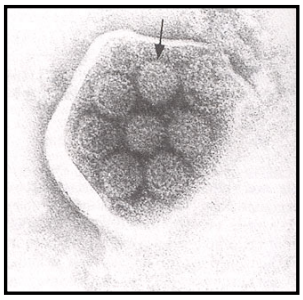
United States Department of Agriculture

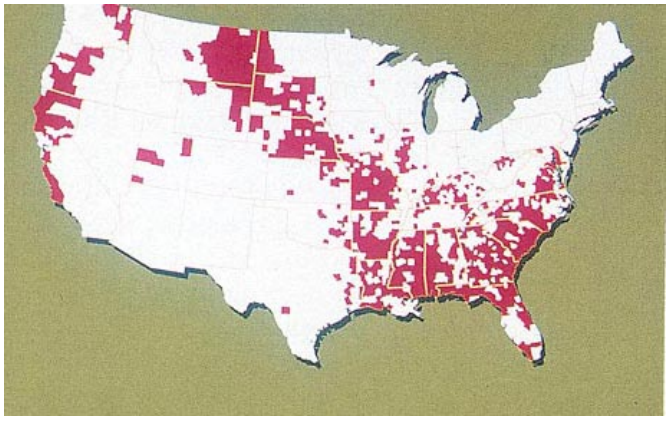
Agricultural Research Service

December 2013

Orbiviruses Gap Analysis

Bluetongue and Epizootic Hemorrhagic Disease Workshop Report





Mention of trade names or commercial products in this report is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture (USDA).

The Agricultural Research Service conducts research to develop and transfer solutions to agricultural problems of high national priority and provides information access and dissemination to ensure high-quality, safe food and other agricultural products; to assess the nutritional needs of Americans; to sustain a competitive agricultural economy; to enhance the natural resource base and the environment; and to provide economic opportunities for rural citizens, communities, and society as a whole.

To cite this report:

Orbiviruses, Bluetongue and Epizootic Hemmorhagic Disease: Gap Analysis Workshop Report. 2013. U.S. Department of Agriculture, Agricultural Research Service, Washington, DC. http://go.usa.gov/BJ5F

CONTENTS

Group Picture – May 14, 2013

Glossary

ABADRU	Arthropod-Borne Animal Disease Research Unit, ARS, United States
AGID	Agar gel immunodifusion
AHSV	African horse sickness virus
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, Maison-Alfort, France
APHIS	Animal and Plant Health Inspection Service
ARS	Agricultural Research Service
BTV	Bluetongue virus
CMIR	Cell-mediated immune response
CVI	Central Veterinary Institute of Wageningen UR, The Netherlands
DIVA	Differentiating infection in vaccinated animals
EEV	Equine encephalosis virus
EHDV	Epizootic hemorrhagic disease virus
ELISA	Enzyme-linked immunosorbent assay
FLI	Friedrich-Loeffler-Institut, Riems, Germany
IHC	Immunohistochemistry
NAHLN	National Animal Health Laboratory Network
NVSL	National Veterinary Services Laboratories
OIE	World Organisation for Animal Health
PCR	Polymerase chain reaction
PIR	Pirbright Institute, United Kingdom
PPE	Personal protective equipment
RT-PCR	Reverse transcriptase-polymerase chain reaction
rtRT-PCR	Real-time Reverse transcriptase-polymerase chain reaction
SCWDS	Southeastern Cooperative Wildlife Disease Study
UGCVR	The University of Glasgow Centre for Virus Research, Scotland, United Kingdom

EXECUTIVE SUMMARY

The viruses that cause bluetongue (BT) and epizootic hemorrhagic disease (EHD) are of concern to livestock producers in North America because of 1) the emergence of new serotypes, 2) increased reports of spillover and clinical disease in cattle, and 3) increased spread and adaptation to new geographical areas. Accordingly, the United States Animal Health Association (USAHA) passed Resolution 16 in October 2012 requesting the United States Department of Agriculture (USDA) and the United States Department of Interior (DOI) to organize a diverse panel of experts including industry stakeholders, university and federal researchers, and federal and state regulatory agency representatives to determine research needs and identify and prioritize intervention strategies.

In response to USAHA Resolution 16, USDA in collaboration with DOI organized a gap analysis workshop composed of international experts on *Orbiviruses*. The workshop participants met at the Arthropod-Borne Animal Diseases Research Unit in Manhattan, Kansas, May 14–16, 2013, to assess the available scientific information and countermeasures to effectively control and mitigate the impact of an outbreak of an emerging *Orbivirus* with epizootic potential, with special emphasis given to bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV).

In assessing the threats, workshop participants determined that the following countermeasures were important but identified several weaknesses that affect the ability to prevent and control disease outbreaks. These obstacles are summarized briefly below.

Variability in Expression of Clinical Disease

The *Orbiviruses* BTV and EHDV are important because they can cause severe disease outbreaks, but not every susceptible species will show disease following infection with all strains of these viruses because of differences in the susceptibility of animal hosts and in the pathogenic potential of strains of the viruses. Hence, the OIE pragmatically defines cases of BTV and EHDV as infection with these viruses, with or without clinical signs. The basis of differences in species susceptibility to disease is not known. The virulence determinants of *Orbiviruses* are not described, so the viruses cannot be monitored effectively. Comprehensive disease control strategies require basic research to determine these unknown aspects of the virus/host interaction.

Virology

Virology research provides basic information that helps elucidate key aspects of virus-vector-host interactions, pathogenesis, diagnostics, epidemiology, and control strategies. Recent advances in reverse genetics, small and large animal models, genomics, and reagents such as monoclonal antibodies, reference sera, and expressed proteins all contribute to the growing field of *Orbivirus* research. Many gaps in knowledge and research tools remain in areas such as molecular determinants of host and vector specificity, virulence factors, antigenic determinants, epitope mapping, receptors, mechanisms of cell culture adaptation, immune responses of mammalian and vector hosts to infection, transmission mechanisms in vertebrate hosts, transovarial transmission in arthropod vectors, antivirals, genomics, and proteomics, and the criteria for domestic versus exotic serogrouping and topotyping need to be re-examined.

Surveillance

Understanding of the ecosystems supporting the arthropod transmission of BTV and EHDV in different climatic and geographic zones is lacking, as is understanding of the distribution of competent vectors and different strains of viruses in the United States and the factors that influence the expression and incidence of clinical disease within specific climatic and geographic zones for all potential affected mammalian species. An active national surveillance program for BTV or EHDV is needed. Such a surveillance program would require consensus among national and state diagnostic laboratories on the best detection strategies for both disease diagnosis and surveillance testing.

Diagnostics

In general, sheep and cattle producers, veterinarians, and diagnosticians are inadequately informed about the clinical presentations of BT and EHD. Existing diagnostic capabilities are unevenly distributed nationally, with varied availability of validated tests and sample processing capacity. Nationally coordinated, standardized surveillance supported by agreed-upon test methods for detection and characterization of *Orbiviruses* is needed. Such a national system would define the need for and management of rapid field-based diagnostic tests.

Vector Control

Better understanding of midge host attack behavior is necessary, and better insecticides, repellants, and kairomones that can be used to reduce biting attack rates are needed. More complete information regarding developmental sites of key vector species, including the biological attributes of those habitats with attention to biotic (e.g., coexisting macro and microorganisms) and abiotic (e.g., moisture level, pH, or chemical description) features could lead to management technologies that could reduce transmission of virus. As part of this effort, there is a need to determine adult resting sites, sources of natural sugars, where and when adults mate or lay eggs, and how far and quickly the adults disperse. This is important for evaluating local spread of disease or effectiveness of control efforts and ultimately for understanding the potential for long-distance spread of disease (e.g., via wind-aided dispersal of infected midges). Identifying alternate *Culicoides* species that may serve as vectors of *Orbivirus* transmission in specifc climatic conditions or zones, and possibly different seasons of the year, is necessary.

Coordinated efforts are needed to characterize physiological and genetic mechanisms related to vector competence and midgut virus infection (exploiting the *C. sonorensis* genome). This information could be useful in creating innovative intervention and management approached to control BT or EHD.

Vaccines

Although a limited number of vaccines are available internationally for BTV, there are none for EHDV. Issues regarding availability of inactivated BTV vaccines are substantial because some are no longer produced commercially in Europe (e.g., those to BTV serotypes 1 and 8). The only fully licensed vaccines in the United States are attenuated, modified-live vaccines. Autogenous vaccines have been used in the captive cervid industry to immunize deer against EHDV and BTV infection. Success has been limited at best. No peer-reviewed objective data are available to

assess immunogenicity, efficacy, or effectiveness. Attenuated, modified-live vaccines have significant safety issues associated with their potential dissemination by insect vectors and reassortment of genes with those of circulating wild-type virus in the field, vertical transmission, and inherent issues related to either under- or over-attenuation of the vaccine virus. Inactivated vaccines provide only serotype-specific protection but are reasonably efficacious and safe. Because of the safety concerns associated with using attenuated vaccines, inactivated vaccines may be the best option in the face of an epizootic emergency. Current inactivated vaccines usually require two doses and do not typically provide sterilizing immunity, which is important in preventing shed and transmission from infected hosts. Neither inactivated nor live-attenuated BTV nor EHDV vaccines are currently DIVA [Differentiation between Infected and Vaccinated Animals]-compatible, whereas new-generation products could be DIVA-compatible. Standardization of diagnostic procedures within diagnostic laboratories and establishment of routine surveillance are critical components of any *Orbivirus* vaccination control program that may be developed in the future.

Recommendations

Based on our current state of knowledge and available countermeasures, the following intervention strategies are recommended in the case of a disease outbreak caused by an emerging or exotic *Orbivirus*.

Detect Threats Early

1. Deploy the best diagnostic methods and procedures to strengthen laboratory networks.
2. Launch and strengthen *Orbivirus* Biosurveillance between National Veterinary Services Laboratory (NVSL) and National Animal Health Laboratory Network (NAHLN).
3. Define the characterization required of any viral strains detected, the tests to be used to deliver this information, the reference laboratories in the laboratory network that will have the repsonsibilities for such tests, and the obligations of all parties to submit viral strains to reference laboratories for these analyses. Strengthen capabilities for accurate and transparent reporting.
4. Train and deploy an effective biosurveillance workforce.
5. Establish a surveillance program using the World Organisation for Animal Health (OIE) Code, which contains specific guidelines for surveillance.

Respond Rapidly and Effectively

1. Develop an interconnected state-federal network of emergency operations centers and multisectoral response to *Orbivirus* outbreaks.
2. Improve access to effective countermeasures during disease outbreaks.

Vaccination

1. A focused effort to identify potential master seedstocks of North American serotypes of BTV and EHDV should be initiated; inactivated vaccines have been produced against only a limited number of BTV serotypes, and revamping production of an existing commercial vaccine can take several months, but creation of an entirely new one can take 2 years or longer, so the presence of available seedstocks to all 26 serotypes of BTV and all 7 serotypes of EHDV would potentially expedite creation of new vaccines.

2. Use of conventionally attenuated vaccines should be avoided if possible due to known instances of vector transmission and reassorment with wild-type viruses, although these are the only vaccines available currently in the United States, and their use to protect against serotypes 10, 11, and 17 may need to continue for the near future in sheep. These vaccines provide good protection from clinical disease with homologous serotype infections. No licensed vaccines are currently available for EHDV in the United States.

3. If a need arises for rapid development of a vaccine to meet an emerging crisis, development of an inactivated virus vaccine in a conventional adjuvanted formulation containing the correct field virus strain is recommended. With two doses of vaccine, inactivated vaccines can provide substantial immunity to the epizootic serotype. This strategy is similar to that used in the 2006–2008 BTV-8 outbreak in Northern Europe, which provided vaccine to the field by 2008. Regulatory mechanisms to deploy such vaccines in an emergency situation need to be explored and developed.

4. New experimental vaccine approaches that may provide DIVA strategies and improved immunity strategies should be developed. These vaccine approaches are described in the research section below.

Research Priorities

In the long term, the group determined that implementing the research studies described below is critical to address the gaps in our scientific knowledge and advance the availability of effective countermeasures. Workshop participants recommend the research objectives described below to advance scientific knowledge of *Orbiviruses* and development of effective countermeasures. Detailed reviews and descriptions are found in each of the respective sections in the gap analyses.

Virology

A better understanding of the basic virology of BTV, EHDV, and the other *Orbiviruses* will help underpin all of the discussion topics that were included in the gap analysis workshop (vaccines, pathology, immunology, entomology, and epidemiology). Virology data are needed to help model current and future disease outbreaks and to understand the global risks we face from BTV, EHDV, African horse sickness virus, equine encephalosis virus, any of the 25 other *Orbivirus* species, and additional novel species that have the potential to emerge and threaten animal health and productivity as well as national or international livestock trade. A detailed assessment of the knowledge gaps is given on pages 28 to 33.

Diagnostics

Standard diagnostic tests are readily available for BT/EHD viruses, and described tests are published in the OIE Manual. Variation in the diagnostic approach to these viruses is considerable. Commercial BTV enzyme-linked immunosorbent assay test kits are available, and many laboratories are experienced in running these assays. Agar gel immunodifusion is still commonly used for EHDV; however, published and commercial EHDV ELISA kits are available. The NVSL does perform laboratory proficiency evaluation for BTV and EHDV serology annually. PCR-based assays are available for BTV and EHDV. Although experience with BTV rtRT-PCR is considerable, protocols run at various state and federal veterinary diagnostic laboratories have not been compared. Genotyping of *Orbivirus* isolates is an essential aspect of their characterization, and a national reference laboratory (or laboratories) should be designated to maintain real-time monitoring and analyses of circulating viruses. Advanced molecular detection methods, including next-generation sequencing and panviral DNA microarrays, have been demonstrated to be useful tools in rapid genetic characterization of viruses with segmented genomes, including BTV. Application of these methods due to costs of implementation is limited, although they could be benefitial for detecting highly divergent emerging strains and/or various species of *Orbiviruses*.

Epidemiology

Research gaps were classified into two broad categories. The first included gaps related to the overall pattern of transmission of BTV and EHDV in North America. The second included gaps related to the expression and incidence of clinical disease within the epidemiological zones. A number of gaps framed as research questions are listed within each category. The identified gaps include the need to characterize ecosystems supporting arthropod transmission of BTV and EHDV and the need to identify factors associated with disease emergence within the ecosystems (in different geographical and climatic zones). In its initial years, development of a coordinated national surveillance program and the analyses it would yield would be appropriately designated as nationally critical research.

Vector Control

In North America, particularly for domestic animal settings in the United States, the biting midge *Culicoides sonorensis* is regarded as the main vector of BTV and related *Orbiviruses*. In the scientific literature prior to 2000, this species was known as *C. variipennis sonorensis*; it has since been elevated to species status. This midge was incriminated as a transmitter of BTV more than 50 years ago, and subsequent field and laboratory studies have confirmed and built on that foundation. Because this is one of the very few species in the genus that has been colonized, *C. sonorensis* also has been the subject of many laboratory studies, such as vector competence for *Orbivirus* strains, and whether or not they are known to circulate in the United States. A priority going forward is to experimentally investigate physiological and genetic mechanisms related to vector competence and midgut infection. Exploiting the *C. sonorensis* genome, try to address similar concerns in alternate *Culicoides* species, which may involve intensified efforts toward laboratory colonization of those species.

Vaccines

Continued research and development of next-generation vaccines is warranted, particularly if they offer solutions to issues such as cross-serotype protection, DIVA compatibility, non-replicating antigens (no subsequent transmission of viral RNA to insects), and rapid onset of immunity with one dose of vaccine. Research objectives should lead to the development of next-generation vaccines for both EHDV and BTV.

INTRODUCTION AND BACKGROUND

Orbiviruses are members of the Reoviridae family and include bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV). These viruses are the cause of significant regional disease outbreaks among livestock and wildlife in the United States. Some of these outbreaks have been characterized by significant morbidity and mortality. Competent vectors are clearly present in most regions of the globe; therefore, all segments of production livestock are at risk for serious disease outbreaks. Exposure and subclinical infections also serve as reservoirs of infection and often result in significant trade restrictions. The economic and explicit impacts of BTV and EHDV infections are difficult to measure, but infections are a cause of economic loss for producers and loss of natural resources (wildlife).

Bluetongue (BT) and epizootic hemorrhagic disease (EHD) are noncontagious, insect-transmitted diseases of domestic and wild ruminants caused by related but distinct viruses. Both BT and EHD viruses cause hemorrhagic fevers in susceptible ruminants; however, BT is principally a disease of domestic livestock, whereas EHD is principally a disease of certain species of wild ungulates. BTV is the prototype member of the genus *Orbivirus*, family Reoviridae (Mertens et al., 2004). Epizootic hemorrhagic disease virus is also classified in the genus *Orbivirus* and is closely related but distinct from BTV. All reoviruses share distinctive common properties, including segmented genomes of double-stranded RNA (dsRNA) and characteristic virion morphology and structure. There are currently two subfamilies and some 16 distinct genera within the family Reoviridae, which includes pathogens of plants, crustaceans, fish, insects, reptiles, and mammals, including humans (Virus Taxonomy, Report of the IX Meeting of the ICTV, Elsevier, 2012). Some 22 distinct virus species of serogroups are within the genus *Orbivirus*, including BTV and EHDV. To date, 26 distinct serotypes of BTV have been described that all share common group antigens but are distinguished on the basis of serotype-specific virus neutralization assays (VNTs), and at least 7 serotypes of EHDV have been identified (Hofmann et al., 2008; Maan et al., 2011; Savini et al., 2011). Variation among field strains of BTV, even those of the same serotype, is considerable, which reflects differences in the nucleotide sequence of each of the 10 distinct dsRNA segments of the BTV genome (Balasuriya et al., 2008; Bonneau et al., 1999; Legisa et al., 2013; Maan et al., 2012; Maan et al., 2010; Pritchard et al., 2004; Shaw et al., 2013; Shirafuji et al., 2012). Genetic heterogeneity of field strains of BTV occurs as a consequence both of genetic drift and genetic shift, the latter as a result of reassortment of viral genes during mixed infections of either the vertebrate or invertebrate hosts of the virus (Bonneau and MacLachlan, 2004; Coetzee et al., 2012a; Coetzee et al., 2012b; Shaw et al., 2013). Variation in the sequence of individual genes occurs through a complex process of genetic drift and founder effect during alternating passage of BTV in its ruminant and insect hosts (Bonneau et al., 2001). Although less well characterized, similar variation also likely occurs among field strains of EHDV (Cheney et al., 1995; Cheney et al., 1996; Savini et al., 2011).

Bluetongue is principally a disease of certain breeds of sheep, although disease sporadically occurs in cattle and some species of wild non-African ruminants (Maclachlan et al., 2009; Verwoerd, 2012; Verwoerd and Erasmus, 2004). BT virus infection of ruminants occurs throughout much of the temperate and tropical regions of the world, coincident with the distribution of specific species of hematophagous *Culicoides*, biting midges that are biological

vectors of the virus (Gibbs and Greiner, 1994; Maclachlan, 2011; Tabachnick, 2004, 2010). BT typically occurs when susceptible sheep are introduced into areas where virulent strains of BTV circulate, or when virulent strains of BTV extend their range into previously unexposed populations of ruminants. The global distribution of BTV generally has been between latitudes of approximately 40–50°N and 35°S. BTV transiently incurred into southern portions of Mediterranean Europe during the 20th century, but since 1999, multiple serotypes have invaded and spread throughout extensive portions of the continent as well as farther north than previously documented (Gomez-Tejedor, 2004; Mellor et al., 2008; Mellor and Leake, 2000; Rodriguez-Sanchez et al., 2008). During the recent European BTV-8 epidemic, for example, the virus spread throughout Western Europe (Guis et al., 2012; Purse et al., 2008; Purse et al., 2005; Toussaint et al., 2006; Wilson and Mellor, 2008). The global epidemiology of EHDV infection is less precisely defined than that of BTV, but the global range of EHDV is predicted to occur between latitudes 35°S and 49°N, coincident with the distribution of competent biting midge vectors (Savini et al., 2011). Unlike BTV infection, EHDV has not been described to date in Europe, although the virus occurs throughout extensive portions of Asia, Southeast Asia, Australia, Africa, and the Americas (Savini et al., 2011). BT has been recognized in the United States since at least the late 1940s; BTV serotype 10 (BTV-10) was first isolated and characterized in California during the early 1950s (McKercher et al., 1953); and BTV-11, 13, and 17 were identified later (Barber, 1979). BTV-2 was first reported in Florida in 1982, and since 1998, some 10 additional serotypes of BTV have been isolated in the Southeastern U.S. (Gibbs et al., 1983; MacLachlan, 2011). BTV-1 was detected in the Southeastern U.S. in 2004 from a white-tailed deer isolate (Johnson, 2006). BTV-2, which was long considered to be confined to the extreme Southeastern U.S., was recently isolated from cattle in California (Maclachlan et al., 2013). The continual risk of virus introductions into areas with naïve livestock and wildlife populations, as well as the increased distribution of the *Culicoides* vector into northern latitudes due to warming climates, makes BT and EHD a constant threat to livestock producers.

Organization of the *Orbiviruses* Gap Analysis Workshop

An international team of *Orbivirus* experts from public and private research institutions, including industry, academia, and government, were invited to participate by the workshop organizing committee (Organizing Committee, Appendix II, page 78). A total of 37 experts (see Appendix XIII, page 98) accepted the invitation. The workshop was hosted by the Arthropod-Borne Animal Diseases Research Unit (ABADRU), Agricultural Research Service (ARS), with the support of the Center of Excellence for Emerging and Zoonotic Animal Diseases (CEEZAD www.ceezad.org), in Manhattan, Kansas, and the International Alliance for Biological Standardization (IABS - http://www.iabs.org). May 14–16, 2013. Instructions (see Appendix I) were provided by the organizing committee prior to the meeting. The workshop participants were tasked by the organizing committee with assessing the best available countermeasures to rapidly and effectively control and, where feasible, eradicate outbreaks of BT and/or EHD, with a special focus on new and exotic viruses should an outbreak occur in the United States.

Expert Reports

The *Orbivirus* experts who participated in the workshop provided the following report as background information.

Control tools for BTV: DISCONTOOLS Bluetongue gap analysis: http://www.discontools.eu/Diseases/Detail/38.

DEFINITION OF THE THREAT

Bluetongue and EHD outbreaks caused by endemic or exotic strains of BTV or EHDV were recognized as posing a significant threat to animal agriculture and wildlife. The following expert report summaries in this section describe the threats as viewed from agricultural and natural resources perspectives and the obstacles to effective mitigation and/or control.

Livestock Producers

The recent outbreak of BT caused by BTV-8 in Europe (2006–2008) demonstrated that under the right conditions, these viruses can cause extreme clinical diseases with losses due to morbidity, mortality, and reproductive failure in cattle and sheep. In regions where infection is endemic, however, the clinical disease caused by BTV is far more subtle, especially in cattle. These obscure clinical signs (listed below) are sometimes difficult to recognize and both acute and chronic forms of infection are manifest. In the more subtle forms of infection, the losses are somewhat insidious but can be substantial. In North America, epizootic outbreaks are more common in sheep in the Western states. The Northeastern section of the U.S. is historically free from BTV infection, as are portions of the Upper Midwest. Seroconversion of exposed animals has economic consequences in that it restricts export market access for seedstock and semen. The livestock industry would benefit from improved and standardized diagnostic assays, improved surveillance, improved vector control methods, and availability of effective vaccines and immunization programs.

When large-scale epizootics occur in deer, some spillover effects occur in cattle and sheep, which is possible with EHDV infection.Cattle are far more susceptible to the virus and may exhibit acute clinical disease that is much like clinical BT. Infection of whitetail deer (WTD) is endemic in the Southern U.S., and large-scale death loss is less common in this area. It is not completely understood why large-scale outbreaks of hemorrhagic disease occur some years in whitetail populations (e.g., 2007, 2012), but drought, vector density, and relative susceptibility of regional populations may play a role.

The captive cervid industry in the U.S. is large and growing. In years when widespread epizootics of either EHDV or BTV infections occur in wild WTD, these infections may also cause severe losses among captive cervid herds. Losses due to extreme mortality and chronic infections may occur.

Some summary points about these diseases are listed in Appendix XI.

Climate Change and the Emergence of Vector-Borne Animal Diseases

The historical distribution of EHD/BT mortality among ruminants in the U.S. has been relatively stable, with virus activity anchored in the Southern U.S. and extending in a northwesterly direction through the mid-Atlantic and Midwest states and into Montana and Washington, as well as west to California and parts of the intermountain west (Nettles et al., 1992; Stallknecht et al., 2002). Much of the Upper Midwest and Northeast is historically free of BTV and has been largely free of EHDV aside from infrequent incursions. In the past decade, however, outbreaks of hemorrhagic disease in these northern areas are becoming more frequent. Disease records

from several Northern states offer good examples: confirmed EHD outbreaks have occurred in New Jersey in 1955, 1975, 1999, 2007, and 2010–12; Michigan in 1955, 1974, 2006, and 2008–2012; and in New York in 2007, 2010, and 2012. These observations are striking, yet the ecological drivers are yet to be investigated. Two of the most widespread and intense epidemics observed since the monitoring of this disease began occurred in 2007 and 2012. Both epidemics coincided with drought and high temperature and resulted in not only widespread mortality among WTD populations, but also disease in cattle. For instance, during the 2007 epidemic, 11 Midwest and Mid-Atlantic states had counties with more than 100 dead WTD reported, with many counties reporting estimates of >1,000 mortalities (SCWDS, unpublished 2013). During the inter-epidemic period from 2007–2012, EHDV was isolated during most years in multiple Northern states: Michigan (4 years), Indiana (4 years), Pennsylvania (3 years), and New Jersey (4 years) (SCWDS, unpublished 2013). This frequency of isolation of EHDV from northern latitudes in the U.S. highlights our lack of understanding of the epidemiology of EHD, especially with regard to overwintering mechanisms and distribution of competent vector populations.

In 2006 and 2007, BTV-8 spread rapidly through sheep flocks and cattle herds in Northwestern Europe (Guis et al., 2012; Purse et al., 2008; Purse et al., 2005; Toussaint et al., 2006; Wilson and Mellor, 2008). The global epidemiology of EHDV infection is less precisely defined than that of BTV, but the global range of EHDV is predicted to occur between latitudes 35ºS and 49ºN, coincident with the distribution of competent biting midge vectors (Savini et al., 2011). As observed with EHD in the U.S., however, movement of the disease has been northward. All of the causes for this spread of the diseases are not known, but climate change may be a significant contributing factor.

In contrast to EHDV, the range of BTV in North America has apparently been stable over the past several decades. States in the Northeast and Upper Midwest historically have been consistently free of BTV infection. The virus has incurred into the Okanagan Valley of Canada on several occasions (1975, 1987, 1988, 1998, and 2004) but has never persisted there; thus, the northern limit of the virus' range in North America has long been considered to be 50ºN. At least 10 new BTV serotypes have been isolated in the Southeastern United States since 1998, however, in addition to the 5 serotypes that historically have been recognized to be present in the country. Furthermore, although BTV serotype 2 was considered since its original isolation in Florida in 1982 to be confined to the Southeastern United States, this serotype recently was identified in California. The role of climate in mediating these events awaits clarification.

OBSTACLES TO PREVENTING AND CONTROLLING *ORBIVIRUSES*

Obstacles to controlling *Orbivirus* diseases in the U.S. are numerous. The inherent complexities of the virus/vector/host interactions lead to variability in epidemiology and pathogenesis. Complicating factors include specific issues such as difficulties in insect pest management, under-detection and under-reporting of both infections of animals and of disease, human factors affecting dispersal of infections such as animal movements, limited vaccine availability, the possibility of the introduction of exotic serotypes, and the overarching effects of climate change.

BTV and EHDV are immunologically diverse viruses. To date, there are 26 serotypes of BTV and 7 serotypes of EHDV that are not cross-neutralizing, so immunity to one serotype confers little to no protection against another serotype of that virus. Similarly, diagnostic tests have to be targeted to either *Orbivirus* species or serotype detection with in viral species, resulting in the need for multiple tests. As with RNA viruses in general, the *Orbiviruses* show generic drift over time. This can be useful to show potential associations and relationships among isolates but can result in the need to validate diagnostic tests for the genotypes likely present within individual regions and for the genotypes of the viruses predicted to pose incursion threats. Additional factors of complexity include intra-host genetic variation that arises from selective pressures in the animal and especially in the insect vector due to midgut and salivary gland barriers, as well as inter-host genetic variation because of selective pressures exerted on the virus as it moves back and forth between mammalian and insect hosts.

