additional studies are necessary to identify virulence mechanisms and factors in *T. gallinae*, as well as to elucidate the epidemiology of trichomonosis in doves, pigeons, and raptors. (Prepared by Rick Gerhold)

Some Staff Changes

We are pleased to welcome Dr. John A. Bryan, II to our staff. John completed his DVM degree at the University of Georgia’s College of Veterinary Medicine in May 2007 and joined SCWDS on August 1. John is completing work on an MS degree in veterinary pathology while working full time at SCWDS. His primary duties include diagnostic work on cases submitted by SCWDS member state fish and wildlife agencies, and he coordinates chronic wasting disease testing under Dr. Kevin Keel’s direction.

John has an interesting background and brings some valuable experience and skills to SCWDS. After receiving his undergraduate degree from Emory University with a major in political science, John joined the United States Peace Corps and spent the next few years in The Republic of Gabon in Central West Africa, where he was involved in the Rural Development Program as the Director of the Integrated Research Farming Project.

Upon completion of his tour with the Peace Corps, John came back to Georgia and entered vet school. However, because he still has a great interest in the people of Central West Africa, during his matriculation John returned to The Republic of Gabon in Central West Africa, where he was involved in the Rural Development Program as the Director of the Integrated Research Farming Project.

We also welcome Ms. Deena Lopez as another new member of our staff. Deena completed her BS degree at the University of Delaware in May 2007, with a major in animal science and wildlife conservation. While in school, she gained experience as a student researcher in animal science by working in the molecular microbiology laboratory on a pathogenic avian form of the intestinal bacterium *Escherichia coli*. In this work, Deena became skilled in various laboratory procedures and techniques, including the polymerase chain reaction (PCR) test and gel electrophoresis. She gained other valuable experience while in school by serving summer internships at the St. Louis Zoo in 2006 and at the Brookfield Zoo in Illinois in 2007. Her previous experience will serve her well at SCWDS where Deena will be working under the direction of Drs. John Bryan and Kevin Keel processing the thousands of samples submitted to our laboratory from southeastern states for chronic wasting disease testing. Deena portends to be a valuable addition to our staff. Welcome Deena!

Dr. Rick Gerhold has completed his MS degree at SCWDS and has entered a PhD program to do research on *Histomonas meleagridis*, the protozoan parasite that causes blackhead disease in birds. Rick will conduct analyses, as well as experimental infections of domestic and wild galliforms. His co-major professors are Dr. Buffy Howerth in the College of Veterinary Medicine’s Pathology Department and Dr. Larry McDougald in the University of Georgia’s Poultry Science Department. We enjoyed and benefited from having Rick with us and regret to see him leave. We wish him well in his new venture.

Dr. Justin Brown has completed his PhD program in veterinary pathology and has accepted a Post-doctoral Research Associate position at SCWDS to continue his important work on the H5N1 highly pathogenic avian influenza viruses. We are pleased that he has decided to stay with us and give us the benefit of his experience and expertise. He is a hard working, dedicated researcher with a first-rate work ethic. (Prepared by Gary Doster)