Epidemiology and Surveillance

Many *Orbivirus* infections in vertebrate hosts are asymptomatic. Cattle usually do not show disease in response to either BTV infection or EHDV infection, although in some cases disease has been shown with certain strains of both viruses. Sheep may show disease with BTV infection, depending mainly on host and virus factors influenced by environmental factors, but not with EHDV. Some species of deer are more susceptible to *Orbivirus* disease, but not all species; hence, *Orbivirus* surveillance depends mainly on strategies to detect virus infection in the absence of disease, and the OIE defines cases of BTV and EHDV as infection with any strain of the virus regardless of whether clinical signs are observed. In North America, the concern of rural industries centers on the diseases caused by these infections.

Under the particular circumstances in North America, numerous factors have made wild ruminants, namely white-tailed deer (WTD), excellent sentinel animals for *Orbivirus* activity in North America: 1) WTD are extremely susceptible to EHDV/BTV infection and can develop severe disease, 2) the abundance and wide geographic distribution of WTD in North America, 3) passive surveillance of WTD through routine diagnostics by the Southeastern Cooperative Wildlife Disease Study (SCWDS; University of Georgia) and the National Veterinary Services Laboratory (NVSL), and 4) more than 30 years of data from a long-term HD mortality questionnaire-based survey conducted by SCWDS. Results from decades of monitoring hemorrhagic disease mortality among WTD in the U.S. have revealed temporal and spatial patterns of infection and disease (Couvillion et al., 1981; Nettles et al., 1992; Stallknecht et al.,

2002). Although WTD are not the predominant species in some areas where BTV and EHDV infections are common, the frequency of epizootics in certain regions appears to be increasing.

EHD and BT occur seasonally from midsummer to late autumn, with reports of disease and diagnostic submissions peaking in September (Couvillion et al., 1981; Stallknecht and Howerth, 2004); however, latitudinal and physiographic variation in the duration of the transmission season remains poorly defined. In addition, annual variation in disease is difficult to understand, especially given the changing patterns observed in the last decade mentioned above. Hemorrhagic disease outbreaks in wild ruminants historically have occurred in a two- to three-year cycle in enzootic regions and an eight- to ten-year cycle in epizootic regions (Couvillion et al., 1981; Nettles et al., 1992), but again, frequency of epizootics in certain regions appears to be increasing.

The obstacles to epidemiological analysis of *Orbivirus* disease transmission are:

- Lack of understanding of the ecosystems supporting arthropod transmission of BTV and EHDV in different climatic/geographic zones;
- Lack of understanding of the distribution of competent vectors and the distribution of different serotypes and genotypes of viruses (BTV and EHDV) in the United States;
- Factors influencing the expression and incidence of clinical disease within these zones for all potential hosts are unknown;
- Lack of active national surveillance program for BTV or EHDV infections; and
- Lack of formal agreement between national and state diagnostic laboratories on the best detection strategies for either disease diagnosis or surveillance testing, or a nationally coordinated laboratory *Orbivirus* testing network.

Diagnostics

The workshop participants determined that the effectiveness of available diagnostics is high, but several obstacles need to be addressed to ensure diagnostics are available, strategically deployed, and used effectively. Table 1 (Page 69) summarizes the most relevant diagnostic tests that are available now or under development. The U.S diagnostic system for detecting *Orbiviruses* consists of strategic links between the National Animal Health Laboratory Network (NAHLN) and the National Veterinary Services Laboratories (NVSL). The mission of the NAHLN includes surveillance, response (surge), and recovery from high-consequence agricultural diseases, including the introduction of new exotic *Orbivirus* strains. High-throughput semi-automated robotic systems are beginning to be deployed and utilized in the NAHLN laboratories. These systems are compatible with sample processing and rapid nucleic acid detection technologies. In addition, many state diagnostic laboratories perform routine tests for domestic *Orbiviruses*. The NVSL performs proficiency testing for laboratories performing BTV serology testing for export purposes on an annual basis.

Standard diagnostic tests are readily available for BT/EHD viruses, and described tests are published in the OIE Manual. Variation in the diagnostic approach to these viruses is considerable. Commercial BTV ELISA test kits are available, and many laboratories are

experienced in running these assays (IDEXX, IDVet, Invitrogen, and VMRD). There are no commercial rapid pen-side or field-based diagnostic tests for BTV or EHDV, but a serum antibody strip test for BTV has been reported (Yang, Hua et al., 2010). Detection of viral RNA by standard or rtRT-PCR is useful for screening samples prior to virus isolation. The generally preferred diagnostic test method for detection of the presence of BTV or EHDV is rtRT-PCR. There are published and commercial rtRT-PCR tests for BTV and published rtRT-PCR tests for EHDV (Toussaint et al., 2007; Hoffmann et al., 2009; Wilson et al., 2009; Clavijo et al., 2010; Sailleau et al., 2012). A PCR array for BTV serotype determination has also been reported (Maan, Maan et al., 2012). The commercial BTV rtRT-PCR kit is not readily available in the U.S., so various published assays are being used. An as-yet unpublished rtRT-PCR for detecting BTV also has been developed and evaluated by the USDA-APHIS. Although experience with the BTV rtRT-PCR is considerable, comparison of protocols run at various state and federal veterinary diagnostic laboratories has been limited.

An important technology essential for *Orbivirus* preparedness is the ability to rapidly characterize outbreak strains and strains detected by active surveillance using the molecular techniques of RT-PCR and sequencing. Such genotyping usually targets one of the more conserved genome sequences of the *Orbivirus* species concerned and allows inferences to be made regarding its likely geographic origin. In the U.S., this technology has been limited to a few research laboratories. Advanced molecular detection methods, including whole-genome next-generation sequencing and panviral DNA microarrays, have been demonstrated to be useful tools in detection and rapid genetic characterization of viruses with segmented genomes. Applications of these methods due to costs of implementation are limited, although they could benefit the detection of highly divergent emerging strains or species of *Orbiviruses*.

The obstacles to and requirements for detecting and characterizing *Orbiviruses* are:

- Farmers and cattle producers are not widely knowledgable about the disease symptoms;
- Existing diagnostic capabilities are limited in the availability of validated tests and also in sample processing capacity;
- No rapid field-based diagnostic tests for BTV or EHDV are available;
- Lack of standardization of diagnostic procedures among diagnostic laboratories; and
- No nationally coordinated surveillance strategy has been devised to comprehensively describe the epidemiology and molecular evolution of *Orbiviruses*, to analyse trends, and to designate requirements for submissions to national reference centre(s).

Vector Control

In North America, and for domestic animal settings in the U.S. in particular, the biting midge *Culicoides sonorensis* is regarded as the main vector of BTV and related *Orbiviruses* (Gibbs and Greiner, 1994; Tabachnick, 1996; Mellor et al., 2000). In literature prior to 2000, this species was known as *C. variipennis sonorensis*; it was elevated to species status by Holbrook et al. (2000). This midge was incriminated as a transmitter of BTV over 50 years ago (Foster et al., 1963), and subsequent field and laboratory studies have confirmed and built on that foundation.

Because this is one of the very few species in the genus that has been colonized (Hunt et al., 1999), *C. sonorensis* also has been the subject of many laboratory studies on aspects such as vector competence for *Orbivirus* strains, whether known or unknown from the U.S.

This vector species also is regarded as an inhabitant of manure-polluted, open, silty mud at the margins of habitats such as dairy wastewater ponds (Mullens and Rodriguez, 1988; Tabachnick 1996). This is not entirely true, as larvae of this species can be found in many very clean habitats such as the edges of desert mountain streams in Southern California (Mullens, personal observation). However, the success of this species in polluted waters is quite remarkable; the most productive wastewater ponds can contain densities in the range of 10,000 larvae per 30 ml of shoreline mud (Mullens and Rodriguez, 1988). It is useful to keep in mind that people, or aggregations of domestic or sometimes wild ruminants, typically tend to create the conditions that favor large populations associated with virus transmission by this species.

Europe recently experienced a difficult lesson in how rapidly a new vector-virus association can appear, as *C. obsoletus* and *C. pulicaris*-group midges began persistent and multi-year transmission of BTV-8 in areas of Northern Europe that were previously free of infection (Carpenter et al., 2009). Something similar could happen here, as demonstrated by a recent dramatic increase in detection and possible transmission of new virus serotypes in parts of the U.S. (e.g., Deep South, Upper Midwest)(Gibbs et al., 2008). In some of these areas, *Culicoides* species other than *sonorensis* already are strongly suspected as vectors (Smith and Stallknecht, 1996; Becker et al., 2010).

Some *C. sonorensis* management strategies exist, but most have been inadequately tested, and the techniques might not be helpful for other vector species. We cannot automatically assume that *C. sonorensis* is the main, or only, vector in all regions, even if these areas are known to be generally within the geographic distribution of *C. sonorensis*. Integrated control approaches require that certain proximal control approaches (e.g., animal protection using pesticides or repellents) must be supplemented by more long-term approaches to pest control that reflect continuing advances in basic knowledge of pest biology (e.g., dispersal, microbial ecology).

The obstacles to vector control are:

- Lack of understanding of host attack behavior;
- Need for better insecticides, repellants, and kairomones that can be used to reduce biting rates;
- Lack of information regarding developmental sites of key vector species including the biological attributes of those habitats with attention to biotic (e.g., coexisting macro and microorganisms) and abiotic features (e.g., moisture level, pH, or chemical description) that may be amenable to manipulation to reduce transmission of virus;
- Need to characterize physiological and genetic mechanisms related to vector competence and midgut virus infection exploiting the *C. sonorensis* genome;
- Need to dentify alternate *Culicoides* species that may serve as vectors of *Orbivirus* transmission; and

- Need to determine adult resting sites, sources of natural sugars, where and when adults mate or lay eggs, and how far and quickly the adults disperse, which is important for evaluating local spread of disease or effectiveness of control efforts and ultimately for understanding of the potential for long-distance spread of disease (e.g., via wind-aided dispersal of infected midges).

Vaccines

A limited number of modified live attenuated vaccines for BTV are available in the United States. The only vaccine approved for national use is against serotype 10 and is produced by the Colorado Serum Company. Attenuated vaccines against BTV serotypes 10, 11, and 13 are produced on behalf of the California Wool Growers by Poultry Health Laboratories; use of these vaccines is limited to sheep in California. These vaccines are capable of generating an effective immune response with one dose and are effective for preventing clinical BT disease. There are numerous potential adverse consequences to the use of attenuated BTV vaccines in livestock, including reduced milk production in lactating ewes as well as abortion, early embryonic death, and teratogenesis when used in pregnant females. The risk of spread through vectors with reversion to virulence and gene reassortment is considerable. The combination of perceived efficacy issues (cross-serotype protection and incomplete immunity) and safety issues (reversion to virulence, incomplete attenuation, and vector spread with gene reassortment) also contribute to less than enthusiastic use of the attenuated vaccines.

Licensed inactivated vaccines have not been available in the United States, presumably because the estimated market has been small. Autogenous vaccines have been produced using inactivated BTV and EHDV. These vaccines have been used extensively in sheep and the captive cervid industry with mixed reports of effectiveness. No published peer-reviewed data are available for evaluation of these autogenous vaccines. Most of the autogenous vaccines contain multiple serotypes of EHDV and BTV as well as mixed bacterial fractions/toxoids. Although the autogenous vaccines are perceived to be relatively safe, efficacy and effectiveness are questionable at best.

Development of vaccines engineered to DIVA and companion diagnostic tests for the vaccines described above are incomplete. With traditional attenuated vaccines, such development may be difficult, but new-generation vaccines using combinations of antigens (like the VLPs and recombinant expression vectors) clearly offer the advantage of DIVA capability allowing the use of infection-associated antibody responses to core structural proteins such as VP7.

Three major concerns are associated with the use of BTV vaccines. These concerns are, in part, technical issues associated with the biology of the disease agent-host interaction and/or a need for additional research and development.

1. The onset of immunity with any particular vaccine formulation must be defined. The rapid antibody response kinetics and field protection observed with both inactivated and attenuated vaccines suggests a very reasonable and acceptable onset of immunity. But clinical studies to determine the onset of immunity of inactivated and attenuated vaccines could be very useful in constructing immunization programs in the field. This is particularly important in the case of a vector-borne disease where the *Culicoides* vectors may spread disease (in terms of distance of spread and numbers of animal exposures) very rapidly.

2. Immunity to BTV is immunologically complex. With both attenuated and inactivated vaccines, sustained protection from viremia, specificity of virus-neutralizing antibody responses, and field effectiveness are effectively serotype-specific. Antibody and cellular interactions with critical viral structures require interactions with a complex set of linear and conformational epitopes. This problem will require continued research to define the nature of protective antigen structures and development of unique formulations and methods for delivery. This research will also define better serological and cellular assays as correlates of protective immunity.

3. On a global basis, support for developing BTV vaccines has been lacking. Most regions of the world deal with endemic disease with few or no attempts to vaccinate susceptible animals. Occasional bursts of disease activity revive some interest in related research. Funding is required to provide for developmental research to improve vaccine efficacy and safety profiles, which, in turn, increase the availability of relevant vaccines. The likelihood of future disease outbreaks is high, especially with climate change and increased global commerce.

The obstacles to vaccination are:

- Although a limited number of vaccines are available internationally for BTV, there are none for EHDV. Issues regarding availability of inactivated BTV vaccines are substantial because some are no longer produced commercially (e.g., those to BTV serotype 8).

- Autogenous vaccines have been used in the captive cervid industry to immunize deer against EHDV infection. Success has been limited at best. No peer-reviewed objective data are available to assess immunogenicity, efficacy, or effectiveness.

- A focused effort to identify potential master seedstocks of North American serotypes of BTV and EHDV should be initiated. Inactivated vaccines have been produced against only a limited number of BTV serotypes and revamping production of an existing commercial vaccine can take several months, but creation of an entirely new one can take 2 years or longer, so the presence of available seedstocks to all 26 serotypes of BTV and all 7 serotypes of EHDV would potentially expedite creation of new vaccines.

- Attenuated, modified-live vaccines have significant safety issues associated with their dissemination by insect vectors and reassortment of genes with those of circulating wild-type virus in the field, vertical transmission, and inherent issues related to either under- or over-attenuation of the vaccine virus.

- Inactivated vaccines provide only serotype-specific protection but are reasonably efficacious and safe. Because of the safety concerns associated with using attenuated vaccines, they may be the best option in the face of an epizootic emergency.

- Current inactivated vaccines require two doses and do not typically provide “sterilizing immunity;” that is, current vaccines may not prevent virus transmission following infection.

- Inactivated vaccines are more costly to produce.

- Neither inactivated nor live-attenuated BTV vaccines are DIVA-compatible, whereas new-generation products could be (similarly for EHDV).

GAP ANALYSIS OF THE AVAILABLE SCIENTIFIC INFORMATION

Epidemiology and Surveillance

Bluetongue

Virus serotypes. Worldwide, 24 serotypes of BTV (Mertens et al., 2005; Maan et al., 2007) are currently recognized, and proposed 25th and 26th serotypes were recently detected (Hofmann et al., 2008; Maan et al., 2011). In the U.S., BTV was initially isolated from sheep during a 1952 outbreak (Hardy and Price, 1952), and BTV-2, -10, -11, -13, and -17 were isolated from U.S. livestock in the next 30 years, representing the historically endemic BTV serotypes in the U.S. (Barber, 1979; Gibbs et al., 1983). However, the number of BTV serotypes documented in the U.S. has swelled since 1999 after the detection of 10 historically non-endemic serotypes in the Southeast (Gibbs et al., 2008; Ostlund, 2010).

Distribution in North America. Bluetongue has persisted in North America since its introduction in the early 1950s. It has persisted in a stable epidemiological pattern characterized by high levels of transmission in Southern and Southwestern states and reduced transmission in Northern and Eastern states (Metcalf et al., 1981). Canada remains free of BT with the exception that the virus is occasionally detected in the Okanagan valley of British Columbia (Pare et al., 2012). Serological surveys have indicated low levels of seropositivity in cattle in Montana (Van Donkersgoed et al., 2004, 2006). Serological surveys in Illinois and Indiana also confirm a gradient of reduced incidence with increasing latitude (Boyer et al., 2007), and a similar pattern was seen in herds sampled in North Dakota, South Dakota, and Nebraska (Green et al., 2005). Occasional outbreaks have occurred along the northern edge of the U.S. distribution. Outbreaks have resulted in deaths of pronghorn and mule deer in northern Wyoming (Throne et al., 1988) and in sheep in Wyoming and Montana in 2007 (Miller et al., 2010). These northerly outbreaks are worrisome because they indicate the potential for the disease to expand its range and cause of mortality in susceptible animals that may have not been previously exposed to the virus.

Potential for expansion. Bluetongue expansion in Europe has been occurring since the early 2000s. Expansion in the southern regions bordering the Mediterranean have been associated with expansion of *Culicoides imicola* from Africa, possibly due to climate change (Purse et al., 2005). Coupled with this has been the recent expansion of an African serotype, BTV-8, into Northern Europe during 2006–2008. This outbreak occurred due to the introduction of virus into indigenous vector species that had the competence to transmit BTV (Carpenter et al., 2006, 2008). The mechanism by which the virus was introduced into Northern Europe is still unknown, but expansion by airborne dispersal of infected midges likely occurred and was responsible for introducing the virus to the U.K. (Burgin et al., 2013). The virus overwintered successfully in Northern Europe, possibly due to a combination of moderate winter temperatures and animal housing systems that facilitated vector survival throughout the winter. High animal morbidity was observed in cattle, possibly due to the virus strain or immune/susceptible status of the animals. Although BTV has been present in North America for more than 60 years, the situation in Europe is a reminder of the potential for BTV to expand beyond traditional boundaries, possibly infect novel vectors, and cause increased morbidity when entering susceptible animal populations.

Epizootic hemorrhagic disease (EHD)

In recent years, changes in the pattern of EHDV infection and disease have forced the scientific community to revisit some fundamental areas of research to better understand virus-vector-host interactions and environmental factors that have potentially enabled the observed changes. Fundamental areas of change include: 1) increased recognition/reporting of EHD disease in cattle, 2) expanding geographical distribution of some EHDV serotypes, and 3) northern expansion of EHD outbreaks and an apparent increase in frequency and intensity of outbreaks.

Increased recognition and reporting of EHD in cattle. Epizootic hemorrhagic disease virus has long been recognized as a significant pathogen of wild ruminants in the U.S. (Shope et al., 1960). Although serosurveys and sentinel studies indicate cattle are commonly infected with EHDV, historical accounts of disease are infrequent (Omori et al., 1969; Metcalf et al., 1992; Barnard et al., 1998). In the past decade, however, numerous EHD outbreaks have occurred in cattle: Reunion Island in 2003 and 2009 (Breard et al., 2004; Sailleau et al., 2011), Israel in 2006 (Yadin et al., 2008), Turkey in 2007 (Temizel et al., 2009), Morocco in 2004 and 2006 (EFSA, 2009), Algeria in 2006 (EFSA, 2009), Tunisia in 2006 (Savini et al., 2011), Jordan in 2006 (Savini et al., 2011) and the United States in 2007 and 2012 (Shulaw and Zhang, 2008; Ostlund, 2008; Rodman and Johnson, 2012; Dudley, 2012). Most recently in the U.S., there was an explosive EHD outbreak in cattle throughout the Midwest during the summer and fall of 2012, and disease was reported in many cattle herds (Rodman and Johnson, 2012; Dudley, 2012). Commonly reported clinical signs of EHD in cattle include fever, inappetence, lethargy, coronitis with lameness, oral erosions/ulcers, salivation, and reduced milk yield (Yadin et al., 2008; Temizel et al., 2009; Dudley, 2012). Although not appreciated historically, decreased production and significant economic loss were well documented during a recent EHD outbreak in dairy cattle (Kedmi et al., 2010). In addition to increased reports of EHD in cattle, disease also has been reported in several additional species in recent years, including yaks (Van Campen et al., 2013), American bison (Dudley, 2012), alpaca (DES, personal communication 2013), and pygmy brocket deer (Favero et al., 2013).

Expanding geographical distribution of some EHD serotypes. Seven serotypes of EHDV (1, 2, and 4 through 8) are currently proposed worldwide (Anthony et al., 2009). Although the true global distribution of the individual EHDV serotypes is not well understood, the viruses are generally considered to exist in temperate and tropical climates that support vector populations; thus, EHDV distribution likely mirrors that of BTV (Savini et al., 2011). In North America, EHDV-1 and -2 have caused cyclical epidemics among ruminant populations for more than 60 years, but a non-endemic serotype, EHDV-6, was isolated from dead WTD in 2006 (Allison et al., 2010), and this virus has been isolated each subsequent year over a wide geographic area in the Midwestern, Eastern, and Southeastern U.S. (Allison et al., 2012; Mead, 2012). This virus represents a novel EHDV reassortant of serotypes 2 and 6, with the L2 and M5 gene segments originating from EHDV-6 and the remaining gene segments originating from EHDV-2 (Allison et al., 2010). Phylogenetic analysis of EHDV-6 strains from the U.S., Australia, South Africa, and Bahrain suggest that the U.S. strain of EHDV-6 is most closely related to the Australian prototype strain (Allison et al., 2012). Interestingly, a non-reassortant strain of EHDV-6 was isolated in the Caribbean in 2010, but genetic analysis indicates that it is not the immediate parental strain of the U.S. isolates (Allison et al., 2012).

Outside of North America, EHDV incursions also have occurred in cattle in many North African and Middle Eastern countries (Temizel et al., 2007; Yadin et al., 2008, Savini et al., 2011). These virus detections, along with the occurrence of disease in cattle and associated production loss (Kedmi et al., 2010), highlighted the potential for EHDV introduction into Europe. The European Food Safety Authority subsequently completed a *Scientific Opinion on Epizootic Hemorrhagic Disease* in 2009 (EFSA, 2009). It was concluded that cattle in the EU likely represent a susceptible population and that the risk of EHDV introduction via wind dispersal of infected vectors is high.

Northern expansion of EHD outbreaks and an apparent increase in frequency and intensity of outbreaks. The historical distribution of EHD/BT mortality among wild ruminants in the U.S. has been relatively stable, with virus activity anchored in the Southern U.S. and extending in a northwesterly direction through the Mid-Atlantic and Midwest and into Montana, Washington, and parts of the Intermountain West (Figure 1; Nettles et al., 1992; Stallknecht et al., 2002). Much of the Upper Midwest and Northeast have been free of EHDV, aside from infrequent incursions, but outbreaks in these areas have become more frequent in the past decade. Disease records from several Northern states offer good examples: confirmed EHD outbreaks occurred in New Jersey in 1955, 1975, 1999, 2007, and 2010–12; Michigan in 1955, 1974, 2006, and 2008–2012; and in New York in 2007, 2010, and 2012. These observations are striking, yet the ecological drivers are yet to be investigated. Two of the most widespread and intense epidemics observed since the monitoring of this disease began occurred in 2007 and 2012 (Figures 2 and 3). Both of these epidemics coincided with drought and high temperature and resulted in not only widespread mortality among WTD populations, but also disease in cattle. For instance, during the 2007 epidemic, 11 Midwestern and Mid-Atlantic states had counties with more than 100 dead WTD reported, with many counties reporting estimates of >1,000 mortalities (SCWDS, unpublished). During the inter-epidemic period from 2007–2012, EHDV was isolated during most years in multiple Northern states: Michigan (4 years), Indiana (4 years), Pennsylvania (3 years), and New Jersey (4 years) (SCWDS, unpublished). This frequency of isolation of EHDV from northern latitudes in the U.S. highlights our lack of understanding of the epidemiology of EHD, especially overwintering mechanisms and competent vector populations.

Insect vectors

Early literature on virus transmission can be confusing if the subspecies is not mentioned or if the historical context of the species name is not considered. The principal vector of BT in the North America is now considered to be *Culicoides sonorensis*. This species was originally considered one of five subspecies of the *Culicoides variipennis* complex (Wirth and Jones, 1957) that included *C. v. variipennis, C. v. occidentalis, C. v. sonorensis, C. v. albertensis,* and *C. v. australis.* Subsequent workers proposed the synonymy of *C. v. sonorensis* and *C. v. australis* (Atchely, 1967) as well as *C. v. sonorensis and C. v. albertensis* (Downes, 1978). Jorgensen (1969) elevated *C. v. occidentalis* to full species status, and this was also proposed by Downes (1978) with *C. occidentalis occidentalis* and *C. occidentalis sonorensis* as subspecies. These were renamed *C. variipennis variipennis, C. v. occidentalis*, and *C. v. sonorensis* (Wirth and Morris, 1985) and were later elevated to full species status (Holbrook et al., 2000). Other confirmed BTV vectors include *C. insignis* in Florida and the Caribbean (Tanya et al., 1992), and *C. debilipalpis* has been considered a vector (Becker et al., 2010). Other species may be possible vectors (Mullens and Dada, 1992, Gibbs and Greiner, 1989). *C. sonorensis* has been confirmed

as a vector of EHDV, but the possibility exists for other species to act as vectors. Expansions of EHDV into Michigan include areas that are not considered part of the *C. sonorensis* normal range. It is unclear if the vector is expanding in addition to the virus or if the virus is being transmitted by other species. In the Southeastern U.S., *C. debilipalpis* (formerly *lahellei*), *C. stellifer, C. spinosus, C. obsoletus, C. biguttatus, C. niger,* and *C. paraensis* warrant further investigation as potential vectors of EHDV and BTV for WTD (Mullen et al., 1985; Smith and Stallknecht, 1996; Smith et al., 1996a,b) based on midge abundance, seasonal occurrence, and host preference.

Criteria for incriminating an arthropod as a vector of a disease agent include 1) repeated virus recovery from field wild-caught specimens free of visible blood, 2) demonstration of ability to become infected by feeding on a viremic vertebrate host or an artificial substitute, 3) demonstration of the ability to transmit biologically by bite, and 4) accumulation of field evidence confirming the significant association of infected arthropods with the appropriate vertebrate population in which disease or infection is occurring (WHO, 1967). Items 2 and 3 allow estimating vector competence; i.e., the genetic capability of a vector species to become infective after ingesting a viremic bloodmeal. This type of work has been focused on *C. sonorensis* but should be expanded to other species that have a high host affinity for the various ruminant species affected by these viruses.

The ability of a vector species to transmit a pathogen can be compared using vectorial capacity. This incorporates more information than vector competence that is listed as items 2 and 3 in the preceding paragraph. Vectorial capacity also considers field and life history components of the vector species that can influence its potential to transmit a pathogen (Lysyk and Danyk, 2007). The field components are functions of the vector population's biology and abundance and include the host biting rate (bites/host/day), host preference (proportion), and vector competence (proportions). The life history component includes aspects of the vector's life history in relation to the extrinsic incubation period of the virus. These include the frequency of feeding (1/gonotrophic cycle duration), survival over the virus extrinsic incubation period, and the expectation of life of the vector. Many of the components of the life history factor are temperature-dependent and show a latitudinal correlation with incidence of BTV (Mullens et al., 2004). The life-history component can vary 8-fold as temperatures increase from 10 to 30°C (Lysyk and Danyk, 2007). Host preference and vector competence range from 0 to 1, and the host biting rate can show substantial variation; in fact, high levels of vector feeding can more than compensate for low vectorial capacity or host preference. Unfortunately, these parameters have been defined only for *C. sonorensis* in a few geographic locations. Many of the risk assessments and modeling exercises for BTV transmission use parameters defined for *C. sonorensis,* even in Europe where it does not occur.

Transmission of BTV and EHDV show distinct seasonal trends in North America, with seroconversion primarily occurring in the late summer and fall. In northern areas, transmission stops when adult vectors cease activity prior to the onset of winter. Late summer seroconversion also is the norm in southerly locations (Gerry and Mullens, 2000, Gerry et al., 2001) where adults may be present in low numbers year-round, although at greatly reduced levels. Overwintering of the viruses remains a mystery, particularly in northern areas where cold precludes persistence in the adult and there is no evidence of transovarial transmission and persistence in overwintering larval populations. Overwintering in southern locations may be in

the adult vector because they are capable of surviving cooling temperatures for extended durations, and transmission may occur at levels too low for detection. Other possibilities include overwintering in the host, as well as annual reintroductions from endemic to non-endemic areas through windborne dispersal of the vector. The latter deserves more consideration in North America as a mechanism for annually reintroducing virus into non-endemic areas.

Patterns of BT and EHD in North America

Understanding the incidence of disease in a zone. Numerous factors have made wild ruminants, namely white-tailed deer, excellent sentinel animals for *Orbivirus* activity in North America: 1) they are extremely susceptible to EHDV/BTV infection and can develop severe disease, 2) the deer are abundant and widely geographically distributed in North America, 3) Southeastern Cooperative Wildlife Disease Study (SCWDS; University of Georgia) and the National Veterinary Services Laboratory (NVSL) conduct passive surveillance of WTD through routine diagnostics, and 4) SCWDS has more than 30 years of data from a long-term HD mortality questionnaire-based survey. Results from decades of monitoring hemorrhagic disease mortality among U.S. WTD have revealed temporal and spatial patterns of infection and disease (Couvillion et al., 1981; Nettles et al., 1992; Stallknecht et al., 2002).

Epizootic hemorrhagic disease and BT occur seasonally from midsummer to late autumn, with reports of disease and diagnostic submissions peaking in September (Couvillion et al., 1981; Stallknecht and Howerth, 2004), but latitudinal and physiographic variation in the duration of transmission season remains poorly defined. In addition, annual variation in disease is difficult to understand, especially given the changing patterns observed in the last decade mentioned above. Hemorrhagic disease outbreaks in wild ruminants historically have occurred in a two- to three-year cycle in enzootic regions and an eight- to ten-year cycle in epizootic regions (Couvillion et al., 1981; Nettles et al., 1992), but again, frequency of epidemics in certain regions appears to be increasing.

Figure 1 shows the distribution of hemorrhagic disease in wild ruminants in the U.S. from 1980–2012 (Stallknecht, unpublished 2013). Within this distribution are endemic and epidemic disease patterns. In endemic regions, such as coastal plain in the Southeastern U.S. where midge activity can persist much of the year and viruses circulate nearly annually, many infections are subclinical or animals survive acute HD to develop the chronic form of disease (Nettles et al., 1992; Stallknecht et al., 2002). Moving inland into the Piedmont and Appalachian Mountains in the Southeast and in portions of the Midwest, epidemics occur less frequently and high levels of mortality can be observed (Stallknecht et al., 2002). Epidemics are even more infrequent (historically) in more northern latitudes and in the Western states but often result in significant mortality when they occur (Nettles and Stallknecht, 1992; Stallknecht et al., 2002). Reports of EHD and BT in livestock, especially cattle, typically coincide with large-scale epidemics in wild ruminant species. In addition to these endemic and epidemic cycles, a third pattern of infection exists in WTD in certain parts of the U.S. (Stallknecht et al., 1996, Stallknecht et al., 2002, Flacke et al., 2004). For instance, in Western and Central Texas, WTD coexist with *Orbiviruses* in a state of endemic stability where prevalence of antibodies to EHDV and BTV among WTD can approach 100%, but reports of clinical disease are extremely rare (Stallknecht et al., 1996). This endemic stability may be the result of acquired immunity via frequent exposure to the viruses, high subsequent passive immunity in fawns, and unknown mechanisms of innate immunity via co-evolution of the host and pathogen (Stallknecht et al., 2002). These mechanisms

of immunity are potentially lost when captive deer from non-endemic regions are imported to endemic regions, possibly explaining the high rates of morbidity and mortality encountered among some captive herds (Roughton, 1975). Evidence to support some of the mechanisms mentioned above have been demonstrated during previous controlled experiments (Shope et al., 1960; Quist et al., 1997; Gaydos et al., 2002a, 2002b, 2002c). A better understanding of the seasonality, abundance, and competence of regional vector populations and their interactions with the environment is necessary to better understand these apparent patterns of infection and disease observed on the landscape.

Compared to cases in wild ruminants, reports of EHD or BT in domestic livestock in the U.S. are less frequent. Thus, when attempting to understand epidemiologic patterns in livestock, there is more reliance on traditional serological surveys, sentinel animal studies, import/export diagnostic sample submissions, and individual animal or herd-level clinical case submissions. Although domestic cattle are not as susceptible as WTD to EHD and BT morbidity and mortality, serological surveys in North America have generally supported the presence of endemic and epidemic disease zones observed in wild ruminants. For instance, serological surveys of cattle for antibodies against EHDV (Odiawa et al., 1985; Shapiro et al., 1991; Boyer et al., 2008) and BTV (Odiawa et al., 1985; Shapiro et al., 1991; Metcalf et al., 1981; Ostlund et al., 2004; Green et al., 2005; Boyer et al., 2007) suggest that EHDV and BTV seroprevalence rates are latitudinally/regionally stratified. For instance, during 1977–78 and 1983–85, regional surveys of U.S. cattle for anti-BTV antibodies were performed yielding seroprevalence rates of <1% in 18 Northern/Northeastern states, 18–53% in 9 central and Southwestern states, 6–39% among 13 Southeastern states, and 80% in Puerto Rico (Pearson et al., 1992; Metcalf et al., 1981). Subsequent followup surveys of the Northern and Northeastern states performed in the 1990s and early 2000s helped to further define the distribution of BTV in the Northern U.S. (Ostlund et al., 2004). Collectively from 1977–2002, market cattle surveys in New England, Michigan, Minnesota, New York, and Wisconsin revealed a seroprevalence of <2.0%.

Although it is clear that there is a gradation of BTV/EHDV activity and that endemic, epidemic, and incursive disease zones exist in North America, these regional zones are yet to be systematically investigated and defined. Although the BTV-free zone in parts of the Northern and Northeastern U.S. has been well investigated, given the changes observed in the past decade, we should not assume this remains unchanged. Much of our current understanding of the North American zones is based on disease reports, surveys, and the distribution of *C. sonorensis.* These zones can be more clearly defined not only through additional serological surveys and strategic sentinel animal studies, but also investigation of additional *Culicoides* species. Better understanding of the ecology and life history of *Culicoides* species in different physiographic regions is necessary; for instance, the seasonal abundance, vector competence, host preference, larval habitat, and adult habitat utilization of different *Culicoides* species will provide insight into patterns of infection and disease. Considering that the disease zones in the U.S. may be different for EHDV and BTV is important because unknown differences in transmission may exist.

Factors influencing BT and EHD incidence. Epidemiological studies examining the relationship between EHDV and/or BTV infection and various environmental and climatic variables in North America are limited (Sellers and Maarouf, 1989, 1991; Sleeman et al., 2009; Boyer et al., 2010; Mayo et al., 2012; Xu et al., 2012; Berry et al., 2013) but have revealed some interesting

associations that warrant further investigation. Recent spatial statistical modeling of WTD morbidity and mortality from 1980–2007 revealed that wetland cover is likely a critical driver of HD morbidity (Berry et al., 2013). The factors underlying this association were not investigated, but likely relate to WTD habitat utilization and larval development sites for *Culicoides* species. Other studies have found significant clustering, or patchiness of infection or outbreaks (Xu et al., 2010; Boyer et al., 2010). In a three-year study by Boyer et al. (2010), temperature was associated with EHDV and BTV seropositivity in cattle during all three years of the study, although spatial distributions between viruses differed. Furthermore, forest patchiness was associated with EHDV seropositivity in cattle for two of three seasons, and heavy spring rainfall was hypothesized to play a role in the lack of association during the third year, which is supported by other studies (Sleeman et al., 2009). Interestingly, no association was observed between BTV seropositivity and forest patchiness. Variation in research findings and field observations, such as those mentioned above, suggest that arbitrarily lumping EHDV epidemiology with BTV epidemiology should be done cautiously because differences in the ecology of these diseases may be significant. Additional studies will be necessary to better understand the ecology of these diseases in a changing environment and climate.

Molecular epidemiological investigations of field strains of EHDV and BTV in North America are limited (Pritchard et al., 1995; Wilson et al., 2000; Cheney et al., 1996, 2003; Mecham et al., 2003; MacLachlan et al., 2007; Balasuriya et al., 2008; Allison et al., 2010, 2012; Murphy et al., 2005, 2006). Many of these previous studies have relied on partial gene, full gene, or multiple gene segment sequence for analysis and have revealed significant findings regarding novel viruses, outbreak dynamics, and evolution of viruses in North America. With the cost and speed of full-genome sequencing decreasing, however, great potential exists for additional phylogenetic studies. The long-term storage of virus isolates, such as those at the University of Georgia and NVSL, offer an invaluable resource. Phylogenetic analysis of field isolates at various temporal or spatial scales can provide insight into a diversity of epidemiological questions.

Current sources of BT and EHD epidemiological information. At present, neither BT nor EHD are considered or treated as reportable diseases in the U.S. Epidemiological information is not routinely collected through national surveys. What data are collected are maintained at the Center for Epidemiology and Animal Health via the NAHRS (National Animal Health Reporting System). This is an aggregation point for reports from state veterinarians on the presence of OIE-listed diseases in the U.S. Some uncertainty is associated with reporting at the state level because reports are often given as presence of absence of the disease. The Southeastern Cooperative Wildlife Disease Study at the University of Georgia also maintains data on these two diseases, and a number of veterinary diagnostic labs operate throughout the country. These include the National Veterinary Services Laboratory, various state veterinary diagnostic laboratories for domestic animals, university laboratories, SCWDS University of Georgia, and Newport labs and other private labs. A more coordinated effort to collect information could provide a starting point for increasing our understanding of the epidemiology of BT and EHD in the United States.

Summary of Orbivirus *epidemiology research gaps*

Research gaps were classified into two broad categories. The first category included gaps related to the overall pattern of transmission of the two viruses in North America. The second category included gaps related to the expression and incidence of clinical disease within the

epidemiological zones. Within each category are listed a number of gaps framed as research questions. We follow with a list of strategies that could be followed to begin to resolve these gaps.

Research needs. The primary research needs are delineated below.

1. Determine the pattern of ecosystems supporting the arthropod transmission of BTV and EHDV in different climatic/geographic zones.
    a. Determine distribution and diversity of virus serotypes, molecular subtypes, and subgroups within the United States.
    b. Determine the true endemic, epidemic, and incursive zones.
    c. Determine overwintering mechanisms of viruses and the importance of this in delineating the zones as defined under (b).
    d. Determine the transition zone between endemic and non-endemic zones using serological survey followed by establishment of sentinel herds.
    e. Determine where virus could be introduced each year and where it could persist.
    f. Identify the mechanisms that viruses expand their range to identify mechanisms of control. These mechanisms might include infected windborne vectors, movement of infected ruminant hosts, movement of virus from overwintering areas, and examining alternate host-vector pathways to enable virus persistence in different ecological areas.
    g. Establish the current distribution of competent vectors and viruses (BTV and EHDV) in the United States.
        i) Identify hematophagous arthropods feeding on susceptible hosts in various regions of the U.S.
        ii) Conduct laboratory studies on vector competence.
        iii) Conduct long-term studies on vector population dynamics in various regions of the United States and fill gaps in biology of vector species.
    h. Provide training and support for arthropod identification.
        i) Develop identification guides based on collections in Florida State Arthropod Collection.
        ii) Identify molecular barcode of vectors.
    i. Conduct ecological niche modeling.

2. Determine the factors influencing the expression and incidence of clinical disease within these zones for all potential hosts.

    a. Determine the impact of animal movement in or out of zones on disease expression in relation to virus diversity.
    b. Determine the effect of local and regional weather patterns and climatic conditions on outbreaks and incidence.
    c. Develop and evaluate potential vaccination and vector control strategies for routine management and outbreak response.

Strategies to fill research gaps. Filling the research gaps can be accomplished through two overarching strategies, as outlined below.

1. Develop a coordinated, comprehensive national active *Orbivirus* surveillance program for wildlife and livestock. It is possible to consider an incremental approach to grow the system from where we are right now and take advantage of opportunities for association and collaboration by layering existing programs and incorporating future programs as they arise. Consideration should be given to following OIE Code guidelines in establishing a surveillance program.

- Disease surveillance and coordination
  - Observe and record clinical disease from state and federal diagnostic labs and research labs.
  - Collect annual SCWDS Hemorrhagic Disease Surveillance data.
  - Investigate of disease outbreaks.
- Virus surveillance
  - Observe serological distribution within states or regions.
  - Use regional sentinel herds to better understand virus distribution, but also to more clearly define disease zones. Initially, the focus should be to determine the transitional front between epizootic and incursive zones, as well as the Southern U.S. to recognize potential virus introductions. Expansion of sentinel herds into other regions can follow. Utilizing captive ruminants at land grant schools, ARS units, and other research herds, as well as meat processors, taxidermists, and the Deer Management Assistance Program are potential avenues to explore.
  - Conduct molecular epidemiological investigations to understand complexities of virus movement, overwintering, and evolution.
- Vector surveillance
  - Conduct gridded, uniform trapping systems for presence/absence of known and potential vector species in association with sentinel herds. Live animal baited aspirations should be performed wherever possible.

2. Analyze and model current and future data to determine the impact of BT and EHD in North America and predict future events

- Animal impact
  - Perform assessments on the impact of EHD and BT on livestock and wildlife population abundance, health, welfare, and productivity.
- Economic impact
  - Perform retrospective and prospective studies to evaluate the economics of the problem and the potential cost-benefit of control strategies.
- Predictions
  - Create predictive models on the expansion of disease incidence and outbreaks in response to weather events and climate change.

Figure 1. Distribution of hemorrhagic disease mortality in the United States, 1980–2012. Compiled by the Southeastern Cooperative Wildlife Disease Study, University of Georgia.

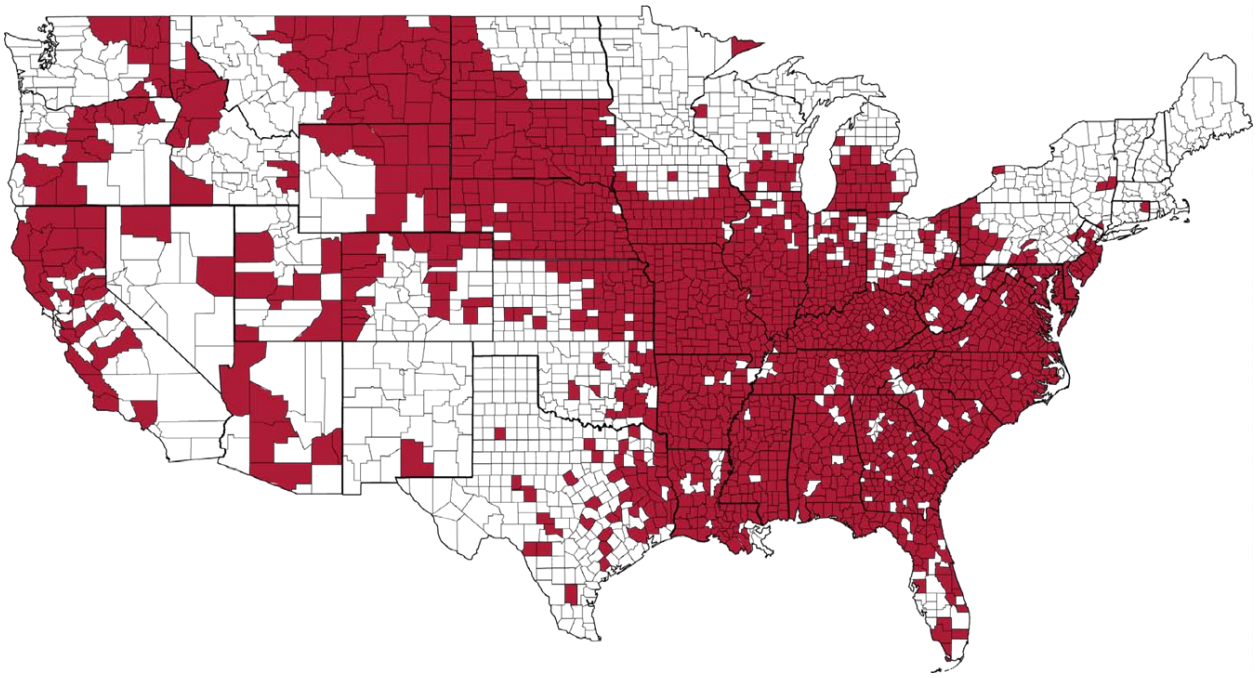


Figure 2. Distribution of hemorrhagic disease mortality among wild ruminants during the 2007 epizootic. Compiled by the Southeastern Cooperative Wildlife Disease Study, University of Georgia.

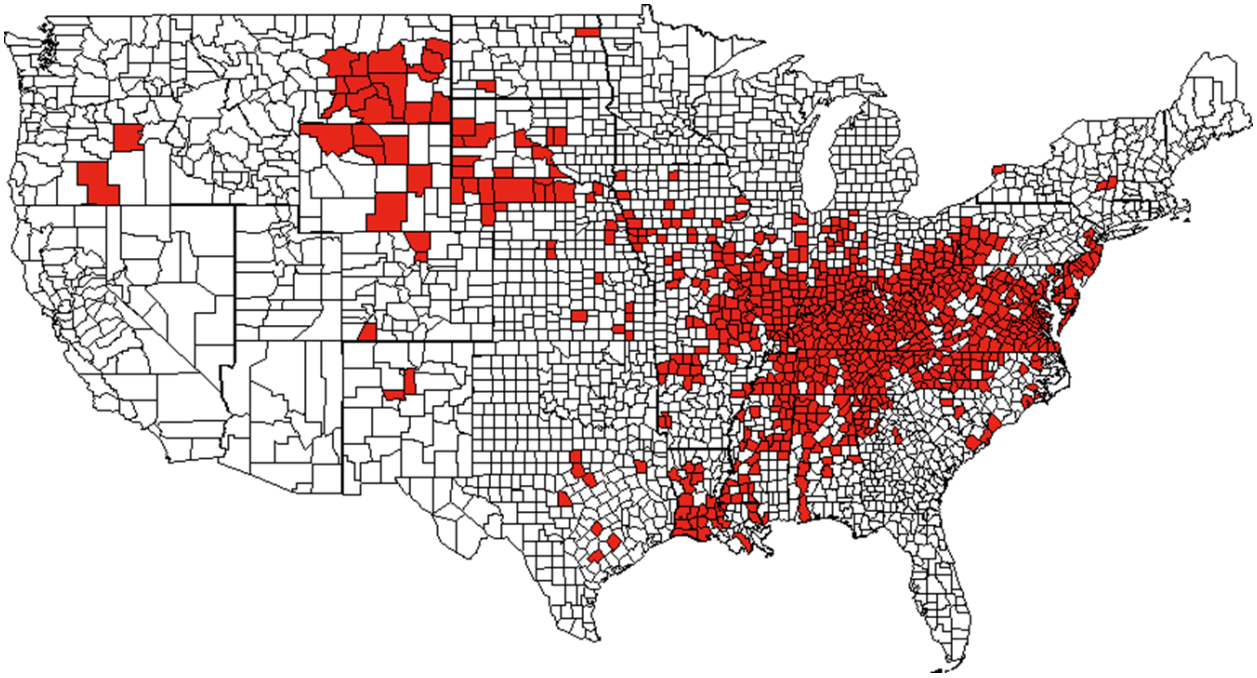
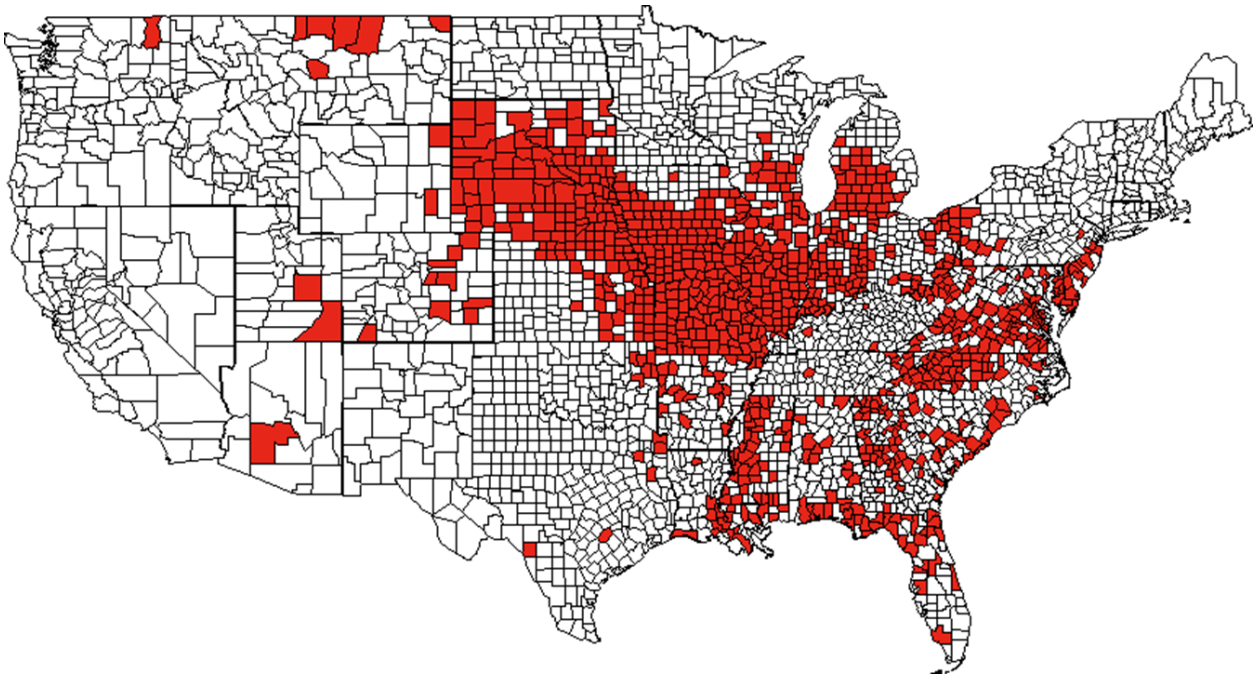


Figure 3. Distribution of hemorrhagic disease mortality among wild ruminants during the 2012 epizootic. Compiled by the Southeastern Cooperative Wildlife Disease Study, University of Georgia.



Virology

The Virology Breakout Group had a wide-ranging and productive discussion concerning recent research developments, new opportunities, and current questions for *Orbivirus* research. The group identified the need for greater international collaboration and the possibility of a collaborative global network of researchers/organisations to combat these important trans-boundary diseases.

Basic virology includes studies of the interactions between the *Orbiviruses* and both their vertebrate hosts and arthropod vector hosts. It also explores how these viruses replicate and make use of the interactions between their hosts and vectors at the molecular level to spread and cause disease in new individuals and populations.

Virology research provides basic data that help us elucidate and understand key aspects, including:

- Replication cycles and virus assembly;
- Putative receptors and attachment mechanisms;
- The genetic variability and molecular basis for antigenic variation between different *Orbivirus* species, serotypes, and topotypes;
- The existence, characteristics, and distribution of novel *Orbivirus* species and serotypes;
- The molecular mechanisms involved in genome segment selection, packaging, and reassortment and their significance in genetic shifting and emergence of novel strains;
- The viral factors modulating adaptive and innate immune responses to the virus (and the antigens or RNAs involved);
- How immune responses protect the host against infection, clinical disease, and onward transmission;
- How the *Orbiviruses* infect and spread and the mechanisms by which they cause clinical disease within susceptible vertebrate hosts;
- Determinants of host range (which species, breeds, or subpopulations of host species are susceptible to infection and which exhibit clinical signs of disease);
- The mechanisms by which *Orbiviruses* infect, disseminate within, and are spread by their arthropod vector species;
- The response of arthropod vector species to *Orbivirus* infection (including immune responses, such as RNAi silencing or antiviral gene VAGO), related to onward transmission;
- Determinants of vector range (which potential insect vector species or populations are susceptible to infection and can effectively transmit the virus and whether there are differences between different virus strains);
- Determinants of virulence (which genes, domains, epitopes) and how the host environment (vertebrate vs. invertebrate) affects them;
- The potential of transmission by other non-vector routes (e.g., vertical, horizontal, mechanical, or oral transmission);
- Molecular markers (e.g., from full-genome sequence data) that can be developed and used to track virus strain movements (molecular epidemiology, phylogeography); and

- Development of refined/next-generation diagnostic systems (e.g., array-based multiplex diagnostics, next-generation sequencing and metagenomics, RT-PCR assays, monoplex or multiplex ELISA systems, fluorescent bead-based assays).

Tools for basic virology: Current availability and needs

Reverse genetics. The recent successful development of reverse genetic technologies for BTV (Boyce et al., 2008) at the London School of Hygiene and Tropical Medicine (LSHTM) has opened new opportunities for research to explore the precise genetic and molecular basis for the properties of the virus. Since then, research groups at the Central Veterinary Institute of Wageningen UR (CVI), Netherlands; Friedrich-Loeffler-Institut (FLI), Riems, Germany; The Pirbright Institute (PIR), U.K.; LSHTM, U.K.; The University of Glasgow Centre for Virus Research (UGCVR) Scotland, U.K.; and Agence nationale de sécurité sanitaire de l'alimentation (ANSES), Maison-Alfort, France, have implemented these technologies in their research programs to generate reassortants and BTV mutants for fundamental as well as applied research. CVI, FLI, UGCVR, ANSES, and PIR are partners in an international collaboration network within Europe (headed by Prof. Mertens) to use and disseminate these technologies. A similar reverse genetics collaboration network is needed in the U.S. for North American BTV and EHDV serotypes.

Reverse genetics techniques allow specific genetic modification of virulent, nonvirulent, or vaccine virus strains (van Gennip et al., 2012a, Shaw et al., 2012, 2013). These can be used to investigate, manipulate, or change viral RNAs, proteins, or infection processes, and consequently provide information concerning specific characteristics of the virus such as virulence and transmissibility. These technologies can be used to create reassortants in a directed manner, as well as site-specific modifications of the viral RNAs and proteins, both for vaccine development and fundamental research (van Gennip et al., 2012b; Nunes et al., 2012; Shaw et al., 2013). Recent studies have included generation of BTV reassortants of newly discovered serotypes, the original isolates of which (BTV-25) cannot yet be grown in cell culture (van Rijn et al., 2011). By using fluorescent or antigenically tagged viruses generated by reverse genetics technologies, it is possible to follow their distribution and movements in infected cells or tissues (Shaw et al., 2012). It will be important to establish collaborations to create and share these reagents to enhance global *Orbivirus* research.

Small animal models. Small animal models provide cost-effective and simplified systems to generate *in vivo* data that facilitate development and initial testing of *Orbivirus* vaccine candidates for sheep, cattle, deer, horses, and other large animal target species. Interferon receptor negative (IFNAR) mouse models recently developed for both BTV and African horse sickness virus (AHSV) have been used in vaccinology and vaccine challenge studies (Calvo-Pinilla, 2009; Castillo-Olivares, 2011; Jabbar et al., 2013). Current data indicate that the development of neutralising antibodies and protective responses post-vaccination in these mice show good correlation with protective responses against AHSV in horses. An important aspect of vaccine-challenge studies in any system is the availability of virulent challenge strains for each virus serotype that will reliably generate severe or lethal clinical disease in the animal model. Such strains have been identified for some serotypes and are available from the *Orbivirus* collection at Pirbright, but more work will be needed to develop a comprehensive bank of challenge strains. Additional small animal models need to be optimized for EHDV (Eschbaumer

et al., 2012) and developed for other *Orbiviruses*. These models will need further validation by comparisons to the target host species in vaccinology studies.

Target host animal models. Target-animal clinical disease models are also needed to confirm the validity of the small animal models and for transmission, pathology, vaccinology, and immunology studies. These systems should be used for proof-of-concept studies, to confirm vaccine efficacy in the target species, and to explore vector/vertebrate-host interactions. In view of the reported changes in *Orbivirus* isolates on multiple passage in cell culture (as with the small animal models), it will be important to establish virulent challenge strains for each *Orbivirus* species and serotype. Facilities for whole-body scanning to explore the pathology and progress of the disease would be particularly valuable. A standardized clinical scoring system to measure the severity of clinical signs post-challenge and the efficacy of vaccination would be valuable for improving the comparability of results derived from *in vivo* studies. Such a system calculating a clinical reaction index has been developed (Huismans et al., 1987) and was slightly modified and used in trials with several ruminant species, including cattle, sheep, and WTD (Backx et al., 2009; van Gennip et al., 2012a, 2012b; Drolet et al., 2013; Moulet et al., 2011). This system has been accepted for cattle and sheep trials in an international network (headed by P. Roy, LSHTM, U.K.) (Celma et al., 2013).

Monoclonal antibodies and reference antisera. Antibodies represent vital molecular tools for diagnosis and research to identify and track individual proteins and epitopes at cellular, tissue, and at the whole-animal/vector levels. A register, and ideally a repository, of reference polyclonal or monospecific (single-target protein) antisera is needed. In addition, characterised monoclonal antibodies to specific *Orbiviruses*, specific proteins, and known epitopes would provide an important resource. This would support many aspects of fundamental research, analysis of antigenic variation, diagnostic assay development, confocal microscopy, and transmission studies.

Expressed viral proteins. In the same way that monoclonal antibodies against specific *Orbivirus* proteins and epitopes would facilitate research, the availability of individually expressed viral proteins would also facilitate diagnostic assay development, immunology and vaccinology studies, as well as fundamental research into the biochemistry, replication, transmission, and pathology of these viruses. Numerous expression systems have been and are in current use, including bacterial, baculovirus, yeast, mammalian cell, and plant-based systems. A wider availability of specific proteins (e.g., through a collaborative research network) in characterised expression vectors would enhance current research capabilities.

Genomic sequence data. Recent advances in sequencing technologies have led to an explosion in available data for different pathogens. The genomes of over 300 BTV strains already have been fully sequenced. This is rapidly becoming the accepted standard for the characterization of novel outbreak strains (Maan et al., 2008, 2010) and has helped to identify new BTV serotypes and new *Orbivirus* species (Hofmann et al., 2008; Maan et al., 2011; Belaganahalli et al., 2012).

Recent further developments have included the generation of representative sequence data for examples of each of the different recognized *Orbivirus* species. This has led to identification of five novel species and an initial diagnostic assay (by conventional RT-PCR) that will detect any known *Orbivirus* strain (e.g., BTV 26 serotypes). These sequence data are in the process publication, which can be slow. A suitable website including a data exchange portal would

facilitate comparisons of data for novel isolates to help identify novel virus strains more rapidly. Based on these sequence data and representative data for new isolates, it is possible to rapidly identify the *Orbivirus* species to which they belong, as well as serotype, topotype, individual virus lineage, geographic origin, and to detect reassortment events in the evolution of the novel virus.

Virus collections. As part of sequencing and molecular epidemiology studies, it is important to generate data for novel isolates that are well documented in terms of their date, location, host species, and clinical signs at point of collection from the field. These isolates should be centrally stored and available for further study, vaccine development, and as challenge strains from one or more international reference collections, similar to the one established at Pirbright (http://www.reoviridae.org/dsRNA_virus_proteins/ReoID/virus-nos-by-country.htm). Similarly, virus strains for which the biological characteristics have subsequently been determined, or modified by reverse genetics, should also be stored and made more widely available.

Holding all characterized viruses in a single collection is not essential as long as viruses available at different locations are cross-referenced in a single (international) database.

Cell lines. Although BTV and other *Orbiviruses* can be adapted to a range of mammalian cell lines (including BHK 21 cells, BSR cells, and Vero cells), they are frequently difficult to adapt and may take several passages, likely changing the virus properties (particularly virulence). Studies of virus replication in these systems therefore may not accurately reflect the characteristics, properties, and replication of field strains. The use of endothelial cell cultures could be more widely explored.

Recent studies at Pirbright have used a *Culicoides sonorensis* cell line (originally developed by ARS/ABADRU in Laramie, WY, using colonized midges; Wechsler et al., 1989) showing enhanced efficiency of isolation for *Culicoides*-borne *Orbiviruses* (usually within a single passage and with minimal loss of virulence). These cells are valuable to explore the growth characteristics of the virus in an insect host cell and are providing a basis for full genome sequencing, transcriptomics, and proteomics of a known *Culicoides* vector species (*C. sonorensis*). The KC cell line also forms the initial basis for the first full genome sequencing of any *Culicoides* species. Although initial studies conducted at Laramie indicate that *Culicoides* cell lines can be persistently infected with BTV, sequencing and RT-PCR assays targeting most of the genome segments have failed to detect any BTV genome sequences in the KC cell line held at PIR. Two additional cell lines were developed at the Laramie lab from wild-caught *C. sonorensis*. All three *Culicoides* cell lines are available from ABADRU, ARS, in Manhattan, KS.

Further cell lines from other vector species, including other *Culicoides spp.*, mosquitoes, ticks and sand flies, would be valuable and should be developed and made readily available. A collection of tick cell lines (the tick cell biobank) containing lines derived from 16 tick species has been established at the Pirbright Institute.

Vector colonies. Colonies of a non-vector species *Culicoides nubeculosus* and a known BT vector species, *Culicoides sonorensis* (originally derived from the colony developed at Laramie), are held at the Pirbright Institute. Three *C. sonorensis* colonies [established from field-caught midges in Idaho (AK), Colorado (Ausman), and California (van Ryn)] are maintained at ABADRU, Manhattan, KS. The ABADRU van Ryn colony (originally from Dr. Brad Mullens,

UC Riverside, California) was recently used to re-establish the Mullens colony after a colony crash. These colonies provide a vitally important resource for virus infection, replication, and transmission studies in the insect vector.

By selective breeding, the *C. sonorensis* colony that is now maintained at Pirbright was previously used to generate more highly susceptible and refractory lines. These colonies are now being re-evaluated.

Availability of mosquito and tick colonies and development of colonies of other *Culicoides spp*. would help support comparative studies of vector competence of different vectors for individual *Orbiviruses* and different serotypes and topotypes. One such example is *Culicoides imicola*, a major vector species for BTV, EHDV, and AHSV from Africa and the Middle and Far East that has recently expanded its territory to include most of the northern shore of the Mediterranean. Other important vectors from North America, Africa, or Australia include *C. insignis, C. brevitarsis, C. wadai, and C. bolotinos*.

Gaps in virology knowledge or data

Our current lack of knowledge concerning *Orbiviruses* includes many specific questions. The answers to these questions are paramount to predicting disease outbreaks and at-risk populations of animals and understanding the mechanisms of arbovirus maintenance and emergence. These questions are common to diseases that pose threats to both animal and human health.

Molecular determinants of host and vector specificity. The members of different *Orbivirus* species infect only certain host species. The molecular basis for this specificity is poorly understood. The BTV outer capsid protein VP2 (responsible for initial cell attachment during initiation of infection) is thought to be composed of a duplicated structure, possibly derived by concatamerization of an ancestral gene. In contrast, Great Island virus, a tick-borne *Orbivirus*, has an equivalent protein that is only half the size of BTV VP2 and may represent an ancestral form.

Questions:

- If we exchanged protein domains, would it change host preferences?
- Why does EHDV not infect/affect sheep, although it does infect cattle and causes lethal infections in WTD?
- Could we adapt EHDV to sheep and then compare it with the wild-type virus to possibly identify host specific genetic differences?
- Why does BTV usually cause much less severe clinical disease in cattle than in sheep?
- In long-established endemic situations, local BTV strains often cause relatively mild clinical signs of infection in local breeds/populations of ruminants. Introduction of exotic strains, however, can lead to much more severe outbreaks of disease (most noticeably in sheep, as seen in India). What is the basis for these differences?
- African ruminants often show less severe clinical disease on infection with BTV, as is clearly seen in zoo animals. What is the basis for these differences?

Vector range. The episystem hypothesis suggests that there may be some restriction of transmission of exotic BTV strains by indigenous *Culicoides* populations. The spread of multiple BTV strains and serotypes into Europe, the arrival of multiple new serotypes in the U.S., and the arrival of Western topotype strains in India, indicate this is not universally true. None of the recently introduced BTV serotypes isolated in the Southeastern region of the U.S. (BTV-1, 3, 5, 6, 9, 12, 14, 19, 22, 24) have (so far) spread across all of North America, but it is possible that genome segment reassortment will lead to the wider spread of individual genes from these previously exotic strains. All of the BTV strains that have been detected in Northern Europe are Western topotype, originally derived from Africa (BTV-1(w), 8, 6, 11, and 14). In contrast, the Eastern strains that emerged in Southern Europe did not spread to the north (BTV-1(e), 9 and 16). The detection of reassortants between different topotypes and different serotypes in North Africa and Southern Europe suggests that novel strains are emerging that may have novel biological properties. This could include the ability to be transmitted by additional vector species or enhanced virulence.

There is evidence that BTV-26 can be transmitted horizontally between goats, but does not appear to infect adult *Culicoides*. It also fails to grow in KC cells but will grow in mammalian cells (BSR) as well as certain mosquito cell lines. This represents an extreme example of the virus's genetic control over vector competence.

Vector competence unknowns include:

- Which *Culicoides* species (and other arthropod species) can act as effective vectors for BTV (and other *Orbiviruses*)? Are there insect-strain differences?
- What are the molecular and environmental determinants of tropism for *Culicoides* species?
- Do different BTV topotypes, serotypes or strains have significant differences in their abilities to be transmitted by individual vector species or populations?
- Which viral genes/proteins influence vector infection/transmission?
- What role do NS proteins play in insect vs. mammalian cells?
- What is the significance of differences in the relative expression levels of different BTV proteins (e.g., NS3) in insect and mammalian systems?
- What role do the arthropod vectors play in development of clinical disease?
- What is the significance of vector saliva proteins (e.g., modification of virus particles, inflammatory responses in the vertebrate host)?

Virulence factors. The following factors must be considered in association with virulence.

- What are the molecular mechanisms that cause more severe clinical disease in certain host species?
- Would introduction of exotic topotypes of BTV (e.g., European BTV-8) cause more severe disease in North American animals, than the endemic BTV strains?
- Why does BTV cause less severe disease in cattle, than it does in sheep?
- Why does EHDV sometime cause severe disease in cattle?

- What causes the high degree of clinical variation seen with the same viruses in the same host species?
- How do genetic components of the vertebrate hosts affect virulence? (e.g., Texas WTD are refractory to EHDV whereas Pennsylvania WTD are very susceptible.)
- Why are BT outbreaks less severe in endemic regions?

Antigenic determinants. There is evidence for partial cross-protection and the existence of cross-reactive immune responses between different BTV serotypes, particularly after serial infection with two or more different serotypes. Further exploring the identity of viral proteins and sequences, as well as delivery strategies that could generate a protective and cross-reactive cell-mediated immune response (CMIR) would be useful. This could involve identification of viral proteins and delivery. Antigenic unknowns include:

- Which *Orbivirus* proteins can be recognized by a protective CMIR?
- What delivery strategies are required for a protective CMIR?
- Would a CMIR be sufficient, or a useful component of a protective vaccination strategy?

Epitope mapping. There is evidence for existence of cross-reactive neutralizing antibodies between different BTV serotypes, particularly after serial infection with two or more different serotypes. It would therefore be valuable to identify type-specific and cross-reactive neutralizing epitope that may provide a basis for better and/or cross reactive vaccines. Reverse genetics technologies may assist in these studies. Epitope unknowns include:

- What are the cross-reactive and type specific epitope on BTV or EHDV outer-coat proteins?
- Could a cross-reactive vaccine be used to eradicate BT?

Cell culture adaptation. We need a better understanding of the molecular basis for adaptation to cell culture and associated attenuation of virulence.

Protein functions. The functions of individual *Orbivirus* proteins during replication requires further clarification. What proteins and which RNA sequences are involved in *Orbivirus* genome assembly and packaging during replication remains uncertain. This process could potentially represent a target for an antiviral strategy. The recent discovery of novel BTV protein(s) in particular highlights gaps in our knowledge (Belhouchet et al., 2011; Ratinier et al., 2011). Viral protein unknowns include:

- Are there other unidentified *Orbivirus* proteins?
- What are the complete set of functions of each orbiviral protein?
- How does the virus cause cell cycle arrest?
- What is the role of NS4 (e.g., as a membrane protein)?
- What is the mechanism of cell killing?
- What is the basis of differences in different mammalian cells?
- How are some immune system cells persistently infected (e.g., gamma delta T-cells)?

- How are insect cells persistently infected and mammalian cells lytically infected?

Immune responses of mammalian and vector hosts. The way *Orbivirus*es control or evade innate immune responses is poorly understood.

- Some cross protection (e.g., decreased clinical disease) occurs following infection with a second virus type. How is this mediated?
- Multiple sequential infections with different serotypes can generate more broadly cross-reactive neutralising antibodies and protective responses. Which epitopes are involved?
- Is a cross-reactive (cross-serotype) vaccine possible ?
- What is the importance of silencing during *Orbivirus* infection of the insect cell?
- What is the role of the antiviral gene VAGO?
- Are there specific viral anti-silencing genes?
- What are the potential roles for microRNA responses?
- What genetic information can be removed from the virus to make DIVA virus vaccines?

Domestic/exotic/topotype characterization criteria. The following points must be considered in developing characterization criteria.

- What is the significance of reassortment processes (e.g., between live vaccine strains and wild-type viruses, or between endemic and exotic virus strains)?
- It would be extremely valuable if the criteria by which strains/serotypes are classified as exotic or domestic were based on all genome segments, not just VP2.
- Should exotic vs. domestic be determined by a whole-genome percentage similarity? Or just based on similarities of genes known to play a role in virulence or be determined for each genome segment individually (10-digit bar code)?

Receptors. Molecular characteristics for cell surface attachment of BTV and other *Orbiviruses* need to be identified.

- Can different BTV serotypes bind to different cell surface receptors/use different entry mechanisms as suggested by initial data for BTV-1 and 10?
- What role do receptors play in host range, virulence, transmissibility, and clinical disease?

Transmission mechanisms in vertebrate hosts. The mechanisms involved in vertical transmission and teratogenic effects in the vertebrate host are poorly understood.

- What are the determinants of transmission between host and vector?
- Can all BTV strains be transmitted vertically, or is this related to adaptation to cell culture or differences between serotypes/topotypes?
- What are the determinants for vertical transmission?
- Is there quasi-species selection during vertical transmission?

- What is the significance of virulent and avirulent strains mixed together? Would reassortment and selection lead to emergence of novel phenotypes? Do selective pressures favor more virulent strains?
- Is there and what is the significance of quasispecies in the individual animal and during transmission?
- Is there and what is the significance of bottlenecking during transmission between vertebrate and invertebrate?
- What is the significance of genome segment reassortment in control or variation in transmission characteristics?

Transovarial transmission in arthropod vectors. The following points must be considered.

- Can transovarial transmission occur in any of the *Orbivirus* vectors?
- If so, how frequently does it occur?
- Does this ability vary between different *Orbivirus*es, serotypes, topotypes?
- If it does occur, what significance does it have in the field persistence and epidemiology of the virus?
- Is it a mechanism for viral overwintering in the absence of infected animals?

Antivirals. The following points must be considered.

- Based on genomic, proteomic, and molecular characteristics of the virus, is there potential for antiviral strategies?
- Could these molecular types potentially be used in conjunction with vaccination strategies?

Genome analyses. Identifying *Orbivirus* strains by serotype is relevant for implementing vaccination strategies but completely insufficient for characterizing and identifying individual virus lineages and isolates or understanding the relatedness of exotic and endemic serotypes. Genome sequencing done thus far for BTV clearly shows a high degree of segment reassortment between serotypes/isolates resulting in a spectrum of BTV strains circulating in the field. A single virus isolate over time may certainly contain genome segments derived from multiple parental strains, each contributing to the overall phenotype of that isolate, yet by identifying only its serotype, only the VP2 segment is currently used to classify it. A new classification/identification system (possibly bar-coding) is needed to account for each segment in an isolate.

Critical to this classification/identification, we need a single database where genome sequence data for all isolates can be entered and accessed as they are generated. This will facilitate analyses to determine relatedness and may predict important epidemiological information such as expected virulence and vector range. An important outcome of whole-genome sequence comparisons is the ability to determine the relatedness of exotic to endemic isolates and informed policy on the classification of “exotic” as well as the duration of that classification in light of repeated isolations.

Important genomic questions include:

- How are the observed differences in %GC content of *Orbiviruses* from different vector species significant?
- Are there differences in codon usage?
- Do we fully understand the significance of genetic drift and shift for these viruses?
- How long does a specific genotype (containing a specific combination of genome segments) persist in the field?
- Do certain segments form reassortment groups (usually move together between strains; e.g., genome segments encoding the two outer capsid proteins)?
- Can reassortment and new combinations of genome segments lead to novel antigenic properties/serotypes?
- What can segment sequence comparisons tell us about important domains and epitopes that confer virulence, transmissibility, and vector/vertebrate host range?
- What controls the variations in expression level of individual proteins in different mammalian hosts, different mammalian cell types, or between vector and vertebrate host?
- What effect do genetic bottlenecks within the vector and vertebrate host have on virulence, transmissibility, and vector/vertebrate host range?
- How are viral genomes correctly packaged so that exactly one copy of each segment is encapsidated?
- If there are compatability restrictions on reassortment (e.g., blocking reassortment between different *Orbivirus* species), what are the determinants for this? Packaging signals, binding of RNA-RNA, protein-RNA, protein-protein?
- Could information concerning packaging signals (not yet available) be used to construct candidate vaccine viruses that cannot reassort with wild-type strains, thereby reducing the risk of reversion to virulence?
- If specific genes/domains of the novel BTV serotypes (BTV-25 and BTV-26) can be identified that block replication in insect vector cells, could these be incorporated into vaccine strains to prevent their transmission in the field?

Functional genomics. Further data concerning the responses of host RNA synthesis, protein expression, and relative up- or downregulation of specific host genes would provide further information concerning the way in which the virus causes disease. Similar data are needed for the vector.

COUNTERMEASURES ASSESSMENT

The protection of animals against *Orbiviruses* has been a concern of livestock producers for decades. *Orbiviruses* spread rapidly once inserted into an animal population and have complex epidemiological profiles that include several animal species and vectors and therefore require an integrated approach for control and eradication. Availability of effective vector control programs, diagnostics for early detection, and effective vaccines to prevent infections and stop virus transmission and spread of disease are paramount. These objectives can be realized with additional research and development.

Decision Model

For the assessment of diagnostics and vaccines, the workshop participants used the quantitative Kemper-Trego (KT) decision model to assess available countermeasures. Instructions for using the model were provided to the participants prior to the workshop (see Appendix I). The criteria and weight were modified by the working group for the purpose of assessing *Orbiviruses* vaccines and diagnostics (See Appendices III-X).

Criteria

The workshop participants determined that the following criteria constituted the ideal vaccine profiles for preventing and controlling BTV and EHDV disease:

Bluetongue vaccines. The following criteria constitute the ideal vaccine profile for preventing and controlling BT.

- Efficacy
- Cross-protection among serotypes
- <1-week onset of immunity
- No maternal antibody interference
- Two-year shelf life
- Safe
- No high containment required
- DIVA-compatible
- Rapid scale-up
- Reasonable cost
- Short withdrawal period
- Feasibility of registration

- Add new antigens
- Accelerated delivery

Epizootic Hemorrhagic Disease Vaccines. The following criteria constitute the ideal vaccine profile for preventing and controlling EHD.

- Efficacy
- Cross-protection among serotypes
- <1-week onset of immunity
- No maternal antibody interference
- Two-year shelf life
- Safe
- No high containment required
- DIVA-compatible
- Rapid scale-up (>10 million doses)
- Reasonable cost
- Short withdrawal period
- Feasibility of registration
- Add new antigens
- Accelerated delivery

Diagnostics. The following constitute ideal diagnostic criteria.

- Sensitivity
- Specificity
- Validation to purpose
- Speed of scale-up
- Throughput
- Pen-side test
- Rapid result
- Need for a confirmatory test

- Need for serological test to show recovery (absence of circulating virus)
- DIVA-compatible
- Easy to perform
- Cost to implement

Weight

Each criterion was weighted to allow a quantitative comparison of the impact of the selected interventions (see Appendices III–X).

Product profile

To ensure a consistent and meaningful assessment, the desired product profile (i.e., the benchmark) that would enable the control and eradication of an *Orbivirus* disease outbreak was identified for vaccines and diagnostics.

Ideal Orbivirus *vaccine profile.* The following criteria constitute the ideal profile for an *Orbivirus* vaccine.

1. Highly efficacious: prevents transmission in all major target animal species; efficacy in young animals
2. Cross-protection within serotypes
3. Cross-serotype protection (cross-protection against all serotypes)
4. One dose with >1-year duration of immunity
5. One week or less onset of immunity
6. No maternal antibody interference
7. Two-year shelf life
8. Safe: non-abortegenic; all species; pure
9. No reversion-to-virulence or insect transmission if live vaccine
10. No high containment required for manufacturing
11. DIVA-compatible
12. Rapid speed of production and scale-up
13. Reasonable cost
14. Short withdrawal period for food consumption (21 days or less)
15. Feasibility of registration (environmental release of a recombinant)
16. Ability to rapidly incorporate emerging viral antigens

Ideal diagnostic test profile. The following criteria constitute the ideal profile for an *Orbovirus* vaccine.

1. Direct tests (e.g., antigen, nucleic acid) for control and eradication
2. Indirect tests for post-control monitoring/detection sub-clinical cattle, captive cervids and wildlife
3. Rapid test
4. >95% specificity
5. >95% sensitivity
6. Pen-side test
7. DIVA-compatible
8. Field-validated
9. Easy to perform/easily train NAHLN's personnel
10. Scalable
11. Reasonable cost
12. Detect all *Orbivirus* virus strains

Values

The values assigned by the workshop participants for each of the interventions reflect the collective best judgment of members of the vaccine and diagnostic breakout groups (see Appendices III–X).

Diagnostics

The workshop showed clearly that in the U.S., diagnostic testing for *Orbiviruses* is undertaken by a range of Federal and State government agency research and diagnostic laboratories as well as university laboratories. All have various capabilities, but because they are not under common management or quality assurance systems, it is a matter of trust that all produce equivalent results. The USDA National Veterinary Service Laboratory (NVSL) does standardize and perform proficiency testing for laboratories performing *Orbivirus* testing for export purposes. The National Animal Health Laboratory Network provides surge capacity in case of an exotic serotype outbreak, no strategy is published for *Orbivirus* surveillance, monitoring, or preparedness. In a structured approach to national *Orbivirus* preparedness management, it could be useful to designate which laboratories are to be considered part of that national preparedness *Orbivirus* network and manage these as a network with delegation of different responsibilities. Where appropriate, harmonization of test methods and QA support across the participating laboratories should be considered. With the national network of laboratories that contribute to the national *Orbivirus* capability, more formally defined coordinated management and

communication practices could be adopted. Based on information gathered at this workshop, establishing coordination between these facilities would be an extremely useful initiative.

Evaluation of the process outputs of the workshop

The committee was satisfied with the criteria and the weightings, probably with individual differences of opinion, but with no great issues that resulted failure to achieve consensus. In some cases it is useful to have sufficient differentiation to better inform policy for test selection for intended purposes. For the purposes of the workshop, available tests, tests in the pipeline, and desired tests were comprehensively evaluated.

Fitness of tests for purpose

An important consideration for each test is fitness for purpose; hence, the tests were evaluated not only as tests *per se,* but with respect to the purposes for which they would be deployed. This is absolutely appropriate. A comparison of scores for the same tests in the spreadsheets for different purposes shows that the process has not led to major differences in scoring for individual tests. The available tests, groupings of individual tests, and the various purposes of tests were evaluated when relevant.

Available tests for detection of the infectious agent

Decision Model Analysis addressed such tests for the purposes of detecting EHDV, detecting BTV, prevalence, and freedom from infection (no vaccination). The tests considered in this section of the Decision Model Analysis were listed as virus isolation, nested RT-PCR (nRT-PCR), quantitative (real-time) RT-PCR (rtRT-PCR), typing PCR (with sequencing of the amplicon), electron microscopy (EM), and DNA microarrays. The virus neutralization (VNT) was noted as the available test for characterization (serotyping), but due to cross-reaction with this test, it is unclear how many labs utilize this test. The NVSL utilizes a standard multiplex RT-PCR that determines U.S. serotypes based on amplicon size (Johnnson et. al, 2000). A BTV rtRT-PCR is commercially available, but there are no USDA licensed rtRT-PCR kits.

Initial diagnosis of BT is by the clinical signs (Elbers, Backx et al., 2008); however, diagnostic tests for BTV and EHDV are necessary for accurate disease diagnosis and for trade regulations. BTV and EHDV likely share common vectors, infect similar animal species, often co-circulate in given geographical locations, and produce similar clinical signs of disease in susceptible animals. Because these two viruses have significantly different effects on trade regulations, it is important to distinguish between them. Current diagnostic procedures for both BTV and EHDV rely on virus isolation, antigen, nucleic acid, or antibody detection (Pearson, Gustafson et al., 1992; Mecham and Wilson, 1994; Mecham, 1997).

Virus isolation. Discussion of this topic has been considerable. The standard as listed in the OIE Manual for many years has been intravenous inoculation of embryonated chicken eggs (ECE) (Fernandes, 1959; Foster and Luedke, 1968), with the inoculum prepared from the specimen followed by a passage in mosquito cells (C6/36 cells) (*Culicoides* cells weren't available when the technique was developed) and then one to two passages in a mammalian cell line for the detection of CPE. Any of a number of such indicator cells can be used. The susceptibility of various mammalian cell lines to BTV infection has been evaluated (Wechsler and McHolland, 1988). The USDA ABADRU has on two occasions published the

establishment of cell lines derived from a *Culicoides* sp., and these are being used for virus isolation internationally (Wechsler, McHolland et al., 1989; McHolland and Mecham, 2003). The original cell line is no longer recommended by ABADRU because this line contains low-level contamination (Wilson, unpublished data). The newer cell lines are available through a simple material transfer agreement. Separately, certain U.S. university laboratories are using bovine endothelial cells, either cell lines or primary cultures, for the initial isolation step rather than ECE. It should be noted that the isolation method of choice (ECE or cell culture) is likely strain-dependent.

Issues: Our analyses of virus isolation resulted in determination that it is sub-optimal in sensitivity for both BTV and EHDV virus detection. However, virus isolation has been found to be as sensitive as possible in controlled situations. For example, the Berrimah Veterinary Laboratory (Weir, personal communication) in Northern Australia has been conducting virus isolation on groups of sentinel cattle (30 to 50 head) weekly for periods of 6 months during the arbovirus transmission season for in excess of three decades. This laboratory is arguably the most experienced laboratory internationally in the isolation of arboviruses from naturally infected ruminants, so its experience is valuable. This laboratory reports isolation an average of 300 viruses per year, 200 of which are BTV, using the ECE primary passage system. When lab personnel cross-check their BTV isolations with seroconversion, they report a success rate approaching 99% on the first attempt. If serology indicates that a BTV has been missed, a repeat of the procedure is usually successful.

Proposal: Virus isolation should be ranked more highly in the Decision Model Analysis with respect to sensitivity.

- Discussions clearly indicated variability in implementation of the ECE isolation system. The Berrimah Veterinary Laboratory processes all embryos through the subsequent cell culture steps, and this is an important factor in their success rate. The lab advised that up to 25% of their BTV isolates would be from non-hemorrhagic embryos (it processes haemorrhagic separately from non-hemorrhagic). Non-hemorrhagic embryos are not usually replicates of a hemorrhagic embryo; rather, some strains of BTV appear not to cause hemorrhagic embryos during the isolation process. As an aside to interested readers, Berrimah Veterinary Laboratory has said that it would not consider not processing all embryos, since the time saved would be minimal because the egg has to be opened anyway and the extra work to harvest the embryo is minimal in an efficient processing and passaging system (Weir, personal communication). U.S. experience differs from that of Australia. U.S. labs have decades of *Orbivirus* isolation work in which BTV was never isolated from an inoculum that didn't yield at least one hemorrhagic egg (Ostlund, personal communication). Perhaps this difference is due to strain differences between the two countries.

- Pirbright has stopped using ECE for BTV isolation and uses *Culicoides* cells provided by the U.S. instead. Unfortunately, no structured comparison has been made of test performance using *Culicoides* cells or bovine endothelial cells as the first passage in BTV or EHD isolation.

Gap: A structured comparison of virus isolation test performance using *Culicoides* cells or bovine endothelial cells as the first passage in BTV or EHD isolation.

- The OIE BTV chapter in review states: “Highest recovery rates are achieved by primary isolation of virus in ECE, followed by passage in AA cells or mammal cells for further replication of virus. Successful virus isolation has also been reported using primary isolation in cells derived from *Culicoides sonorensis* free of BTV and *Culicoides* viruses and designated as KC or CuVa cells (Wechsler et al., 1989; MacHolland and Mecham, 2003). In case of passage in AA, KC, or CuVa cells, additional passages in mammalian cell lines such as BHK-21 or Vero are usually performed.” The NVSL methods are in compliance with this description of ECE followed by passage in mammalian lines. Other U.S. labs, especially universities, serving wildlife interests or the livestock industries use other approaches such as first passage in endothelial cells.

- Concern over the natural transmission of strains of BTV derived from live, attenuated vaccines or of BTV that contain reassorted gene segments from such vaccine strains was noted. It could be expected that such strains may be more adapted to cell culture, and that they will possibly be more likely to be isolated in cell culture systems than wild-type strains. (Note that there has been no suggestion to date that naturally transmitted BTV in Northern Australia have been derived from vaccine strains.)

Gap: No national network exists for detection and monitoring of *Orbiviruses*. The national laboratory network (NAHLN) does not have any specific responsibility for BTV/EHDV. No funded USDA APHIS are programs related to national *Orbivirus* preparedness. A science-based standard operating procedure or guidelines for virus isolation among the U.S. laboratories that perform *Orbivirus* isolation, especially if a national *Orbivirus* network be developed, would be an important part of national *Orbivirus* preparedness.

Standard and nested RT-PCR. Detection of viral RNA has been used for some time, but the method of choice is RT-PCR. These genetic-based diagnostic tests have been developed for BTV and EHDV (Dangler, de Mattos et al., 1990; Akita, Chinsangaram et al. 1992; Wilson, Archer et al., 1992; Akita, Glenn et al., 1993; Katz, Alstad et al., 1993; Katz, Gustafson et al., 1993; Wilson and Chase, 1993; Shad, Wilson et al., 1997).

Issues: With the nested RT-PCR technique, the possibility of contamination of the laboratory environment with amplified PCR product is unavoidable. The resulting risk of false positive test results is mitigated in well-managed and -constructed laboratories with rigidly enforced work practices based on separation of the steps in the test procedure; however, not every laboratory has been designed with the work places necessary to effectively enforce such work practices, and work practices are still susceptible to human error. Hence, some laboratory scientists argue that the nested RT-PCR technique as a front line or routine test for the detection of *Orbivirus* species in poorly suited to the modern laboratory. Nonetheless, the nested RT-PCR test has been given the highest possible ranking in both the analyses for detecting BTV and EHDV in the Decision Model Analysis.

Given the acknowledged inherent threat in test performance for false positives in the nested RT-PCR, with the risk mitigated to greater or lesser effectiveness depending on the laboratory, the ranking of this test for specificity in the Decision Model Analysis was downgraded due to this issue.

Recommenation: Only laboratories that have the ability to control contamination should use the nested RT-PCR test for the detection of BTV and EHDV.

RtRT-PCR. Quantitative rtRT-PCR (rtRT-PCR) assays also have been developed for these viruses (Orru, Santis, et al., 2004; Wilson, Stallknecht, et al., 2004; Shaw, Monaghan, et al., 2007; Hoffman, Eshbaumer, et al., 2009). These PCR-based diagnostic procedures are exquisitely sensitive and theoretically can be configured to any desired specificity if the sequence of the viral gene of interest is known. Some caution is needed in interpretation of rtRT-PCR results because viral RNA can be detected up to 16–20 weeks post-infection, but only 2–8 weeks by virus isolation (MacLachlan et al., 1994). A review of available real-time assays for BTV in 2009 summarizes the design and strategies of the assay at that time (Hoffmann, Beer et al., 2009). Since then, new assays have been developed, including an improved multiplex rtRT-PCR assay that detects and distinguishes between BTV and EHDV (Wilson, Hindson et al., 2009). In addition, an as-yet unpublished rtRT-PCR for detecting BTV has been developed and evaluated on field diagnostic samples and virus isolates of 24 serotypes of BTV by the NVSL (McIntosh, et al., unpublished). Fewer rtRT-PCR assays are available specifically for EHDV (Wilson, O'Hearn et al., 2009; Clavijo, Sun et al., 2010; Eschbaumer, Wernike et al., 2012).

An often overlooked aspect in evaluation of rtRT-PCR is selection of the sample and nucleic acid extraction method. Nucleic acid extraction methods must be evaluated for the animal source (Brito, Gardner et al., 2011) and specimen chosen (Vanbinst, Vandenbussche et al., 2010). EDTA blood represents the most common sample type for molecular detection of BTV (Maclachlan, Drew et al., 2009); however, PCR inhibitors such as immunoglobulin G and hemoglobin can have significant negative effects on sensitivity of RT-PCR-based tests (Al-Soud and Radstrom, 2001). In this regard, molecular test validation with regard to sample type and species have highlighted the importance of test validation and the need for continued improvement in methods for RNA purification from tissues. Improved RNA purification methods are needed to reduce the impact of such inhibitors to minimize the potential for variable test performance between different species and tissue types.

rtRT-PCR has become a standard test for the detection of RNA viruses in biological (diagnostic) specimens in many laboratories. Initial problems with the published rtRT-PCR tests, in that they did not detect all the BTV strains in some episystems, have been overcome. Reference laboratories in many of the different episystems globally have tested available rtRT-PCR test methods and either adopted published methods or adapted these to their particular circumstances. This is the case in the U.S. The NVSL has completed RT PCR /rtRT PCR comparisons and contributed to the selection of a specific PCR for the updated OIE chapter on BTV. These comparative data have not yet been published and do not compare the assay performance in multiple institutions. The Decision Model Analysis for both BTV and EHDV detection and for assessment of prevalence (surveillance) scored this test most highly of the agent detection tests.

Recommendation: A selected panel of US laboratories that perform detection of *Orbivirus* strains as part of their routine diagnostic panel should evaluate the available rtRT-PCR tests to provide guidance and standardization of this test.

Issues:

- Published and accepted information clearly shows that rtRT-PCR will detect viral nucleic acid long after viable virus is no longer present in a specimen, so positive rtRT-PCR test results must be interpreted accordingly. A positive test result will indicate that an animal has been infected or vaccinated at some time prior to the collection of the specimen. The animal may not necessarily remain infectious to *Culicoides* insects and hence capable of sustaining transmission of the virus. Such considerations can be of consequence in the national movement or international trade in animals, but such purposes of testing were not included within the scope of the workshop Decision Model Analysis.

- Different laboratories may perform rtRT-PCR testing in different ways, using different equipment, extraction methods, primer/probe sequences, and reaction chemistries. For national EAD purposes, confidence is required that all laboratories performing the test will give equivalent results. A useful first step is standardizing the test method and ensuring that participating labs are part of a single quality assurance program (see earlier comments in section B on this topic).

Recommendations:

1. The USDA should coordinate the comparison of commercial, published, and rtRT-PCR tests.

2. The USDA should coordinate a process of standardization of BTV and EHD rtRT-PCR test methods for adoption by all U.S. laboratories that have a mandated responsibility in the national laboratory network for the detection of *Orbivirus* strains as part of national *Orbivirus* preparedness.

3. The USDA should nominate a proficiency test provider for *Orbivirus* rtRT-PCR and require that the nominated facility provide PT testing in accordance with the requirements of the international standard ISO17043.

4. The USDA should develop a system of networked quality assurance based on the continuous use of a low positive control as a network calibrator or network QA reagent. This network QA reagent should be used as a control agent every time the test is performed at all laboratories conducting rtRT-PCR testing in the national laboratory network for the detection of *Orbivirus* strains as part of national *Orbivirus* preparedness. The result of the test should then be reported to a central national coordinator for the purposes of analysis of test performance across the network.

Gap: A coordinated *Orbivirus* monitoring and preparedness network is needed. This network requires standardization and quality assurance of *Orbivirus* rtRT-PCR methodologies used in the U.S.

Typing PCR (with sequencing of the amplicon). Rapid detection of an orbiviral species (BTV or EHDV) is a necessary first step in an outbreak investigation or a surveillance program but is usually insufficient. The detection (by rtRT-PCR, or possibly by virus isolation) can lead to focused studies aimed at characterizing the agent by determining the serotype, the genotyping aspects of interest, and hence the epidemiological relationship between the agent and previously transmitted viruses in the study area. At present, genotyping of U.S. isolates for epidemiology

purposes is limited to a few research laboratories. There are serotype-specific standard RT-PCR assays for BTV (Johnson, Wilson et al., 2000; Maan, Maan et al., 2012) and EHDV (Aradaib, McBride et al., 1995; Aradaib, Wilson et al., 1998). The Decision Model Analysis ranked this item highly for both BTV and EHD. Although a high degree of capability exists among U.S. labs, the extent of typing assays outside the national laboratories is unclear. Standardization is lacking.

Issues:

- Serotyping can be addressed by PCR and sequencing, as can genotyping (especially topotyping) and molecular epidemiological assessments. However, there are other approaches to serotyping, and the PCR that allows serotyping may not be the preferred PCR for other assessments such as topotyping.

- Not all laboratories conducting front line rtRT-PCR for the rapid detection of *Orbiviruses* may be suitable for maintaining any or all of the methods that may be required for sequential follow up of detections.

- A high level of expertise exists in research laboratories, but how this can be harnessed in a coordinated national response may not be clearly defined.

- Newer technologies to be discussed later will have an impact on the test methods of choice. It is yet to be determined whether experimental procedures are ready for deployment.

Recommendations:

1. Algorithms of sequential investigations to be conducted on detection of *Orbiviruses* under different circumstances should be developed.

2. The preferred test method for the determination of each piece of required information should be discussed and agreed upon, and responsibilities should be allocated to relevant laboratories.

3. The likely timeframe for replacement by current approaches with "experimental" test methods should be assessed and the change-over of methods planned, likely resulting in cost savings to the network.

Gap: A clearly defined national strategy for the characterization of detected *Orbiviruses* that specifies the information needed, the tests to be used to deliver the information, the specific laboratories where those tests will be maintained, and the national requirements for referral of specimens and reporting of results.

Electron microscopy (EM). Traditionally, EM has been useful in the diagnosis of diseases of unknown etiology because the morphology of the virion is useful in indicating the viral family of the infectious agent.

Issues:

- EM is most useful where there is an isolate grown to high titre because this facilitates visualization of the agent; hence, a time delay usually occurs between collection of specimens to reporting of EM results.

- Molecular techniques are being discovered to give information more quickly, and with a higher level of specificity.

- The Decision Model Analysis for EM indicated only a moderate level of usefulness of this technique. Even so, for criteria such as "Rapid Result" and "Virus Characterization," EM may have been over-ranked, especially for detection of *Orbiviruses* and their differentiation. (EM may be more useful for other diseases such as FMD or poxvirus infections.)

Recommendations: The focus in allocating resources for *Orbivirus* disease preparedness should be directed at other technologies.

DNA Microarrays. This methodology was included in the section of established tests in the Decision Model Analysis, because some U.S. laboratories have multi-pathogen or pan-viral DNA microarrays suitable for detection of *Orbiviruses* (Barrette et al., 2009; Jack et al., 2009; Palacios et al., 2007; Quan et al., 2007). Because such DNA microarrays capture viral DNA from a sample using many different oligonucleotide features targeting conserved genetic loci that are cross-reactive within or among specific virus family, genera, or species, their "purpose" is more suited to detection rather than diagnostics or genetic characterization. These design features, including diversity and multiplicity of well conserved genetic detection targets, make DNA microarrays well suited to detection of emerging or previously unknown pathogens, as well as for detection of multiple agents that may be associated with disease syndromes. The ability to capture and positively select virus-derived nucleic acid from host nucleic acid also serves as a useful companion tool to conventional or next-generation sequence analysis. DNA microarrays that target all segments of segmented viral genomes such as the USDA pan-viral microarray (Barrette et al., 2009) are particularly useful companion tools to sequence analysis. Mapping of positive microarray features during data analysis may be exploited further to identify candidate primer and probe binding sites for the design of new or modified RT/rtRT-PCR methods that may be needed to survey and/or diagnose newly detected divergent *Orbiviruses*.

This technology is only available in a few U.S. laboratories; thus, the analysis did not give them a weighted score nearly as high as PCR techniques.

Issues:

- Have arrays been developed to their full potential, or are they to some extent still experimental, even for the detection of *Orbivirus* species? Are we expecting improvements in the Decision Model Analysis of this technology in the near future? Is refining arrays for this purpose an optimum use of resources?

- Typing arrays have been listed as an experimental test method. It is unclear how advanced these are for serotyping and how robust the procedure can be made given that standard molecular techniques show regional differences among serotypes.

- It is likely that a more useful array configuration would deliver *Orbivirus* detection and serotyping in a single analysis.

Recommendations:

1. DNA microarrays offer a distinct advantage over RT/rtRT-PCR based diagnostics in detection of emerging or diverging *Orbiviruses*; however, their purpose along with other advanced molecular detection (AMD) technologies should be defined as detection tools rather than diagnostic tools at this time.

2. Development of *Orbivirus*-specific DNA microarrays that combine detection and characterization features could be purposed and validated for diagnosis and characterization of known species of *Orbivirus* and could be useful for detection and characterization of emerging strains or species of *Orbivirus*; however, the costs of such development should be weighed in comparison to the cost of implementation of other AMD technologies, including next-generation sequencing (NGS) or high-throughput sequencing (HTS).

3. The utility of DNA microarrays as well as other AMD or sequence-based detection methods for diagnosis, detection, and/or characterization of *Orbiviruses* will be highly dependent upon improvement of publicly available genetic sequence information for *Orbiviruses*. The international community, including the ICTV, should be consulted on the improvement of genome sequence collections and annotation of the NCBI nucleotide database for viruses.

4. Continued development of bioinformatics software to interpret and exploit AMD technologies, including DNA microarrays and HTS, can improve the implementation of these methods for early detection, characterization, and response to emerging diseases by *Orbiviruses*.

Gap: Several methods are in development for unbiased pathogen detection, including pathogen arrays. USDA should coordinate a systematic evaluation of these methodologies.

Experimental tests for detection of the infectious agent

The tests considered in this section of the Decision Model Analysis were listed as deep sequencing or high-throughput sequencing (HTS), immunohistochemistry or immunofluorescence (IHC/FA), in situ hybridization, topotyping, multiplexed PCR for BTV and EHDV detection, typing arrays, Luminex for nucleic acid detection, Luminex for antigen detection, alternative amplification techniques, and pen-side tests.

The questions that were used in evaluating these methodologies were:

- Is there a need for the technology? (Which deficiency or opportunity is it addressing?)

- If it replaces an existing technology, what is the comparative advantage? (e.g., speed, sensitivity, specificity, comprehensiveness of information.)

- How would the technology be deployed in the existing or foreseeable national laboratory network?

- How would the technology be utilized in the existing or foreseeable regulatory environment?

Deep sequencing or high-throughput sequencing (HTS). Many diagnostic scientists consider this methodology to be the primary diagnostic platform of the future. This technology for *Orbiviruses* is well established in at least three federal laboratories in the world. New underpinning methodologies are being developed, and new equipment and costs of both equipment and reagents are decreasing. The approach is so affordable in some commercial packages that many laboratories are investing to develop familiarity with the opportunity, even in less developed countries. Early adaptors will likely take the lead in establishing and developing approaches. The OIE has established a working group to develop approaches to validating HTS methods for diagnostic work.

Issues:

- The technology produces large amounts of data, so data management and data analysis (bioinformatics) are potentially limiting factors to uptake.

- A national system of data management is needed, with standards for archiving of raw and processed data and the means to perform these tasks.

- Data management must include accessibility to the epidemiological and laboratory submission information regarding specimens and the animals, herds, or situations from which the specimens are collected.

- The ideal HTS data management system includes easy access to the bioinformatic analysis tools required for the particular task.

Needs and Opportunities:

- Processing of specimens will theoretically give all the molecular sequence information around a specimen in one run: identification of viral species in the tested sample, molecular serotyping, topotyping, identification of reassortants, and analyses of the likely origin of reassorted genes. As the knowledge of molecular markers for various phenotypic traits of the *Orbiviruses* improves, the data to assess these also could be expected to be available for analysis from the one initial run; hence, the opportunity is to derive all required molecular information efficiently and quickly.

Deployment and utilization:

- Providing the outputs of HTS are accepted as validated, no regulatory problems should occur in deployment.

- Which U.S. laboratories will deploy this technolocy and have a mandated responsibility in the national laboratory network for the detection of *Orbivirus* strains as part of national *Orbivirus* preparedness will be a matter for discussion and policy decision.

Recommendation: The USDA should prioritize development of HTS and evaluate it as a future diagnostic technology, including consideration of the issues of bioinformatics capability, validation, quality assurance, and positioning in the national laboratory network.

Gap: A policy for HTS development that comprehensively addresses issues and opportunities

Immunohistochemistry or immunofluorescence (IHC/FA). IHC/FA is an established and mature technology rather than a new technology but finds its main use in experimental (pathogenesis) studies. It may have diagnostic uses in specialized situations, such as linking a lesion to a detected infectious agent in circumstances where the presence of the agent might be incidental to the disease. This could happen in orbiviral infections of cattle, in particular, where the duration of viraemia can be several weeks. During the period of viraemia, an animal may coincidently suffer from a different disease, but the orbiviral infection will be detected in laboratory testing. Such situations would not be easily interpretable because the agent is not necessarily found in the lesion in orbiviral infections. Hence, the technology complements the full pathological investigation of the case rather than giving a definitive result of itself.

Recommendation: That IHC/FA continues to be maintained in major national network laboratories with a histopathology capability.

In-situ *hybridization.* This technology can be considered the molecular equivalent of IHC, in that it detects viral genome in tissues, and hence potentially in pathological lesions, whereas the latter detects the presence of viral antigen. It may be useful in diagnostic situations in linking a detected infectious agent to the pathological lesions, as discussed more fully under IHC/FA above. IHC can be of particular value in linking virus detection to pathological disease in instances where a new or emerging *Orbivirus* may be detected using AMD technologies such as DNA microarrays and/or HTS. It is an experimental tool.

Recommendation: Maintain *in situ* hybridization in major national network laboratories with a histopathology capability.

Topotyping. Topotyping should now be considered an essential component of the molecular characterization of *Orbiviruses* detected in outbreaks or surveillance programs. Its basis is the observation that different orbiviral strains in differing episystems evolve locally. Thus, when the molecular sequences of apparently similar serotypes from different geographical locations are compared, the differences are detectable. After knowledge of the sequences in different areas has been developed, it is possible to postulate whether a given isolate is likely an introduction into the area where it was detected, and even to postulate from which geographic area it may have been derived. National and international reference laboratories develop the databases and the knowledge on which these analyses depend.

Issues:

- The orbiviral genome comprises 10 gene segments, so it must be decided on which segment to standardize. An important consideration is that the analyses can be done on a number of the segments to a greater or lesser degree. Standardizing on a segment widely used for such work internationally is recommended.

- Because orbiviral species undergo reassortment in the field during the processes of natural transmission among vector insects and vertebrate host animals, any assessment of a detected virus on the basis of just one gene segment may not give the full epidemiological picture. Hence, although techniques that have been in use for over two decades are useful, the regulatory and national disease management requirements are likely to require more information in the future.

Recommendations:

1. Topotyping of detected Orbiviral infections should be included in the detection and characterization of *Orbivirus* strains as part of national *Orbivirus* preparedness.
2. Discussion and a policy decision are needed regarding which laboratories in the national laboratory network maintain the capability for this technique and should be supported by clearly communicated expectations of referral of specimens.
3. A national strategy for the development and deployment of HTS as identified in Section D.ii.a above should have as one of its objectives delivery of a topotyping capability.

Gaps:

1. A clearly developed and implemented national strategy for topotyping of orbiviral strains detected in the U.S. using existing technology, and the national collation and monitoring of that information.
2. A national strategy for the comprehensive development of HTS-based orbiviral characterization, as also identified in Section D.ii.a above.

Multiplexed PCR for BT and EHD detection. Testing for multiple agents in one test is usually attractive, and multiplexing PCR addresses this opportunity. The techniques are established, so the challenge is to develop the reagents and test conditions for each diagnostic situation.

Issues:

- Agent detection in a multiplexed reaction is sometimes less sensitive that in a single rtRT-PCR.
- Co-circulation of BTV and EHDV occurs in endemic states, so the slight loss of sensitivity versus the advantage of performing simultaneous testing in a single assay is beneficial. The assay can be run in singleplex format when needed, but both the single and multiplexed tests would have to be validated and maintained under the laboratory QA system.

Recommendation. The multiplexed rtRT-PCR for BTV and EHDV detection should be considered for states where co-circulation is common.

Typing arrays. This opportunity has been discussed under the section above dealing with DNA microarrays (section D.i.f). If resources for test development are limited, investing in HTS technology may be more rewarding. One reason for not focusing entirely on HTS technology

might be to avoid having only one technology under development. The risks of adopting such a focus would have to be assessed in a structured risk analysis framework.

Luminex for nucleic acid detection and antigen detection. Luminex is a fluorescent microsphere approach to multiplexing diagnostic testing that is being investigated experimentally in a number of laboratories. Where research teams have developed appropriate expertise, particularly in designing and producing the reagents for the multiple separate detections in the system, the results have been encouraging.

As with the array technology, Luminex can be designed as an unbiased or syndromic detection system. Some of the comments in the sections on arrays are applicable to Luminex. The Luminex systems are early in development, so it is difficult to know if they will yield acceptable test performance characteristics. The technology is currently suitable only for a laboratory setting and likely deployable only in state, regional, or federal diagnostic laboratories. The assays are quite flexible and can be developed to meet a variety of needs from basic serology to molecular characterization and serological characterization of isolates. After development and evaluation, whether this technology provides advantages to alternative approaches, where should the technology be based, how quality assurance would be managed, and with what cost need to be determined.

Luminex is easily adapted to analyzing serological responses in a multiplex format allowing for syndromic testing. Luminex serology based methods were not evaluated because there was not enough information on the development to make an evaluation.

Recommendation. Luminex is a promising new technology and instrumentation is available, so the USDA should continue to develop it.

Alternate amplification techniques and pen-side tests. The promise of such techniques has a high profile. They are particularly the focus of research in the private sector, and perhaps are supported from funding sources other than agriculture.

Should animal health agencies be monitoring developments with the intention of specifically assessing promising technology platforms for disease preparedness purposes?

The decision criteria suggested at the beginning of this Section D.ii could be considered.

Is there a need for the technology? (Which deficiency or opportunity is it addressing?)

- Implicit in both these approaches is the idea that testing could be done at the point of collection of the specimen before transport to the laboratory. In outbreaks of some disease this could be useful, perhaps even for outbreaks of orbiviral disease such as for the BTV 8 outbreak in Europe.

- Would a point-of-sampling test allow regulatory responses to be initiated? Have the test performance criteria to allow such actions been defined?

If point-of-sampling tests replace an existing technology, what is the comparative advantage? (e.g., speed, sensitivity, specificity, comprehensiveness of information)

- As asked above, assuming the tests are of comparable or acceptable sensitivity and specificity and offer the advantage of speed of test result, can action be taken on the result?

- Would confirmatory testing still be needed in a laboratory, with the extra cost implications?

- Have the criteria under which confirmatory testing would not be required been established?

How would the technology be deployed in the existing or foreseeable national laboratory network?

- If such tests are to have regulatory credibility, by whom could they be used? What training would be required?

- What validation would be required, how would it be done, and by whom? (The OIE has developed a system; has the USDA assessed that for its own purposes?)

- What quality assurance processes would be required, and how would these be accredited?

How would the technology be utilized in the existing or foreseeable regulatory environment?

- See questions above.

Recommendation: The USDA should establish a technical working group to evaluate issues around and make recommendations on the use of point-of-sampling tests. Such tests include tests for antibody detection, antigen detection, and nucleic acid detection and characterization, either pen-side or in an onsite mobile testing unit or station. Issues that should be considered include:

- The purposes for which such tests might be used, including reference to specific diseases;

- The criteria, for each type of test, by which it might be considered validated as fit for purpose;

- Recommendations for regulations regarding the use of such tests, including personnel performing the test, their training, and quality assurance of the test procedure;

- The likely costs of each test, the shelf life, and recommendations on the logistics of ensuring test availability if the test is clearly advantageous.

Gap: The issues around the use of testing away from the laboratory using portable devices for regulatory purposes in disease outbreak and surveillance situations should be comprehensively addressed to both a) inform potential test developers of the performance criteria required, and b) to allow the USDA to develop the management strategies that would provide the framework for the use of such devices.

Based on a rigorous analysis of the outcome of such deliberations, the USDA could then have objective criteria to decide whether to invest in the evaluation of any particular platform for any particular purpose.

Available tests for detection of antibodies to infectious agents

The Decision Model Analysis addressed such tests for the purposes of Prevalence, Protective Immunity, Serology, and Freedom from Infection (no vaccination).

The tests considered in this section of the Decision Model Analysis were listed as the BTV C-ELISA, the VNT, the CFT, the BTV AGID, and the EHD AGID. BTV C-ELISA and EHDV blocking ELISA kits are commercially available, but published data on their performance are limited.

In diagnostic investigations focused on BT or EHD, positive diagnostic results and compatible clinical signs are needed to confirm a case. Agar Gel Immunodifusion (AGID) and positive ELISA results indicate only the sero-positive status of an animal that may be from vaccination, previous exposure, or acquired from maternal immunity. BTV/EHDV cross-reactivity with the AGID test also adds significant complexity to the diagnosis of BT or EHD. A number of enzyme-linked immunosorbent assays (ELISA) have been developed for the detection of antibody to BTV (Afshar, Thomas et al., 1987; Afshar, Thomas et al., 1987; Drolet, Mills et al., 1990; Mecham and Wilson, 1994; Zhou, Riva et al., 2001). These tests have been shown to be more specific than the AGID test in not cross-reacting with antibody to EHDV (Afshar, Thomas et al., 1989; Afshar, Anderson et al., 1991; Afshar, Trotter et al., 1993).

BTV C-ELISA. The BTV C-ELISA has been used for more than 2 decades and has been evaluated extensively. Although versions based on different monoclonal antibodies exist in different countries, it is accepted that most have equivalent performance characteristics that allow the important detection of antibodies to BTV and not to EHDV. The specificity of the BTV C-ELISA for this purpose is widely accepted. The USDA-APHIS requires labs conducting BTV ELISA for export testing use a standardized USDA-licensed test kit.

As for the standard test for detection of agent, the rtRT-PCR, consideration could be given to aspects of the management of the test across the proposed national network. Those aspects were discussed in the relevant section in D.i.c above.

Recommendations:

1. The USDA-APHIS coordinates quality-assured laboratories conducting BTV C-ELISA for export testing, but this does not include testing for routine diagnostic purposes. Although privacy concerns will have to be addressed, the development of a quality-assured network to monitor BTV activity would be advantageous

Gap: Standardization and quality assurance of the BTV C-ELISA tests implemented across the state and regional diagnostic labs for routine diagnosis is needed.

Virus neutralization test (VNT). The VNT is the standard approach for detecting the serotype-specific antibody for both BTV and EHDV species and is useful in epidemiological studies, in measuring response to vaccination, in demonstrating that vaccination has occurred. No attempt

is made to correlate VNT titre with level of protection from either vaccination or natural exposure. Antibody is usually taken to be protective. In the OIE Terrestrial Code Chapter on BTV, reference is made to the movement of animals under certain circumstances providing they have detectable antibody to the serotypes known to be present in the country or zone of origin. This is a measure that facilitates trade and the movement of live animals under some farming systems and is not currently relevant to the U.S.

Interpretation of antibody responses can be a little problematic, particularly where animals have been infected with more than one serotype. Such animals may develop neutralizing antibody to a serotype(s) different from the infecting serotypes, so results must be interpreted with respect to the epidemiological situation.

Because the VNT requires propagation of live virus in culture, laboratories offering the procedure face biorisk management issues. It is common practice for laboratories in national networks to offer the VNT only for serotypes known to be transmitted in the geographic area they service, which removes the requirement of facilities for the handling of foreign animal disease agents. Sera requiring further evaluation are sent to the national reference laboratory.

Recommendation: The USDA should designate U.S. laboratories in the proposed national laboratory network that would be responsible for offering BTV and EHDV VNT in support of national *Orbivirus* preparedness.

Gap: Lack of a documented national strategy for BTV and EHDV VNT, with designated laboratory facilities for endemic disease testing other than the USDA, APHIS national reference laboratories.

Complement fixation test (CFT). The CFT suffers from cross-reactions between antibodies to the BTV and EHDV species or serogroups. It was not ranked in the Decision Model Analysis, and consensus that there is no need for this test is probable.

Recommendation: The CFT test should not be accepted in the U.S. for the purposes of detecting antibodies to either BTV or EHDV.

BTV and EHD agar gel immunodiffusion test. The BTV AGID and EHDV AGID tests suffer from cross-reactions between antibodies to the BTV and EHDV species or serogroups. In the Decision Model Analysis, they were ranked low for specificity and moderately low for sensitivity but moderately high for validation for purpose, perhaps reflecting that the performance criteria are well known. This has perhaps led to the tests scoring more favorably overall than they should, even allowing for ease of performance and cheapness.

Recommendation: That the BTV AGID and EHDV AGID tests be phased out in the U.S., to be wholly replaced by C-ELISAs, and that there be a fixed timeline beyond which AGID test results are no accepted in the U.S. for the purposes of detecting antibodies to either BTV or EHDV.

Gap: Lack of a documented national strategy for replacement of BTV and EHDV AGID tests with C-ELISA tests in U.S. laboratories that have a mandated responsibility in the national laboratory network for national *Orbivirus* preparedness.

Experimental tests for the detection of antibodies to infectious agents

The tests considered in this section of the Decision Model Analysis were listed as EHDV cELISA, Luminex for antibody detection, and pen-side tests.

Again, for agent detection, certain concepts may be applicable to consideration of experimental tests: the need for the technology or test, its comparative advantage(s), how the technology would be deployed in the national laboratory network, and how the technology would fit with regulatory requirements.

EHD cElisa. This test has been published and in use in other countries for almost as long as the BTV cELISA. There is one commercially available blocking ELISA test, but little information on this test is available, and there is no USDA, APHIS licensed EHDV ELSIA test. The EHDV cELISA has not been widely available in the U.S. and has not received nearly as much evaluation as the BTV cELISA internationally. Nonetheless, it is reported to offer the same advantages of specificity for EHDV serogroup antibodies as the corresponding BTV test. In this aspect, it is potentially a much more precise tool for *Orbivirus* diagnostics that the AGID test. The EHDV AGID test is a USDA, APHIS licensed test, and thus is well established.

Issues:

- Monoclonal antibodies on which to base the test format may not be as widely available or as well characterized as they are for the BTV cELISA.
- The test has not been extensively validated or compared internationally.

Recommendations:

1. The USDA should collaborate with the Australian BTV reference laboratory and other laboratories having an EHDV cELISA for the purposes of designing and implementing an international comparison and validation exercise.
2. When the USDA is satisfied that the cELISA is sufficiently validated (irrespective of the previous recommendation) and consistent with the recommendation with respect to AGID test above, the EHDV AGID test, although a standard and inexpensive test, should be discouraged in the U.S..

Gap: Lack of a documented national strategy for the adoption of the EHDV cELISA as the national test for the detection of antibodies to EHDV in U.S. laboratories that have a mandated responsibility in the national laboratory network for national *Orbivirus* preparedness.

Luminex for antibody detection. The discussion and recommendations in the section for nucleic acid detection and antigen detection above (see page 70) is applicable.

Pen-side tests for serology. The discussion and recommendations in the section for alternative amplification techniques and pen-side tests above (see page 70) is applicable.

Table 1: *Orbivirus* diagnostic tests available or under development

Test	What does it detect?	Development status	Capability	Utilization
AGID	Antibodies to BTV and/or EHDV	Available	Inexpensive, easy-to-run serology assay but lacks specificity	Primarily diagnosis but can be used for serosurvey
Indirect ELISA	Antibodies to BTV and/or EHDV	Available	Slightly more complex serology assay to run but increased specificity	Diagnosis and sero-prevalence survey
Competitive ELISA	Antibodies to BTV and/or EHDV	Not readily available in the U.S.	Slightly more complex serology assay to run but with internationally accepted serogroup specificity, a recommended OIE test	Diagnosis and sero-prevalence survey
Competitive ELISA using recombinantly produced proteins	Antibodies to BTV and/or EHDV	Not readily available in the U.S.	Slightly more complex serology assay to run but with internationally accepted serogroup specificity	Diagnosis and sero-prevalence survey
Antigen Capture ELISA	BT and/or EHD Viral proteins	Available	Deployable by laboratories with ELISA technology, largely replaced by real-time PCR internationally	Detecting virus (for diagnosis and active surveillance)
Virus isolation by ECE	Infectious virus	Available	Standard recommended OIE method	Detecting virus (for diagnosis and active surveillance)
Virus isolation by cell culture	Infectious virus	Available	Published as an equally sensitive isolation method to ECE if using *Culicoides* cells, otherwise less sensitive	Detecting virus (for diagnosis and active surveillance)
Standard RT-PCR	BTV or EHDV Viral RNA	Available	Largely replaced by real-time RT-PCR internationally	Detecting virus (for diagnosis and active surveillance)
Nested RT-PCR	BTV or EHDV Viral RNA	Available	Largely replaced by real-time RT-PCR internationally	Detecting virus (for diagnosis and active surveillance)
rtRT-PCR	BTV or EHDV Viral RNA	Available	The most commonly accepted PCR method internationally	Detecting virus (for diagnosis and active surveillance)

Multiplex RT-PCR	BTV and EHDV Viral RNA	Published	Equivalence data relative to the real time RT-PCR should be available where used	Detecting virus (for diagnosis and active surveillance)
Serotype rtRT-PCR	BTV and EHDV Viral serotype-specific RNA	Published for some viral episystems	Yet to be validated in all episystems (including North America?)	Detecting virus (for diagnosis and active surveillance)
Serotype RT-PCR array	Viral RNA from L2	Published		Detecting virus (for diagnosis and active surveillance)
Topotyping PCR, with sequencing	Likely geographic origin of a viral isolate	Published		Detecting virus (and characterizing it for diagnosis and active surveillance)

Diagnostics Research Goals

Standard diagnostic tests are readily available for BT/EHD viruses and described tests are published in the OIE Manual. Variation in the diagnostic approach to these viruses is considerable. Commercial BTV ELISA test kits are available, and many laboratories are experienced in running these assays. The AGID is still commonly used for EHDV, but there are published cELISAs and a commercial EHDV ELISA. The National Veterinary Services Laboratories do perform laboratory evaluation for BTV and EHDV serology annually.

No commercial rapid pen-side or field-based diagnostic tests are available for BTV or EHDV; however, a serum antibody strip test for BTV has been reported.

Detection of viral RNA by standard or rtRT-PCR is useful for screening samples prior to virus isolation. The preferred diagnostic test method for detection of the presence of BTV or EHDV is rtRT-PCR. There are published and commercial rtRT-PCR tests for BTV and published rtRT-PCR tests for EHDV. The commercial BTV rtRT-PCR kit is not readily available in the U.S., so various published assays are being used. In addition, an as-yet unpublished rtRT-PCR for detecting BTV has been developed and evaluated by the NVSL. Although experience with the BTV rtRT-PCR is considerable, protocols run at various state and federal veterinary diagnostic laboratories have not been compared.

For diagnosis and surveillance, further characterization of detected viral strains is essential, both with respect to serotype and for topotyping (i.e., genotyping for the purposes of molecular epidemiology). Serotyping traditionally has been done by neutralization tests with standard antisera, but rtRT-PCR tests have been described internationally. These require verification for use in different episystems. Further genetic characterization by sequence analysis for molecular epidemiology (such as for distinguishing isolates as being from continental North America, the Caribbean, or elsewhere) is usually performed by PCR and sequencing on a nationally agreed-upon viral RNA segment. As a minimum capability, this should be available in a designated national *Orbivirus* reference laboratory.

Advanced molecular detection methods, including next-generation sequencing and panviral DNA microarrays, have been demonstrated to be useful tools in rapid genetic characterization of viruses with segmented genomes, including BTV. Application of these methods due to costs of implementation are limited but could benefit detection of highly divergent emerging strains or species of *Orbiviruses*.

Vector Control

In North America, and for domestic animal settings in the U.S. in particular, the biting midge *Culicoides sonorensis* is regarded as the main vector of BTV and related *Orbiviruses* (Gibbs and Greiner, 1994; Tabachnick, 1996; Mellor et al., 2000). In literature prior to 2000, this species was known as *C. variipennis sonorensis*; it was elevated to species status by Holbrook et al. (2000). This midge was incriminated as a transmitter of BTV over 50 years ago (Foster et al., 1963), and subsequent field and laboratory studies have confirmed and built on that foundation. Because this is one of the very few species in the genus that has been colonized (Hunt et al., 1999), *C. sonorensis* also has been the subject of many laboratory studies on aspects such as vector competence for *Orbivirus* strains, whether known or unknown from the U.S.

This vector species also is regarded as an inhabitant of manure-polluted, open, silty mud at the margins of habitats such as dairy wastewater ponds (Mullens and Rodriguez, 1988; Tabachnick, 1996). This is not entirely true, as larvae of this species can be found in many clean habitats such as the edges of desert mountain streams in Southern California (Mullens, personal observation). However, the success of this species in polluted waters is quite remarkable; the most productive wastewater ponds can contain densities in the range of 10,000 larvae per 30 ml of shoreline mud (Mullens and Rodriguez, 1988). People or aggregations of domestic or sometimes wild ruminants typically tend to create the conditions favoring large populations associated with virus transmission by this species.

Europe recently experienced a hard lesson in how rapidly a new vector-virus association can appear when *C. obsoletus* and *C. pulicaris*-group midges initiated persistent and multi-year transmission of BTV-8 in areas of Northern Europe that were previously free of infection (Carpenter et al., 2009). Something similar could happen here, and a recent dramatic increase has occurred in detection and possible transmission of new virus serotypes in parts of the U.S. (e.g., Deep South, Upper Midwest; Gibbs et al., 2008). In some of these areas, *Culicoides* species other than *sonorensis* already are strongly suspected as vectors (Smith and Stallknecht, 1996; Becker et al., 2010).

In this report, we expose knowledge gaps critical to successful vector (and hopefully, then, disease) control. Throughout, the ideas are based on the following main points.

1. Some *C. sonorensis* management strategies exist, but most have been inadequately tested, and the techniques might not be helpful for other vector species.
2. We cannot automatically assume that *C. sonorensis* is the main, or only, vector in all regions, even if these areas are known generally to be within the geographic distribution of *C. sonorensis*.
3. Integrated control approaches require that certain proximal control approaches (e.g., animal protection using pesticides or repellents) must be supplemented by more long-term approaches to pest control that reflect continuing advances in basic knowledge of pest biology (e.g., dispersal, microbial ecology).

Methods of Culicoides *vector control*

This report is too brief to cover all methods of control. Fortunately, a fairly recent article covers much of what is known of *Culicoides* control methodology (Carpenter et al., 2008). It is useful,

however, to think of control tactics in four general categories: chemical, biological, cultural, and molecular. Integrated control often would use these in combination to reduce vectorial capacity.

1. Chemical control would involve things such as pesticide applications to the host or perhaps the environment. Example: spray ruminants with insecticides or repellents to keep adult midges from biting.

2. Biological control involves the actions of natural enemies (predators, parasites, pathogens, or competitors) to negatively affect the pest. It also includes actions of other organisms such as bacterial symbionts that can impact vectorial capacity traits such as competence for infection by an *Orbivirus*. Example: encourage or introduce mermithid parasites into new *C. sonorensis* habitats.

3. Cultural control involves altering the environment or farming methods to disfavor the pest in some way. Example: alter water source edge slopes (steeper) or nutrients (lower) to make them less favorable for *C. sonorensis*.

4. "Molecular" control involves potential alterations of the pest itself (physiology or genetic makeup), such that vectorial capacity is negatively affected. Example: elucidate and drive genes into vector populations that make them refractory to BTV infection or reduce their survival.

Vector control versus disease control

Vectorial capacity is a population-level parameter that comprehensively describes the ability of a vector population to transmit a pathogen. It absolutely operates in the context of the environment; for example, temperature has huge influence on it. Several ecological or physiological factors that govern vectorial capacity are our "points of attack" for control:

1. Biting rates (reduce vector density or rates of attack on the host of concern);
2. Vector competence (reduce susceptibility of the vector to infection by the *Orbivirus*); and
3. Survival (reduce longevity of the vector because *Orbiviruses* require a period of infection/replication before they can be transmitted).

It is vital to keep in mind that we are not especially interested in vector control *per se*. Rather, our goal is to reduce or eliminate disease agent transmission. This is a related, but separate, question. It is also probably much harder to achieve.

Gaps and recommendations

The vector control breakout group decided that the best approach for reporting gaps and recommendations was to provide general actions to correct key deficiencies in our vector control knowledge base. Several gaps are linked. Approaches are listed from more proximal to more ultimate, recognizing that BOTH types of contributions are critical to long-term success.

Gap	Recommendation
Too little information is available on effectiveness of pesticides/repellents applied to ruminants for biting vector protection, particularly including their value in preventing pathogen transmission.	Conduct more experimental on-animal field testing, particularly in areas of active *Orbivirus* transmission. Structure studies that include controls and are long-term enough to determine whether the interventions will both reduce vector biting and impact pathogen transmission.
We lack information on how, when, and in what combinations such animal-applied control materials should be tuned against particular target vectors; for example, when during the day/night and where on the body (dorsal, ventral etc.) does a particular target vector bite, or what are their host preferences?	Conduct more field and laboratory evaluations to study host attack behavior and identify better materials (including toxicants, repellents, kairomones, etc.) that can be used to reduce biting rates. These ultimately would be incorporated in field tests as above.
Many methods, even those for *C. sonorensis* in defined, man-made habitats such as dairy wastewater ponds, have never been tested outside the initial studies, or for their ultimate effects on ambient adult (biting) populations.	Conduct scientifically controlled field studies to determine if manipulations such as water source elimination or modification, parasite introduction, etc. actually cause the desired level of vector (and disease) suppression.
For most parts of the U.S., we still lack information on what is attacking large ruminants and objective assessments of which vector species are responsible for *Orbivirus* transmission; this is especially true in wildlife habitats.	Utilize bait animals and blood meal identification of field-collected midges to determine which are biting ruminants in good numbers. Simultaneously use traps to establish some relationship between easier-to-use monitoring tools (traps) and predicted animal biting rates. Encourage targeted surveys in regions of particular interest for expanding *Orbivirus* ranges (e.g., Upper Midwest for EHDV, Deep South for BTV).
Our knowledge of immature *Culicoides* habitats is extremely poor, and it is the abundance and nature of such habitats that most determine vector distributions and densities in nature. If we don't know where they are coming from or what the immatures need, our integrated management capabilities are severely limited.	Conduct field surveys and adjunct laboratory testing to determine where developmental sites of key vector suspects occur. Once found, study the biological attributes of those habitats, with attention to biotic (e.g., coexisting macro and microorganisms) and abiotic features (e.g., moisture level, pH, or chemical description) that may be amenable to manipulation.
We still don't understand enough about how *Orbiviruses* utilize their vector hosts, or how	Explore genetic mechanisms responsible for hematophagy, reproduction, and immunity and

vector immunity and physiology might be manipulated to disfavor this for control. We also lack information on the microbial ecology of the vectors, an area that is expanding rapidly in other vector-disease systems.	link those to studies of vector competence and vectorial capacity. Survey natural field populations for microbial associates that might subsequently be tested for impact on vectorial capacity traits.
We do not yet fully understand the physiological and genetic bases of vector competence, especially midgut barriers to infection. These require intensified investigation, both for *C. sonorensis* and other species, and mechanisms only now are becoming resolvable using modern molecular techniques and genomics.	Experimentally investigate physiological and genetic mechanisms related to vector competence and midgut infection, hopefully exploiting the *C. sonorensis* genome. Specifically, try to address similar concerns in alternate *Culicoides* species, which may involve intensified efforts toward laboratory colonization of those species.
We have almost no field data on how low we would need to get vector populations to actually reduce disease agent transmission. We need targets to guide control efforts.	Conduct field trials, probably with sentinel ruminants and careful vector monitoring, in areas of *Orbivirus* transmission, to relate vector activity and virus infection level (entomological inoculation rates) to transmission. Initially these would be correlational studies (time series), but prospective field studies that incorporate designed experimental vector control efforts are also needed.
Critical aspects of adult vector ecology that relate directly to control possibilities are lacking. These include adult resting site selection (where adult midges spend almost all their time when not searching for blood or oviposition sites), role of sugar feeding, mate and mating site location, how oviposition sites are found and utilized, or most aspects of dispersal.	Conduct field surveys to determine adult resting sites (may vary seasonally and have a role in virus overwintering), use and location of natural sugar sources (may be helpful in designing sugar bait control approaches), where and when adults mate or lay eggs, and how far and quickly they disperse. The latter is proximally important for evaluating local spread of disease or effectiveness of control efforts and ultimately for gaining a better understanding of potential for long-distance spread (e.g., via wind-aided dispersal of infected midges).

Vaccines

Effective immunological prophylaxis for the control of *Orbiviruses* is a complex problem facing animal health authorities worldwide and therefore requires significant background information before an assessment of available vaccines and vaccine technologies can be completed and understood. The following section provides specific information on the history and breakthroughs in *Orbivirus* vaccine development and a detailed analysis of available commercial and experimental vaccines.

History of Orbivirus *vaccine development*

An attenuated, sheep-adapted monovalent BTV vaccine was developed and first used in 1906 in South Africa by Sir Arnold Theiler (Theiler, 1908). By the 1950s, multiple BTV serotypes were isolated and attenuated as live-attenuated (modified live virus (MLV)) vaccines by serial passage in embryonated chicken eggs (Alexander and Haig, 1951). The attenuation process was later modified to include plaque selection (purification) and propagation in cell culture rather than in embryonated eggs, as reviewed by Dungu and colleagues (Dungu et al., 2004a; Dungu et al., 2004b). These attenuated MLV vaccines have been produced and used for many years in Southern Africa, and a polyvalent vaccine containing some 15 serotypes was eventually developed. The current MLV vaccine formulation used in Southern Africa delivers a series of three pentavalent immunizations (five serotypes per immunization) individually administered at three-week intervals, although immunity to all serotypes is incomplete (when delivered as a combination vaccine)(Dungu et al., 2004a; Dungu et al., 2004b). Similar MLV vaccines have been produced and used since the 1950s in the United States and Israel. The embryonated egg-passaged BTV vaccine that was originally developed in California in the 1950s (McKercher et al., 1957) was withdrawn from the marketplace some 20 years later because of the ability of the vaccine virus to cross the placenta and induce teratogenic defects in fetal ruminants (Shultz and DeLay, 1955; Maclachlan et al., 2000; MacLachlan and Osburn, 2008). Attenuated South African vaccine viruses were used briefly in portions of the Mediterranean basin following the incursion of BTV to the region in the late 1990s (Savini et al., 2008), but there were serious concerns related to potential reversion to virulence of these vaccines as well as their documented potential to be abortigenic and naturally transmitted by vector midges and reassort gene segments with wild-type BTV in the field (Batten et al., 2008; Ferrari et al., 2005; Savini et al., 2008: Savini et al., 2013). Although these vaccines were generally efficacious and effective, safety concerns led to development and use throughout much of Europe of inactivated vaccines to several BTV serotypes during the recent BT epidemic, notably serotype 8 after incursion into Northern Europe (Savini et al., 2008; Zientara and Sanchez-Vizcaino, 2013).

Vaccine formulations

Several combinations of attenuated BTVs have been used for attenuated vaccines; in fact, the history of using multivalent vaccines in Africa is long. Although these vaccines have a history of generating effective immunity, concerns regarding safety are considerable (as described above). One formulational limitation has been vaccine interference resulting from using multiple fractions of different serotypes in the same vaccine. When the number of serotypes included in one dose exceeds five, incomplete immunity may result; therefore, the more clinically effective BTV attenuated vaccines contain five or fewer serotypes.

These vaccines are prepared as lyophilized, standardized formulations of viable virus that are rehydrated just prior to use. The attenuated vaccines are relatively inexpensive to manufacture. One dose is protective against the homologous serotype of BTV. The vaccine viruses do replicate in sheep and cattle, so midges may become infected when feeding on vaccinated animals.

Inactivated viral antigen stocks are generated by multiple processes, including formalin, beta-propriolactone, binary ethylenimine, and irradiation. All of these methods have been used successfully, although incomplete inactivation has been reported.

Multiple traditional adjuvants have been used that are similar to what is used in other sheep and cattle vaccines. The manufacture of inactivated vaccine is relatively expensive and requires use of adjuvants and large doses of viral antigen. Inactivated formulations require two doses of vaccine to stimulate stable protective antibody responses.

Recombinant virus-like particles have been used to generate single virus proteins (VP2), core-like (single-coat), and virus-like particle (VLP, double-coat) structures. High doses of VP2 are protective in sheep, but combinations of VP2 and VP5 proteins require a lower dose of antigen for protection. Divalent to pentavalent combinations of VLPs have been tested. These VLP suspensions in adjuvant are protective and even provide some cross-serotype protection. The recombinant BTV proteins are produced in baculovirus-based systems and have been produced on a very large scale. Similarly, recombinant vector-expressed vaccines that utilize pox and other viruses to express VP2, with or without VP5, can induce protective immunity against BTV infection of livestock.

Differentiating infected from vaccinated animals

Development of vaccines engineered to DIVA and their companion diagnostic test for any of the vaccines described above is incomplete. With traditional attenuated vaccines, such development may be difficult, but new-generation vaccines using combinations of antigens (such as VLPs) clearly offer the advantage of DIVA capability, considering use of infection-associated antibody responses to core structural proteins such as VP7, for instance.

Immunogenicity and efficacy—vaccine-associated immunity

Vaccine-induced and convalescent, infection-driven immune responses to *Orbiviruses* include neutralizing antibody responses (directed at the VP2 protein) and non-neutralizing antibody to other structural and nonstructural viral proteins. The neutralizing antigenic determinants of *Orbiviruses* reside on the VP2 outer capsid protein, but the other outer capsid protein (VP5) can influence the conformational nature of individual epitopes on VP2 (DeMaula et al., 2000; MacLachlan et al., 1992; Rossitto and MacLachlan, 1992; White and Eaton, 1990). The different serotypes of BTV segregate into clusters that sometimes demonstrate at least partial cross-neutralization by sera from naturally exposed animals. The degree of cross-recognition may increase with subsequent exposure of animals to multiple serotypes of BTV (Dungu et al., 2004a; Dungu et al., 2004b; Heidner et al., 1990; MacLachlan et al., 1992; Thomas, 1985). In addition to humoral immune responses to BTV, there is also stimulation of multiple classes of T-lymphocytes that generate cytokines, interferons, and chemokines that regulate and facilitate effective immunological maturation and memory (MAPA, 2006; Perez de Diego et al., 2012;

Savini et al., 2006a; Stott et al., 1985; Stott et al., 1990). These T-lymphocytes include regulatory (helper) and effector (killer cell) populations. Further study of this aspect of host responses to orbiviral infections is warranted to allow continued improvement of vaccines and to potentially overcome the inherent problem of serotype specificity of protective immunity. Furthermore, additional characterization of the response of dermal macrophages and plasmacytoid dendritic cells as part of the early pathogenesis of *Orbivirus* infection is warranted. Early host responses mediated by these cells (Type I interferons, chemokines, and other cytokines) has been demonstrated (Chauveau et al., 2012; Darpel et al., 2012; Ruscanu et al., 2013; Ruscanu et al., 2012). One important consideration of the host immune response to vaccination is that immunization should reduce the extent and duration of peak viremia to prevent infection of feeding midges, which essentially prevents transmission of virus to susceptible animals (Savini et al., 2006b).

Limitations of vaccine-associated immunity and clinical use

Three major concerns are associated with the use of BTV vaccines. These concerns are, in part, technical issues associated with the biology of the disease agent-host interaction and/or a need for additional research and development.

1. The onset of immunity with any particular vaccine formulation must be defined. The rapid antibody response kinetics and field protection observed with both inactivated and attenuated vaccines suggests a very reasonable and acceptable onset of immunity. But clinical studies to determine the onset of immunity of inactivated and attenuated vaccines could be useful in constructing immunization programs in the field. This is particularly important in the case of a vector-borne disease where the *Culicoides* vectors may spread disease (in terms of distance of spread and numbers of animal exposures) very rapidly.

2. Immunity to BTV is immunologically complex. With both attenuated and inactivated vaccines, sustained protection from viremia, specificity of virus-neutralizing antibody responses, and field effectiveness are effectively serotype-specific. Antibody and cellular interactions with critical viral structures require interactions with a complex set of linear and conformational epitopes. This problem will require continued research to define the nature of protective antigen structures and development of unique formulations and methods for delivery. This research will also define better serological and cellular assays as correlates of protective immunity.

3. On a global basis, support for developing BTV vaccines has been lacking. Most regions of the world deal with endemic disease with few or no attempts to vaccinate susceptible animals. Occasional bursts of disease activity revive some interest in related research. Funding is required to provide for developmental research to improve vaccine efficacy and safety profiles, which will in turn increase the availability of relevant vaccines. The likelihood of future disease outbreaks is high, especially with climate change and increased global commerce.

Vaccine availability and use in the United States

Modified live vaccines. A limited number of modified live attenuated vaccines for BTV are available in the U.S. The only vaccine approved for national use is against serotype 10 and is

produced by the Colorado Serum Company. Attenuated vaccines against BTV serotypes 10, 11, and 13 are produced on behalf of the California Wool Growers by Poultry Health Laboratories; use of these vaccines is limited to sheep in California. These vaccines are capable of generating an effective immune response with one dose and are effective in preventing clinical BT disease. Using attenuated BTV vaccines in livestock carries numerous potential adverse consequences, including reduced milk production in lactating ewes as well as abortion, early embryonic death, and teratogenesis when used in pregnant females. The risk of spread through vectors with reversion to virulence and gene reassortment is considerable. The combination of perceived efficacy issues (cross-serotype protection and incomplete immunity) and safety issues (reversion to virulence, incomplete attenuation, and vector spread with gene reassortment) also contribute to less than enthusiastic use of the attenuated vaccines.

Inactivated vaccines. Licensed inactivated vaccines have not been available in the United States, presumably because the estimated market has been small.

Autogenous vaccines. Autogenous vaccines have been produced using inactivated BTV and EHDV. These vaccines have been used extensively in sheep and the captive cervid industry with mixed reports of effectiveness. No published peer-reviewed data are available for evaluation of these autogenous vaccines, most of which contain multiple serotypes of EHDV and BTV as well as mixed bacterial fractions/toxoids. Although the autogenous vaccines are perceived to be relatively safe, efficacy and effectiveness are questionable at best.

Vaccine availability and current use in international markets

Japan. Both attenuated and inactivated vaccines are commercially available in Japan for Ibaraki Disease, which is caused by EHDV serotype 2, formerly serotype 7. Because EHDV-2 is clearly present in North America, use of similar vaccines could be considered in the U.S. Ibaraki, although a distinct strain, is a typical EHDV and is virulent in cattle and capable of causing fatal infections.

Europe. Use of attenuated vaccines in the Mediterranean basin is currently limited and mostly in sheep and goats. The inactivated BTV-1 and BTV-8 vaccines that were used successfully circa 2008–2010 are not currently in production.

Africa. The traditional multivalent vaccines are produced and available.

Vaccine research for new-generation BTV vaccines

Several experimental recombinant vaccines have been described, and they clearly have numerous inherent potential benefits, including rapid onset of immunity, lack of transmissibility, potential for DIVA, and even a polyvalent strategy. A recombinant vaccinia virus that expressed both VP2 and VP5 of Australian BTV serotype 1 induced variable titers of neutralizing antibody in sheep and afforded protection against homologous challenge (Lobato et al., 1997). There are reports of other similar experimental vaccines (Calvo-Pinilla et al., 2009). Both virus-like and core-like particle vaccine candidates have been efficacious (Lourenco and Roy, 2011; Perez de Diego et al., 2011; Stewart et al., 2010; Stewart et al., 2012; Stewart et al., 2013). A recombinant capripoxvirus expressing VP7 was shown to provide partial protection against heterologous BTV challenge (Perrin et al., 2007; Wade-Evans et al., 1996), but like the recombinant vaccinia BTV vaccine, its development was not continued. Finally, a recombinant canarypox virus-VP2/VP5

vaccine was recently described that induced highly effective protective immunity in sheep (Boone et al., 2007). This vaccine has a major inherent advantage in that the existing VP7 competitive ELISA assay would be DIVA compatible, and utilizes an expression vector that is incorporated in several vaccines already in use in the EU and elsewhere. The vaccine is in development. Other recombinant viruses have been evaluated experimentally for antigen delivery of BTV antigens (Ma et al., 2012).

Other vaccine candidates are in developmental research evaluation stages. They include replication-deficient monovalent and multivalent viruses, combinatorial subunit antigens for prime-boost delivery, VLP vaccines using cowpea mosaic virus capsid proteins and multiple recombinant subunits in adjuvants, recombinant antigens as subunit vaccines as well as expressed in plants (Anderson et al., 2013; Calvo-Pinilla et al., 2012; Calvo-Pinilla et al., 2009; Celma et al., 2013; Jabbar et al., 2013; Thuenemann et al., 2013). Continued research and development of these approaches is warranted, particularly if they offer solutions to issues such as cross-serotype protection, DIVA compatibility, non-replicating antigens (no subsequent transmission of viral RNA), and rapid onset of immunity with one dose of vaccine.

Summary of current status and obstacles to vaccinating against Orbiviruses

1. Although a limited number of vaccines are available internationally for BTV, none are for EHDV. Availability issues for inactivated BTV vaccines are substantial because some are no longer are produced commercially (e.g., those to BTV serotype 8).

2. Autogenous vaccines have been used in the captive cervid industry to immunize deer against EHDV infection. Success has been limited at best. No peer-reviewed objective data are available to assess immunogenicity, efficacy, or effectiveness.

3. A focused effort to identify potential master seedstocks of North American serotypes of BTV and EHDV should be initiated; inactivated vaccines have been produced against only a limited number of BTV serotypes, and revamping production of an existing commercial vaccine can take several months, but creation of an entirely new one can take 2 years or longer, so the presence of available seedstocks for all 26 serotypes of BTV and all 9 serotypes of EHDV would potentially expedite creation of new vaccines.

4. Attenuated, modified-live vaccines have significant safety issues associated with their dissemination by insect vectors and reassortment of genes with those of circulating wild-type virus in the field, vertical transmission, and inherent issues related to either under- or over-attenuation of the vaccine virus.

5. Inactivated vaccines provide only serotype-specific protection but are reasonably efficacious and safe. Because of the safety concerns associated with using attenuated vaccines, they may be the best option in the face of an epizootic emergency.

6. Current inactivated vaccines require two doses and do not typically provide “sterilizing immunity;” that is, current vaccines may not prevent virus transmission following infection.

7. Inactivated vaccines are more costly to produce.

8. Neither inactivated nor live-attenuated BTV vaccines are DIVA-compatible, whereas new-generation products could be (similarly for EHDV).

9. Standardization of diagnostic procedures within diagnostic laboratories and establishment of routine surveillance are critical components of any *Orbivirus* control program.

Assessment of commercial vaccines

Vaccination is currently central to the response of most at-risk countries to any BT outbreak (see Appendix X.) Vaccination, however, can be problematic given the plurality of BTV serotypes, coupled with apparent serotype-specific immunity in livestock; thus, effective vaccines potentially must be developed for all 26 currently recognized BTV serotypes. Furthermore, the lack of choice in currently available commercial vaccines for BTV is glaring. Live, attenuated MLV vaccines are routinely used to prevent BT among sheep in the United States, South Africa, and Israel, and MLV vaccines were used for compulsory vaccination of cattle and sheep in Italy following incursion of BTV into that country in 1999. MLV vaccine viruses clearly can be acquired and transmitted by insect vectors, then circulate as field strains, and they can reassort gene segments with field viruses to generate novel progeny. MLV vaccines also have the capacity to cross the placenta to infect the fetus.

Inactivated BTV vaccines, which were not commercially available at the beginning of the European epizootic, enjoy several potential advantages over MLV vaccines. Specifically, inactivated vaccines cannot revert to virulence, reassort genes with field or MLV viruses, or cross the placenta to cause reproductive losses. Inactivated vaccines were used exclusively in response to the outbreak of BTV-8 in Europe; however, inactivated vaccines suffer from relatively slow onset of immunity compared with MLV vaccines and the lack of commercial products for most serotypes.

Use of attenuated vaccines should be avoided if possible, although these are the only vaccines available currently in the United States and their use to protect against serotypes 10, 11, and 17 may need to continue for the near future in sheep. These vaccines do provide good protection from clinical disease with homologous serotype infections. No currently licensed vaccines are available for EHDV in the United States.

Assessment of experimental vaccines

New-generation products such as baculovirus-expressed virus-like particles (VLPs) and recombinant vectored vaccines, including a canarypox virus recombinant expressing the VP2 and VP5 outer capsid proteins of BTV, have been shown to be effective experimentally, but their inherent cost and limited market potential have prevented their commercial use to date. Baculovirus-expressed subunit vaccination strategies are clearly viable for BTV because the neutralization epitopes are clustered on VP2, although the expression of immunogenic VP2 can be challenging given the conformational nature of individual epitopes. See Appendix S.

If rapid development of a vaccine is needed to combat a disease outbreak with a new and emerging strain, development of an inactivated virus vaccine in a conventional adjuvanted formulation will be required. With two doses of vaccine, inactivated vaccines can provide substantial immunity to the epizootic serotype. This strategy is similar to that used in the 2006–2008 BTV-8 outbreak in Northern Europe, which provided vaccine to the field by 2008.

Exploration and development of regulatory mechanisms to deploy such vaccines in an emergency situation are needed. Future vaccine approaches are described in the research needs section that follows the Recommendations below.

Disinfectants

Spray disinfecting solutions are available to disinfect and clean walls, ceilings, floors, decks, container surfaces, vehicles, wheels, waterproof footwear (such as rubber boots), livestock equipment, utensils, and instruments (see Table 2). Instruments and other small items may be submersed in respective solutions. Specific instructions for mixing and applying solution and appropriate safety precautions should be followed per the manufacturers' recommendations and specific Material Safety Data information.

Table 2: *Orbivirus* disinfectants available now

Disinfectants	Application	Development status	Capability	Utilization
Quaternary ammonium compounds (Roccal D Plus@) Didecyl dimethyl ammonium chloride, 9.2% Alkyl dimethyl benzyl ammonium chloride, 9.2% Alkyl dimethyl benzyl ammonium chloride, 4.6% bis-n-tributyltin oxide, 1.0% Inert Ingredients, 76.0%	Surface	Available	Facility surfaces, working surfaces, equipment	Surface disinfection, tyically 15–20 minutes
Alcohol, 70% ethanol in water	Surface	Available	Facility surfaces, working surfaces, equipment	Surface disinfection, typically 15– 20 minutes
Virkon-S@ Potassium peroxymonosulfate, 20.4% Sodium chloride, 1.5% Other ingredients, 78.1% Total, 100% Equivalent to 9.75% available chlorine Powder, used as a 1% solution in water	Surface	Available	Facility surfaces, working surfaces, equipment	Surface disinfection, typically 15–20 minutes
Laundry chlorine bleach, 10% in water	Surface	Available	Facility surfaces, working surfaces, equipment	Surface disinfection, typically 15–20 minutes

CONCLUSIONS AND RECOMMENDATIONS

Endemic or exotic BT and EHD outbreaks are recognized as significant threats to animal agriculture and wildlife. Significant BT and EHD outbreaks have occured in North America in the last decade. These events have affected domestic livestock and WTD populations, resulting in both death and production losses. Similar to the recent outbreaks of BT disease caused by BTV-8 in Europe (2006–2008), these viruses can cause extreme clinical diseases with losses due to morbidity, mortality, and reproductive failure in cattle and sheep. In regions where infection is endemic, however, the clinical disease caused by BTV is far more subtle, especially in cattle. Obscure clinical signs are sometimes difficult to recognize, and acute and chronic forms of infection are observed. In the more subtle forms of infection, the losses are somewhat insidious but can be substantial. In North America, epizootic outbreaks are more common in sheep in the Western states. The Northeastern section of the U.S. has generally been free from BTV infection. Seroconversion of exposed animals has economic consequences in that it restricts export market access for seedstock and semen. The livestock industry would benefit from improved and standardized diagnostic assays, improved surveillance, improved vector control methods, and availability of effective vaccines and immunization programs.

With EHDV infection, when large-scale epizootics occur in deer, there may be some spillover effect in cattle that can result in acute clinical disease that is much like clinical BT disease. Infection of WTD is endemic in the Southern part of the U.S., and large-scale death loss is less common in this area. Why large-scale outbreaks of hemorrhagic disease occur in WTD populations in some years (e.g., 2007, 2012) is not well understood, but drought, vector density, and relative susceptibility of regional populations may play a role. The captive cervid industry in the U.S. is large and growing. In years with widespread epizootics of either EHDV or BTV infections in wild WTD, these infections may also cause severe losses among the captive herds. Losses due to extreme mortality and chronic infections may occur.

Orbivirus infections constitute a significant threat to the livestock industry in the United States. Workshop participants recommend the following actions to employ the best control and intervention countermeasures to prevent or respond to a disease outbreak caused by a new and emerging *Orbivirus*.

Immediate Response Countermeasures

Diagnostics

- Adopt standardized diagnostic tests that are readily available for BT/EHD viruses and described in the OIE Manual. The diagnostic approach to these viruses varies considerably. Commercial BTV ELISA test kits are available, and many laboratories are experienced in running these assays. The National Veterinary Services Laboratories perform laboratory evaluation for BTV and EHDV serology annually.

- For detection of viral RNA, standard or rtRT-PCR should be used for screening samples prior to virus isolation. The preferred diagnostic test method for detection of the presence of BTV or EHD is rtRT/PCR. Published and commercial rtRT-PCR tests are available for BTV and published rtRT-PCR tests are available for EHDV. The commercial BTV rtRT-

PCR kit is not readily available in the U.S., so various published assays are being used. An unpublished rtRT-PCR (rtRT-PCR) for detecting BTV also has been developed and evaluated by the NVSL. Although experience with the BTV rtRT-PCR is considerable, protocols run at various state and federal veterinary diagnostic laboratories have not been compared.

- Genotyping of *Orbivirus* isolates from either outbreak diagnosis or active surveillance is an essential component of *Orbivirus* characterization. A national laboratory or laboratories should be designated to undertake this work on viruses isolated nationally every year with the intention of mapping the changing pattern of genotypes over time and detecting the occurrence of new genotypes (possible exotic incursions) and new reassortants.

Vector control

Control methods can be summarized in four general categories: chemical, biological, cultural, and molecular. Integrated control often would use these in combination to reduce vectorial capacity.

- Chemical control: spray ruminants with insecticides or repellents to keep adult midges from biting during peak vector cycles.
- Biological control: introduce mermithid parasites into new *C. sonorensis* habitats.
- Alter the environment or farming methods to disfavor the pest in some way, such as by altering water source edge slopes (steeper) or nutrients (lower) to make them less favorable for *C. sonorensis*. This would include management of watering equipment and systems to minimize the development of midge larvae.

Vaccines

- Use of attenuated vaccines should be avoided if possible, although these are the only vaccines available currently in the U.S. Therefore, their use in the near future may need to continue to protect sheep against serotypes 10, 11, and 17. These vaccines provide good protection from clinical disease with homologous serotype infections. No currently licensed vaccines are available for EHDV in the U.S.
- If rapid development of a vaccine is necessary to meet an emerging crisis, development of an inactivated virus vaccine in a conventional adjuvanted formulation is recommended. With two doses of vaccine, inactivated vaccines can provide substantial immunity to the epizootic serotype. This strategy is similar to that used in the 2006–2008 BTV-8 outbreak in Northern Europe, which provided vaccine to the field by 2008. Exploration and development of regulatory mechanisms to deploy such vaccines in an emergency situation are needed.
- New experimental vaccine approaches to provide alternative strategies should be developed.

Research to Address Gaps in Scientific Knowledge

The gaps in our scientific knowledge and available countermeasures to control a disease outbreak have significant gaps and require improvements that can be achieved only through a

coordinated research agenda. Workshop participants recommend the implementation of the following research objectives to advance our knowledge of *Orbiviruses* and the availability of effective countermeasures.

Virology

A better understanding of the basic virology of BT and the other *Orbiviruses* will help to underpin all of the discussion topics that were included in the gap analysis meeting (vaccines, pathology, immunology, entomology, and epidemiology). The virology data generated will also help us to model current and future disease outbreaks and understand the global risks we face from BTV, EHDV, AHSV, EEV, and any of the 23 other *Orbivirus* species, or additional novel species, that have the potential to emerge and threaten animal health and productivity, national or international livestock trade, and even human health.

Specific research goals include:

- Replication cycles and virus assembly;
- The putative receptors and attachment mechanisms;
- The genetic variability and molecular basis for antigenic variation between different *Orbivirus* species, serotypes, and topotypes;
- The existence, characteristics, and distribution of novel *Orbivirus* species and serotypes;
- The molecular mechanisms involved in genome segment selection, packaging, and reassortment and their significance in genetic shifting and emergence of novel strains;
- The viral factors modulating adaptive and innate immune responses to the virus (and the antigens or RNAs involved);
- How immune responses protect the host against infection and onward transmission;
- How the *Orbiviruses* infect and spread, and the mechanisms by which they cause clinical disease within susceptible vertebrate hosts;
- Determinants of host range (which species, breeds, or sub-populations of host species are susceptible to infection and which exhibit clinical signs of disease);
- The mechanisms by which *Orbiviruses* infect, disseminate within, and are spread by their arthropod vector species;
- The response of arthropod vector species to *Orbivirus* infection, related to onward transmission;
- Determinants of vector range (which potential insect vector species or populations are susceptible to infection and can effectively transmit the virus—are there differences between different virus strains?);
- Determinants of virulence (which genes, domains, epitopes) and how the host environment (vertebrate vs. invertebrate) affects them;
- The potential and mechanisms involved in transmission by other “non-vector” routes (e.g., vertical, horizontal, mechanical, or oral transmission);
- Molecular markers (e.g., from full-genome sequence data) that can be developed and used to track virus strain movements (molecular epidemiology); and
- Development of refined/next-generation diagnostic systems (e.g., array-based diagnostics, next-generation sequencing and metagenomics, RT-PCR assays, monoplex or multiplex ELISA systems, “bead”-based assays).

Diagnostics

Continued development of better serological and molecular tools as well as diagnostic approaches will improve the accuracy and power of both disease diagnostic and epidemiological surveillance efforts. The following research goals should be realized to achieve needed improvements.

- Comparison of commercial, published, and new BTV rtRT-PCR assays;
- Optimization, evaluation, and validation of the BTV, EHDV single-plex or multi-plex rtRT-PCR assays using standard protocol(s) and instrumentation in the National Animal Health Laboratory Network (NAHLN);
- Optimization, evaluation, and validation of the recombinant antigen-based ELISA antibody test kits;
- Development of a pen-side test with a commercial manufacturer;
- Development and validation of DIVA companion diagnostic test kit for new vaccines when available;
- Optimization of *Orbivirus* genetic characterization (deep sequencing, DNA microarrays, or RT-PCR arrays); and
- Validation, standardization, and availability of BTV PCR assays: The technology exists for these tests, and reliable and robust real-time PCR assays are used elsewhere in the world and sporadically in the U.S., but diagnostic laboratory standardization and initiation of routine surveillance using these reliable assays is a critical need. Validation, standardization, and availability of EHDV serology and PCR assays: There is a critical need for continued research to develop diagnostic assays and subsequent diagnostic laboratory standardization and initiation of routine surveillance.

Epidemiology

To more effectively understand the processes of vector biology, environmental maintenance of the viruses, disease transmission, and disease pathogenesis, the following objectives should be completed.

- Determine the pattern of ecosystems supporting the arthropod transmission of BTV and EHDV in different climatic/geographic zones.
    - Determine distribution and diversity of virus serotypes, molecular subtypes, and subgroups within the United States.
    - Determine the true endemic, epidemic, and incursive zones.
    - Determine overwintering mechanisms of viruses and the importance of this in delineating the zones.
    - Determine the transition zone between endemic and non-endemic zones using serological survey followed by establishment of sentinel herds.
    - Determine where virus could be introduced each year and where it could persist.
    - Identify the mechanisms through which viruses expand their range to identify

mechanisms of control, which might include infected windborne vectors, movement of infected ruminant hosts, movement of virus from overwintering areas, and examining alternate host-vector pathways to enable virus persistence in different ecological areas.
    - Establish the current distribution of competent vectors and viruses (BTV and EHDV) in the United States:
        - Identify hematophagous arthropods feeding on susceptible hosts in various regions of the U.S.
        - Conduct laboratory studies on vector competence.
        - Conduct long-term studies on vector population dynamics in various regions of the United States and filling gaps in biology of vector species.
    - Provide training and support for arthropod identification:
        - Develop identification guides based on collections in Florida State Arthropod Collection.
        - Identify molecular barcode of vectors.
    - Perform ecological niche modeling.

- Determine the factors influencing the expression and incidence of clinical disease within these zones for all potential hosts:

    - Determine the impact of animal movement in or out of zones on disease expression in relation to virus diversity.
    - Determine the effect of local and regional weather patterns and climatic conditions on outbreaks and incidence.
    - Develop and evaluate potential vaccination and vector control strategies for routine management and outbreak response.

Vector control

As more complete information becomes available regarding the biology of the midge vectors of BTV and EHDV, the chemical, biological, and molecular control of the vector should reduce the exposure of susceptible animals to virus infection. To achieve more effective control of the vector, the following research work (including evaluation of experimental control techniques) should be completed.

- Conduct more experimental on-animal field testing, particularly in areas of active *Orbivirus* transmission. Structure studies that include controls and are long-term enough to determine whether the interventions will both reduce vector biting and impact pathogen transmission.

- Conduct more field and laboratory evaluations to study host attack behavior and identify better materials (including toxicants, repellents, kairomones, etc.) that can be used to reduce biting rates, which ultimately would be incorporated in field tests as above.

- Conduct scientifically controlled field studies to determine if manipulations such as water source elimination or modification, parasite introduction, etc., actually cause the desired level of vector (and disease) suppression.

- Utilize bait animals and blood meal identification of field-collected midges to determine which are biting ruminants in good numbers. Simultaneously use traps to establish some relationship between easier-to-use monitoring tools (traps) and predicted animal biting rates. Encourage targeted surveys in regions of particular interest for expanding *Orbivirus* ranges (e.g. Upper Midwest for EHDV, Deep South for BTV).

- Conduct field surveys and adjunct laboratory testing to determine where developmental sites of key vector suspects occur. Once found, study the biological attributes of those habitats, with attention to biotic (e.g., coexisting macro and microorganisms) and abiotic features (e.g., moisture level, pH, or chemical description) that may be amenable to manipulation.

- Explore genetic mechanisms responsible for hematophagy, reproduction, and immunity, and link those to studies of vector competence and vectorial capacity. Survey natural field populations for microbial associates that might subsequently be tested for impact on vectorial capacity traits.

- Experimentally investigate physiological and genetic mechanisms related to vector competence and midgut infection, hopefully exploiting the *C. sonorensis* genome. Specifically try to address similar concerns in alternate *Culicoides* species, which may involve intensified efforts toward laboratory colonization of those species.

- Conduct field trials, probably with sentinel ruminants and careful vector monitoring in areas of *Orbivirus* transmission, to relate vector activity and virus infection level (entomological inoculation rates) to transmission. Initially these would be correlational studies (time series), but prospective field studies that incorporate designed experimental vector control efforts are also needed.

- Conduct field surveys to determine adult resting sites (may vary seasonally and have a role in virus overwintering), use and location of natural sugar sources (may be helpful in designing sugar bait control approaches), where and when adults mate or lay eggs, and how far and quickly they disperse. The latter is important proximally for evaluating local spread of disease or effectiveness of control efforts, and ultimately for gaining a better understanding of potential for long-distance spread (e.g., via wind-aided dispersal of infected midges).

Vaccines

In view of the current limitations on vaccine efficacy, delivery platforms, safety, availability, and DIVA compatability, these research recommendations should be undertaken.

1. A focused effort to identify potential master seedstocks of North American serotypes of BTV and EHDV should be initiated; inactivated vaccines have been produced against only a limited number of BTV serotypes and revamping production of an existing commercial vaccine can take several months, but creation of an entirely new one can take two years or longer, so the presence of available seedstocks to all 26 serotypes of BTV and all 7 serotypes of EHDV would potentially expedite creation of new vaccines.

2. Develop new generation vaccines for both EHDV and BTV that provide:
    - Rapid onset of immunity in the face of an emerging outbreak;
    - No transmission of vaccine virus by insect vectors (in the case of MLV vaccines);
    - Cross-serotype protection;
    - Non-replicating antigen technology; and
    - DIVA capability.

APPENDIX I: *ORBIVIRUS* GAP ANALYSIS WORKSHOP INSTRUCTIONS

Decision Model

We will use a decision model to assess potential countermeasures to control disease outbreaks caused by new and emerging *Orbiviruses*. These countermeasures include diagnostics and vaccines that must significantly improve our ability to control, and, where feasible, eradicate a disease outbreak in the United States. The decision model is a simple tool that will allow us to focus on critical criteria and rank the available interventions relative to each other. The decision model is available as a Microsoft Excel spreadsheet and has been prepared to quantitatively assess the rankings we assign to a set of selected criteria that will lead to the selection of the highest cumulative option. We can use as many criteria as we want, but the objective is to get down to the ones that will make or break success. The criteria for each intervention will be selected by the diagnostic and vaccine working groups on May 16, 2013, but a preliminary set has been identified to expedite the process.

Criteria

If a vaccine is going to be used as a emergency outbreak control tool, then we need to know: 1) is it efficacious (does it effectively eliminate virus amplification or just reduce amplification); 2) does it work rapidly with one dose (probably do not have time for a second dose); 3) is available today from the perspective of having a reliable and rapid manufacturing process (need to know it can be up and running rapidly and will yield a predictable amount of vaccine; 4) can we get the product to the outbreak site rapidly and safely; 5) once at the site, can we get it into the target population rapidly (feedlot, cow-calf segment); 6) type of administration—mass or injected, people and equipment to do the job become important); and 7) are diagnostics available to monitor success and/or DIVA-compliant. Although cost is important, the cost of the vaccine in an outbreak will be small in comparison to the other costs. In addition, how fast the product can be made is important because that will have a huge impact on how large of a stockpile will be needed. Accordingly, you will see from the Excel sheets that have been prepared for vaccines that the following critical criteria and assignment of weights for each criterion are proposed.

Weight	Critical Criteria
10	Efficacy
2	Safety
8	One dose
10	Speed of scaleup
2	Storage
6	Distribution
8	Mass administration
4	All ruminants
6	DIVA-compatible
8	Dx available
4	Cost to implement

Cyril Gerard Gay, D.V.M., Ph.D.

Senior National Program Leader, Animal Health, ARS, USDA

APPENDIX II: WORKSHOP AGENDA

Gap Analysis and Countermeasures Assessment *Orbiviruses* Workshop
May 14–16, 2013
Agenda

Introduction

Bluetongue and Epizootic Hemorrhagic Disease viruses are of concern to livestock producers in North America because of 1) the emergence of new serotypes, 2) increased reports of spillover and clinical disease in cattle, and 3) increased spread and adaptation to new geographical areas. Accordingly, the United States Animal Health Association (USAHA) passed Resolution 16 on October 2012, requesting the United States Department of Agriculture (USDA), and the United States Department of Interior (DOI) to arrange a diversified blue-ribbon panel (including industry stakeholders, university and federal researchers, federal and state regulatory agencies) to determine research needs and identify and prioritize intervention strategies.

In response to USAHA Resolution 16, the USDA, in collaboration with the DOI, organized a gap analysis workshop comprised of international experts on *Orbiviruses*. The *Orbivirus* Working Group (OVWG) will meet at the Arthropod-Borne Animal Diseases Research Unit, Manhattan, Kansas, on May 14–16, 2013, to conduct a gap analysis of the available scientific information and assess countermeasures to effectively control and mitigate the impact of an outbreak of an emerging *Orbivirus* with epizootic potential, with special emphasis given to Bluetongue virus (BTV) and Epizootic Hemorrhagic Disease (EHD) virus. The OVWG will prepare a report that will 1) define the threat, 2) provide a gap analysis of our knowledge of animal *Orbiviruses*, 3) identify priority research needs, and 4) provide an in-depth analysis of available countermeasures to contain and mitigate the threat.

Workshop Objectives

1. Gap analysis
2. Countermeasures assessment
3. Research priorities
4. Workshop Report

Venue

Arthropod-Borne Animal Diseases Research Unit, Center for Grain and Animal Health Research, 1515 College Ave, Manhattan, Kansas, 66502.

Organizing Committee

Cyril G. Gay, D.V.M., Ph.D. (Chair)
Senior National Program Leader
Animal Production and Protection
Agricultural Research Service
United States Department of Agriculture
Beltsville, Maryland
Phone: (301) 504-4786
cyril.gay@ars.usda.gov

Randall L. Levings, D.V.M., Ph.D.
National Center for Animal Health Emergency Management
Veterinary services, Animal and Plant Health Inspection Service
United States Department of Agriculture
Ames, Iowa
randall.l.levings@aphis.usda.gov

N. James MacLachlan, D.V.M., Ph.D.
Department of Pathology, Microbiology, and Immunology
Center for Vectorborne Diseases
College of Veterinary Medicine
University of California, Davis
njmaclachlan@ucdavis.edu

Scott McVey, D.V.M., Ph.D.
Arthropod-Borne Animal Diseases Research Unit
Center for Grain and Animal Health Research (CGAHR)
Agricultural Research Service
United States Department of Agriculture
Manhattan, KS
Phone: (785) 537-5561
Scott.Mcvey@ars.usda.gov

Juergen A. Richt, D.V.M., Ph.D.
Regents Distinguished Professor
Kansas State University
Diagnostic Medicine/Pathobiology Dept.
College of Veterinary Medicine
K224B Mosier Hall
Manhattan, KS 66506-5601
Phone: (785) 532-4408
jricht@vet.k-state.edu

Chris Whitehouse, Ph.D.
National Wildlife Health Center
United States Geological Survey
Department of the Interior
Phone: **(**608) 270-2460
cwhitehouse@usgs.gov

Agenda

May 14, 2013
Knowledge and Gaps

09:00–09:10
Welcome
Ralph C. Richardson, D.V.M.
Dean, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas

09:10–09:15
Thomas Shanower, Ph.D.
Director, Center for Grain and Animal Health Research, Manhattan, KS

09:15–09:30
Workshop goals, objectives, methods, and outcomes
Cyril G. Gay, D.V.M., Ph.D.
Senior National Program Leader, Animal Production and Protection, USDA-ARS

09:30–11:30
Introduction: The threat of *Orbiviruses* to animal agriculture and wildlife

09:30–10:00
Climate Change and the Emergence of Vector-Borne Animal Diseases
Timothy J. Lysyk, Ph.D.
Research Scientist, Veterinary Entomology, Agriculture and Agri-Food Canada, Alberta

10:00–10:30
Livestock Producers
Larry Hollis, D.V.M., M.Ag
Professor, Animal Sciences and Industry, Kansas State University

10:30–11:00
Break

11:00–11:30
Deer Farmers
Shawn Shafer
North American Deer Farmer's Association (NADeFA)

11:30–12:00
Wildlife
Jason Sumners, Resource Scientist
Missouri Department of Conservation (MDC)

12:00–13:00
Lunch compliments of Kansas State University

13:00–14:00
Introduction: Obstacles to preventing and controlling *Orbiviruses*

13:00–13:30
Bluetongue
Barbara Drolet, Ph.D.
Arthropod-Borne Animal Diseases Research Unit, USDA-ARS

13:30–14:00
Epizootic Hemorrhagic Disease
Mark G. Ruder, D.V.M., Ph.D.
Veterinary Medical Officer, Arthropod-Borne Animal Diseases Research Unit, USDA-ARS

14:00–17:30
Session 1: Epidemiology – Livestock and wildlife

14:00–15:00
Bluetongue Virus
Paul Gibbs, D.V.M., Ph.D.
Professor Emeritus, College of Veterinary Medicine, University of Florida

Group Discussion: Leader David Dargatz, D.V.M., Ph.D, Center for Epidemiology and Animal Health, USDA-APHIS
- Gaps and research needs

15:00–15:30
Break

15:30 – 16:30
Epizootic Hemorrhagic Disease Virus
Chris Chase, D.V.M., Ph.D.
Professor, Department of Veterinary and Biomedical Sciences, South Dakota State University

Group Discussion: Leader Mark G. Ruder, D.V.M., Ph.D., Arthropod-Borne Animal Diseases Research Unit, USDA-ARS
- Gaps and research needs

16:30–17:30
***Orbiviruses* in Wildlife**
David Stallknecht, Ph.D.
Professor, College of Veterinary Medicine, University of Georgia

Group Discussion: Leader Chris Whitehouse, Ph.D., Branch Chief of Disease Investigations, National Wildlife Health Center, United States Geological Survey
- Gaps and research needs

Adjourn

Agenda
May 15, 2013
Knowledge and Gaps

09:00–10:30
Session 2: Virology – Determinants of virulence, host range, and host-pathogen interactions

09:00–09:45
Determinants of virulence and host range
Peter Mertens, Ph.D.
Head, Vector-borne Viral Diseases, The Pirbright Institute

Group Discussion: Leader Martin Beer, D.V.M., Ph.D., Friedrich-Loeffler-Institut
- Gaps and research needs

09:45–10:30
Host-pathogen interactions
Elizabeth W. Howerth, D.V.M., Ph.D.
Professor, Pathology, College of Veterinary Medicine, University of Georgia

Group Discussion: Leader Piet A. van Rijn, Ph.D., Project Leader of Viral Exotic Diseases, Central Veterinary Institute of Wageningen
- Gaps and research needs

10:30–11:00
Break

11:00–15:00
Session 3: Diagnostics - Surveillance, response, and recovery

11:00–11:45
Bluetongue Virus (sheep, cattle, and deer)
Peter Daniels, D.V.M., Ph.D
Deputy Director, Australian Animal Health Laboratory, CSIRO

Group Discussion: Leader Barbara Drolet Ph.D., Arthropod-Borne Animal Diseases Research Unit, USDA-ARS
- Gaps and research needs

11:45–12:30
Epizootic Hemorrhagic Disease Virus (deer and cattle)
Bill Wilson, Ph.D.
Veterinary Microbiologist, Arthropod-Borne Animal Diseases Research Unit, USDA-ARS

Group Discussion: Leader Mike MacIntosh, Ph.D., Foreign Animal Diseases Diagnostic Laboratory, Plum Island Animal Disease Center, USDA-APHIS

- Gaps and research needs

12:30–13:30
Lunch compliments of Kansas State University

13:30–14:30
Session 4: Vector Control

13:30–14:30
Integrated Management and Control of Culocoides
Bradley A. Mullens, Ph.D.
Professor, Department of Entomology, University of California, Riverside

Group Discussion: Leader Lee Cohnstaedt, Ph.D., Veterinary Entomologist, Arthropod-Borne Animal Diseases Research Unit, USDA-ARS
USDA-ARS
- Gaps and research needs

14:30–15:00
Break

15:00–17:00
Session 5: Vaccines – Ideal vaccine profile, vaccinology, what's in the pipeline

15:00–16:00
Bluetongue Virus
Jim MacLachlan, D.V.M., Ph.D.
Professor, Center for Vectorborne Diseases, College of Veterinary Medicine, University of California, Davis

Group Discussion: Leader Cyril G. Gay, D.V.M., Ph.D., Livestock Protection and Protection, Office of National Programs, USDA-ARS
- Gaps and research needs

6:00–17:00
Epizootic Hemorrhagic Disease Virus
Scott McVey D.V.M., Ph.D., Research Leader**,** Arthropod-Borne Animal Diseases Research Unit, USDA-ARS

Group Discussion: Leader Cyril G. Gay, D.V.M., Ph.D., Livestock Protection and Protection, Office of National Programs, USDA-ARS
- Gaps and research needs

Adjourn – Shuttles to the hotel – Free evening – Walk to Restaurants near University Campus

Agenda
May 16, 2013
Gap Analysis and Countermeasures Assessment

09:00–15:00 (Working lunch)
Breakout Sessions – Epidemiology, Virology, Diagnostics, Vaccines, and Vector Control
Instructions: Provided by **Dr. Cyril G. Gay**
Each breakout group will review available scientific information and countermeasures, identify gaps, assess strength and weaknesses, and identify research priorities to address gaps and weaknesses. For diagnostics and vaccines, the breakout groups will use a decision model to assess potential countermeasures and determine the best tools available. These countermeasures must significantly improve our ability to control, and, when feasible, eradicate new and emerging *Orbiviruses* affecting livestock production and captive cervids. When finished with their objectives, members of each group can join and contribute to the assessment of any other group based on their expertise and core competencies.

Breakout Group 1: Epidemiology
Session leaders: Timothy Lysyk and Mark Ruder

- Transmission
- Susceptible hosts
- Livestock versus captive cervids
- Wildlife-domestic animal interphase
- Research gaps and priorities assessment

Breakout Group 2: Virology

- **Session leaders: Peter Mertens and Piet A. van Rijn**
- Functional genomics
- Determinants of virulence and host range
- Host-pathogen interactions
- Research gaps and priorities assessment

Breakout Group 3: Diagnostics

- **Session leaders: Peter Daniels, and Bill Wilson**
- Review criteria for selecting diagnostics
- Review available and new diagnostics for surveillance, response, and recovery
- Decision model analysis of available diagnostics
- Decision model analysis of experimental diagnostics
- Rank diagnostic tests
- Research gaps and priorities assessment

Breakout Group 4: Vector Control
Session leaders: Brad Mullens and Lee Cohnstaedt

- Review available pesticides
- Review new and improved pesticides in the pipeline
- Review most effective applications and delivery
- Review novel integrated pest management approaches
- Research gaps and priorities assessment

Breakout group 5: Vaccines
Session leaders: Jim MacLachlan and Scott McVey

- Review criteria for selecting vaccines
- Review list of available vaccines
- Review most promising technologies in the pipeline
- Decision model analysis of commercial and experimental vaccines
- Rank vaccines
- Research gaps and priorities assessment

15:00–16:15
Reports from Section Leaders

- **Epidemiology**
- **Virology**
- **Diagnostics**
- **Vector Control**
- **Vaccines**

16:15–16:45
Wrap up, conclusions and next steps
Cyril G. Gay, D.V.M., Ph.D
Senior National Program Leader, Animal Production and Protection, USDA-ARS

Adjourn

APPENDIX III: DIAGNOSTICS FOR *ORBIVIRUS* DETECTION

Diagnostics for *Orbivirus* Detection

Diagnostics For Orbiviruses - May 2013

Rank each Intervention (2,4,6,8, or 10) as to its importance to making a decision, only one "10" rankings allowed

Critical Criteria	B cELISA	VN	CF	E AGID	BTV AGID	BTV ELSA	E cELISA	Luminex	Pen Side	Luminex Ag	Luminex NA	Alt Amplificatio
Sensitivity	8	6	0	6	6	8	8	10	6	8	8	10
Specificity	10	6	0	4	4	8	10	6	4	4	4	4
Validation to purpose	8	8	0	8	8	8	8	2	2	2	2	2
Throughput	8	4	0	6	6	8	8	10	2	10	10	4
Deployable to NAHLN	8	1	0	8	8	8	8	2	8	2	2	2
Rapid Result	8	2	0	4	4	8	8	6	8	6	6	8
Easy to perform	8	6	0	8	8	8	8	6	10	6	6	6
Cost to Implement	6	2	0	8	8	6	8	6	8	4	4	6
Cost to Run	8	6	0	10	10	8	8	4	8	6	6	4

Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

determine - assay not developed

Critical Criteria	B cELISA	VN	CF	E AGID	BTV AGID	BTV ELSA	E cELISA	Luminex	Pen Side	Luminex Ag	Luminex NA	Alt Amplificatio
Sensitivity	80	60	0	60	60	80	80	100	60	80	80	100
Specificity	80	48	0	32	32	32	80	48	32	32	32	32
Validation to purpose	80	80	0	80	80	80	80	20	20	20	20	20
Throughput	64	32	0	48	48	64	64	80	16	80	80	32
Deployable to NAHLN	16	2	0	16	16	16	16	4	16	4	4	4
Rapid Result	64	16	0	32	32	64	64	48	64	48	48	64
Easy to perform	64	48	0	64	64	64	64	48	80	48	48	48
Cost to Implement	24	8	0	32	32	24	32	24	32	16	16	24
Value	472	294	0	364	364	424	480	124	320	328	328	324

Available in laboratories
commercially available (could be stock piled)
"pipeline" technologies in development

* = Lmited or no data available to evaluate

Major Assumptions:
Diagnostic Test Profile
1. Detect all Orbivirus strains
2. Direct tests for control and eradication
3. Indirect tests for post-control monitoring/detection subclinical cattle
4. Rapid test
5. >95% specificity
6. >95% sensitivity
7. Pen-side test
8. DIVA Compatible
9. Field validated
10. Easy to perform/easily train NAHML's personel
11. Scalable
12. Reasonable cost

APPENDIX IV: DIAGNOSTICS FOR BLUETONGE VIRUS DETECTION

Diagnostics for Bluetongue Virus Detection

Diagnostics For BTV - May 2013

Rank each Intervention (2,4,6,8, or 10) as to its importance to making a decision, only one "10" rankings allowed

Weight	Critical Criteria	VI	n RT-PCR	qRT-PCR	Typing PCR	VNT	EM	Arrays	qRT-PCR	deep Seq*	IHC/FA*	in situ Hyb*	topotyping*	multiplex B/E PCR*	Typing Array*	Luminex NA*	Alt Amplification*	Pen Side*	Luminex Ag*
10	Sensitivity	6	8	8	8	6	2	6	8	4	4	6	8	8	4	8	2	6	2
8	Specificity	10	10	10	10	4	4	8	10	8	6	8	10	10	4	4	2	6	2
10	Validation to purpose	8	8	8	8	6	6	4	6	4	2	2	8	4	2	2	2	2	2
8	Throughput	2	4	8	4	2	2	4	8	2	2	2	4	8	2	10	8	6	2
2	Deployable to NAHLN	6	2	8	2	2	2	4	8	6	6	6	2	8	2	2	2	2	8
8	Rapid Result	2	6	8	4	2	8	6	8	4	6	6	4	8	2	6	6	8	10
6	Viral characterisation	8	6	6	8	6	6	8	6	10	8	8	8	6	8	6	6	4	4
8	Easy to perform	6	6	8	6	2	4	6	8	4	6	6	6	8	2	4	4	6	8
4	Cost to Implement	4	6	4	6	2	2	4	4	4	2	2	6	4	2	6	6	4	8
4	Cost to Run	6	4	6	4	6	4	4	6	2	6	6	4	6	2	6	6	4	8

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

Critical Criteria	VI	n RT-PCR	qRT-PCR	Typing PCR	VNT	EM	Arrays	qRT-PCR	deep Seq*	IHC/FA*	in situ Hyb*	topotyping*	multiplex B/E PCR*	Typing Array*	Luminex NA*	Alt Amplification*	Pen Side*	Luminex Ag*
Sensitivity	60	80	80	80	60	20	60	80	40	40	60	80	80	40	80	20	60	20
Specificity	80	80	80	80	32	32	64	80	64	48	64	80	80	32	32	16	48	16
Validation to purpose	80	80	80	80	60	60	40	60	40	20	20	80	40	20	20	20	20	20
Throughput	16	32	64	32	16	16	32	64	16	16	16	32	64	16	80	64	48	16
Deployable to NAHLN	12	4	16	4	4	4	8	16	12	12	12	4	16	4	4	4	4	16
Rapid Result	16	48	64	32	16	64	48	64	32	48	48	32	64	16	48	48	64	80
Viral characterisation	48	36	36	48	36	36	48	36	60	48	48	48	36	48	36	36	24	24
Easy to perform	48	48	64	48	16	32	48	64	32	48	48	48	64	16	32	32	48	20
Cost to Implement	16	24	16	24	8	8	16	16	16	8	8	24	16	8	24	24	16	20
Cost to Run	24	16	24	16	24	16	16	24	8	24	24	16	24	8	24	24	16	20
Value	400	448	524	444	272	288	380	504	320	312	348	444	484	208	380	288	348	252

in use in reference laboratories
commercially available
"pipeline" technologies in development

* = Lmited or no data available to evaluate

Major Assumptions:

Diagnostic Test Profile

1. Detect all Orbivirus strains
2. Direct tests for control and eradication
3. Indirect tests for post-control monitoring/detection subclinical cattle
4. Rapid test
5. >95% specificity
6. >95% sensitivity
7. Pen-side test
8. DIVA Compatible
9. Field validated
10. Easy to perform/easily train NAHML's personel
11. Scalable
12. Reasonable cost

APPENDIX V: DIAGNOSTICS FOR EHD VIRUS DETECTION

Diagnostics for EHD Virus Detection

Diagnostics For EHDV - May 2013

Rank each Intervention (2,4,6,8, or 10) as to its importance to making a decision, only one "10" rankings allowed

Weight	Critical Criteria	VI	n RT-PCR	qRT-PCR	Typing PCR	VNT	EM	Arrays	deep Seq	IHC/FA	in situ Hyb	topotyping	multiplex B/E PCR	Typing Array*	Luminex Ag*	Luminex NA*	Alt Amplification*	Pen Side*
10	Sensitivity	6	8	8	8	6	2	6	4	4	6	8	8	4	8	2	6	2
8	Specificity	10	10	10	10	4	4	8	8	6	8	10	10	4	4	2	6	2
10	Validation to purpose	8	8	6	8	6	6	4	4	2	2	8	4	2	2	2	2	2
8	Throughput	2	4	8	4	2	2	4	2	2	2	4	8	2	10	8	6	2
2	Deployable to NAHLN	6	2	8	2	2	2	4	6	6	6	2	8	2	2	2	2	8
8	Rapid Result	2	6	8	4	2	8	6	4	6	6	4	8	2	6	6	8	10
6	Viral characterisation	8	6	6	8	6	6	8	10	8	8	8	6	8	6	6	4	4
8	Easy to perform	6	6	8	6	2	4	6	4	6	6	6	8	2	4	4	6	8
4	Cost to Implement	4	6	4	6	2	2	4	4	2	2	6	4	2	6	6	4	8
4	Cost to Run	6	4	6	4	6	4	4	2	6	6	4	6	2	6	6	4	8

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

Critical Criteria	VI	n RT-PCR	qRT-PCR	Typing PCR	VNT	EM	Arrays	deep Seq	IHC/FA	in situ Hyb	topotyping	multiplex B/E PCR	Typing Array*	Luminex Ag	Luminex NA*	Alt Amplification*	Pen Side*
Sensitivity	60	80	80	80	60	20	60	40	40	60	80	80	40	80	20	60	20
Specificity	80	80	80	80	32	32	64	64	48	64	80	80	32	32	16	48	16
Validation to purpose	80	80	60	80	60	60	40	40	20	20	80	40	20	20	20	20	20
Throughput	16	32	64	32	16	16	32	16	16	16	32	64	16	80	64	48	16
Deployable to NAHLN	12	4	16	4	4	4	8	12	12	12	4	16	4	4	4	4	16
Rapid Result	16	48	64	32	16	64	48	32	48	48	32	64	16	48	48	64	80
Viral characterisation	48	36	36	48	36	36	48	60	48	48	48	36	48	36	36	24	24
Easy to perform	48	48	64	48	16	32	48	32	48	48	48	64	16	32	32	48	64
Cost to Implement	16	24	16	24	8	8	16	16	8	8	24	16	8	24	24	16	32
Cost to Run	24	16	24	16	24	16	16	8	24	24	16	24	8	24	24	16	32
Value	376	432	480	428	248	272	364	320	312	348	444	484	208	380	288	348	320

in use in reference laboratories
commercially available
"pipeline" technologies in development

* = Lmited or no data available to evaluate

Major Assumptions:

Diagnostic Test Profile

1. Detect all Orbivirus strains
2. Direct tests for control and eradication
3. Indirect tests for post-control monitoring/detection subclinical cattle
4. Rapid test
5. >95% specificity
6. >95% sensitivity
7. Pen-side test
8. DIVA Compatible
9. Field validated
10. Easy to perform/easily train NAHML's personel
11. Scalable
12. Reasonable cost

APPENDIX VI: DIAGNOSTICS FOR SURVEILLANCE

Diagnostics for Surveillance

Diagnostics For Herd Surveillance - May 2013

Rank each Intervention (2,4,6,8, or 10) as to its importance to you in making a decision, no more than one "10" rankings allowed

Weight	Critical Criteria	cELISA	qRT-PCR	Type Specific RT-PCR	Type Specific Sero Assay*	Type Specific qRT-PCR*
10	Validation to purpose	8	8	6	2	2
6	correlation to protection	2	2	10	10	10
8	Throughput	10	10	10	10	10
8	ability to detect multiple strains	8	8	8	6	6
6	Pan-species use	8	8	4	2	2
10	Deployable	8	8	4	6	8
8	Cost to Implement	8	6	4	8	4

Available in laboratories
commercially available (could be stock piled)
"pipeline" technologies in development

* = Lmited data available to evaluate

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

* = Cant determine - assay not developed

Critical Criteria	cELISA	qRT-PCR	Type Specific RT-PCR	Type Specific Sero Assay	Type Specific qRT-PCR
Validation to purpose	80	80	60	20	20
correlation to protection	12	12	60	60	60
Throughput	80	80	80	80	80
ability to detect multiple strains	64	64	64	48	48
Pan-species use	48	48	24	12	12
Deployable	80	80	40	60	80
Cost to Implement	64	48	32	64	32
Value	428	412	360	344	332

Major Assumptions:

Diagnostic Test Profile

1. Detect all Orbivirus strains
2. Direct tests for control and eradication
3. Indirect tests for post-control monitoring/detection subclinical cattle
4. Rapid test
5. >95% specificity
6. >95% sensitivity
7. Pen-side test
8. DIVA Compatible
9. Field validated
10. Easy to perform/easily train NAHML's personel
11. Scalable
12. Reasonable cost

APPENDIX VII: DIAGNOSTICS FOR HERD PROTECTIVE IMMUNITY

Diagnostics for Herd Protective Immunity

Diagnostics For Herd Protective Immunity - May 2013

Rank each Intervention (2,4,6,8, or 10) as to its importance to you in making a decision, no more than one "10" rankings allowed

Weight	Critical Criteria	VNT	Type Specific Sero Assay*
10	Validation to purpose	10	2
10	correlation to protection	6	10
8	Throughput	4	8
4	ability to detect multiple strains	2	6
6	Pan-species use	8	4
6	Deployable	8	4
4	Cost to Implement	2	6

Available in laboratories
commercially available (could be stock piled)
"pipeline" technologies in development

* = Lmited data available to evaluate

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

* = Cant determine - assay not developed

Critical Criteria	VNT	Type Specific Sero Assay*
Validation to purpose	100	20
correlation to protection	60	100
Throughput	32	64
ability to detect multiple strains	8	24
Pan-species use	48	24
Deployable	48	24
Cost to Implement	8	24
Value	304	108

Major Assumptions:

Diagnostic Test Profile

1. Detect all Orbivirus strains
2. Direct tests for control and eradication
3. Indirect tests for post-control monitoring/detection subclinical cattle
4. Rapid test
5. >95% specificity
6. >95% sensitivity
7. Pen-side test
8. DIVA Compatible
9. Field validated
10. Easy to perform/easily train NAHML's personel
11. Scalable
12. Reasonable cost

APPENDIX VIII: DIAGNOSTICS FOR *ORBIVIRUSES* WITHOUT VACCINATION

Diagnostics for *Orbiviruses* Without Vaccination

Diagnostics For Orbiviruses - Freedom from infection without vaccination, May 2013

Rank each Intervention (2,4,6,8, or 10) as to its importance to you in making a decision, no more than one "10" rankings allowed

Weight	Critical Criteria	E AGID	B AGID	B cELISA	E cELISA	EHDV qRT-PCR	BTV qRT- PCR	Luminex*
10	Validation to purpose	8	8	8	8	8	8	2
8	Specificity	4	4	10	10	10	10	4
10	Sensitivity (herd level)	6	6	8	8	8	8	6
8	Throughput	6	6	8	8	8	8	6
6	ability to detect multiple strains	8	8	8	8	8	8	6
8	Pan-species use	8	8	8	8	8	8	6
6	Deployable	8	8	8	8	8	8	2
4	Cost to Implement	8	6	8	8	4	4	2

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

Critical Criteria	E AGID	B AGID	B cELISA	E cELISA	EHDV qRT-PCR	BTV qRT- PCR	Luminex*
Validation to purpose	80	80	80	80	80	80	20
Specificity	32	32	80	80	80	80	32
Sensitivity (herd level)	60	60	80	80	80	80	60
Throughput	48	48	64	64	64	64	48
ability to detect multiple strains	48	48	48	48	48	48	36
Pan-species use	64	64	64	64	64	64	48
Deployable	48	48	48	48	48	48	12
Cost to Implement	32	24	32	32	16	16	8
Value	412	404	496	496	480	480	264

in use in reference laboratories
commercially available
"pipeline" technologies in development

* = Lmited or no data available to evaluate

Major Assumptions:
Diagnostic Test Profile
1. Detect all Orbivirus strains
2. Direct tests for control and eradication
3. Indirect tests for post-control monitoring/detection subclinical cattle
4. Rapid test
5. >95% specificity
6. >95% sensitivity
7. Pen-side test
8. DIVA Compatible
9. Field validated
10. Easy to perform/easily train NAHML's personel
11. Scalable
12. Reasonable cost

APPENDIX IX: ASSESSMENT OF COMMERCIAL BLUETONGE VACCINES

Vaccine Assessments

Assessment of Commercial Bluetongue Vaccines, May 2013

Rank each intervention (2,4,6,8, or 10) according to its importance to making a decision; only one "10" ranking allowed

Weight	Critical Criteria	MLV	Inactivated	MLV-SA	Autogenous
10	Efficacy	6	8	4	2
6	Valency	4	6	6	4
8	Cross-serotype protection	4	6	6	2
6	> 1 duration of immunity	6	4	4	2
6	< week onset immunity	4	2	4	2
4	No maternal antibody	2	2	2	2
4	Two-year shelf life	4	6	4	2
10	Safe vaccine	0	10	0	4
8	No high containment	8	8	8	2
8	DIVA-compatible	0	2	0	2
8	Rapid scale-up	8	6	8	4
10	Reasonable cost	8	6	8	2
4	Short withdrawal	8	8	8	2
6	Feasibility of registration	2	8	2	2
6	Add new antigens	4	6	4	6
6	Accelerated delivery	6	6	6	6

Rank each Criteria 2,4,6,8 or 10 on each criterion; no more than two "10" rankings allowed

Critical Criteria	MLV	Inactivated	MLV-SA	Autogenous
Efficacy	60	80	40	20
Valency	24	36	36	24

Cross-serotype protection	32	48	48	16
> 1 duration of immunity	36	24	24	12
< week onset immunity	24	12	24	12
No maternal antibody	8	8	8	8
Two-year shelf life	16	24	16	8
Safe vaccine	0	100	0	40
No high containment	64	64	64	16
DIVA-compatible	0	16	0	16
Rapid scale-up	64	48	64	32
Reasonable cost	80	60	80	20
Short withdrawal	32	32	32	8
Feasibility of registration	12	48	12	12
Add new antigens	24	36	24	36
Accelerated delivery	36	36	36	36
Value	476	636	472	280

APPENDIX X: ASSESSMENT OF EXPERIMENTAL *ORBIVIRUS* VACCINES

Vaccine Assessments

Assessment of Experimental *Orbivirus* Vaccines, May 2013

Rank each Intervention (2,4,6,8, or 10) according to its importance to making a decision; only one "10" ranking allowed

Weight	Critical Criteria	Inactivated	Baculovirus	CPV
10	Efficacy	8	4	4
6	Valency	8	4	4
4	Cross-serotype protection	8	4	4
8	> 1 duration of immunity	4	2	2
6	< week onset immunity	2	2	2
4	No maternal antibody	2	4	4
4	Two-year shelf life	6	4	4
8	Safe vaccine	10	8	8
8	No high containment	8	10	10
8	DIVA-compatible	2	8	8
8	Rapid scale-up	6	6	6
10	Reasonable cost	6	6	6
4	Short withdrawal	8	6	6
6	Feasibility of registration	8	8	8
6	Add new antigens	6	8	8
6	Accelerated delivery	6	6	6

Rank each Criteria 2,4,6,8 or 10 on each criterion; no more than two "10" rankings allowed

Critical Criteria	Inactivated	Baculovirus	CPV
Efficacy	80	40	40
Valency	48	24	24

Cross-serotype protection	32	16	16
> 1 duration of immunity	32	16	16
< week onset immunity	12	12	12
No maternal antibody	8	16	16
Two-year shelf life	24	16	16
Safe vaccine	80	64	64
No high containment	64	80	80
DIVA-compatible	16	64	64
Rapid scale-up	48	48	48
Reasonable cost	60	60	60
Short withdrawal	32	24	24
Feasibility of registration	48	48	48
Add new antigens	36	48	48
Accelerated delivery	36	36	36
Value	620	576	576

APPENDIX XI: SUMMARY POINTS

Summary Points – BTV and EHDV in Livestock

- Obscure clinical signs:
  - Temporary bull sterility
  - Ulcers/erosions in/around oral cavity
  - Crusty lesions on muzzle
  - Coronary band involvement – foot rot
- Usually not epizootic (except sheep/deer):
  - Solitary abortion
  - Solitary dummy or deformed calf
- Efficient vector transmission in the U.S: One transoceanic flight away from new introduction to U.S.
- One mutation or genetic reassortment away from the generation of a new and emerging strain with the potential for significant impact on the U.S. livestock industry

Summary Points – BTV and EHDV in the Captive Cervid Industry

- EHD/BTV outbreaks historically have been scattered throughout the industry and varied from year to year
- Hit hard in 2007
- Vaccines development
- Back-to-back in 2011 and 2012
- 100% losses !!!
- Many farms with 75–80% loss of deer
- Many deer with multiple strains
- Cattle industry impacted in 2007, 2011, and 2012
- Will Blue Tongue 8 make its way to the U.S.?
- What will we do if it does?

Summary Points – BTV and EHDV in Wildlife

- EHD has been present in the U.S. for a long time
- No long-term effects on any deer herd have been recorded
- Determining distribution and severity of an outbreak are the keys to adaptive population management
- Localized impacts affect hunter satisfaction and short-term decision on population management
- Preventive measures?
  - Avoid concentration of wildlife
  - Guidelines for wildlife watering holes and ponds
  - Vaccination is not a solution of management of HD in free-ranging populations
- Increased frequency of outbreaks
- Repeated impacts in some geographies
- Unpredictable occurrence and impacts
- Evolving population response? (from acute to chronic)

- Introduction of exotic serotypes (difference in virulence?)
- Expanding geography of outbreaks
- Connection with cattle or exotic cervid imports
- Connection with agricultural facilities/environment

Additional considerations for EHDV

- 1959 Japan EHD outbreak
  - Animals present with fever, stomatitis, dysphagia
  - Hemorrhagic and ulcerative lesions
  - In-herd morbidity rates: <0.1%–12.6%
  - EHDV isolated and Koch's Postulates fulfilled (Omori et al., 1969)
    - EHDV-2 (Ibaraki)
  - Additional outbreaks: 1960, 1982, 1987, 1997
  - Early anecdotal reports from U.S.
  - Concurrent with 1955 EHD outbreaks
  - Cases of "muzzle disease" in cattle from PA and DE and "mucosal disease" in a steer from ND
- Antibodies against EHDV have been detected in a variety of species throughout the world
  - Significance and epidemiologic role is not known
  - Experimental EHDV infections
  - WTD, mule deer, black-tailed deer, wapiti, red deer, fallow deer, sika deer, muntjac
  - Cattle, sheep, goat
  - Rabbit, mouse, hamster, pig, dog
- EHDV is widely distributed throughout the world, and cattle are commonly infected
- EHDV has been recognized in new regions in recent years associated with reports of disease
  - Documented production loss (Kedmi et al., 2010)
  - Suspected reproductive loss (Ohashi et al., 1999)
- Events of the past decade have indicated that the distribution of EHDV serotypes is dynamic
- Despite the inability to replicate EHD experimentally, field reports indicate that disease does occur
- WTD are the most susceptible species on the planet
  - Captive WTD are under heavy disease pressure
  - Mortality in captive WTD herds can reach 80–90% during severe EHD outbreaks → increases visibility of EHD

APPENDIX XIII: WORKSHOP PARTICIPANTS

Gary Anderson, D.V.M., Ph.D.
Director, Kansas State Veterinary Diagnostic Laboratory
Professor
Kansas State University College of Veterinary Medicine
Diagnostic Medicine/Pathobiology Dept.
Manhattan, KS 66506-5601
Tel: (785) 532-4454
Fax: (785) 532-4481
ganders@vet.k-state.edu

Michael Beck, Ph.D.
Boehringer Ingelheim Vetmedica, Inc (BIVI)
R & D, Building D
2621 N. Belt Highway
St. Joseph, MO 64506
Tel: (816) 383-8972
michael.beck@boehringer-ingelheim.com

Martin Beer, DVM, Ph.D
Director of the Institute of Diagnostic Virology
Friedrich-Loeffler-Institut
Federal Research Institute for Animal Health
Bundesforschungsinstitut für Tiergesundheit
Institut für Virusdiagnostik
Südufer 10
17493 Greifswald - Insel Riems
Tel: +49 38351 7-1200
Fax: +49 38351 7-1226
Martin.Beer@fli.bund.de

Ariane Cagienard, D.V.M., ECVPH
Emerging Disease Program Manager
Veterinary Public Health
29, Avenue Tony Garnier
BP 7123
69348 Lyon Cedex 07
France
Tel: +33 472 72 70 17
Fax: +33 472 72 31 81
ariane.cagienard@merial.com

Chris Chase, D.V.M., M.S., Ph.D.
Professor
Department of Veterinary and Biomedical Sciences South Dakota State University
PO Box 2175 ADR Rm 125, N. Campus Dr.
South Dakota State University
Brookings, SD 57007
Tel: (605) 688-5652
Fax: (605) 688-6003
Christopher.Chase@sdstate.edu

Lee Cohnstaedt, Ph.D.
Research Entomologist
Arthropod-Borne Animal Diseases Research Unit
Center for Grain and Animal Health Research (CGAHR)
Agricultural Research Service
United States Department of Agriculture
1515 College Ave.
Manhattan, Kansas 66502
lee.cohnstaedt@ars.usda.gov

Peter Daniels, D.V.M., Ph.D
Deputy Director
CSIRO - Australian Animal Health Laboratory
PMB 24
Geelong 3220
Tel: +61 3 5227 5014
Fax: +61 3 5227 5555
Peter.Daniels@csiro.au

David Dargatz, D.V.M., Ph.D.
Epidemiologist
USDA-APHIS-VS, Centers for Epidemiology and Animal Health
2150 Center Ave. Building B
Fort Collins, CO 80526
Tel: 970 494-7231
Fax: 970 494-7228
david.a.dargatz@aphis.usda.gov

Barbara Drolet, Ph.D.
Research Microbiologist
Arthropod-Borne Animal Diseases Research Unit
Center for Grain and Animal Health Research (CGAHR)
Agricultural Research Service, United States Department of Agriculture (USDA)
1515 College Ave.
Manhattan, Kansas 66502
barbara.drolet@ars.usda.gov

Lane Foil, M.S., Ph.D.
Entomology Department
404 Life Sciences Building-LSU
Baton Rouge, LA 70803
Tel: (225) 578-1825
Fax: (225) 578-7307
LFoil@agcenter.lsu.edu

Cyril Gerard Gay, D.V.M., Ph.D. (Chair, Organizing Committee)
Senior National Program Leader, Animal Production and Protection
USDA-REE-ARS, Office of National programs
5601 Sunnyside Ave.
Beltsville, MD 20705
Tel: (301) 504-4786
Fax: (301) 504-5467
cyril.gay@ars.usda.gov

Paul Gibbs, BVSc, Ph.D., FRCVS
Professor Emeritus
College of Veterinary Medicine
University of Florida
Gainesville, FL 32610
Tel: 1-352-294-4182
pgibbs@ufl.edu

Dick Hesse, M.S., Ph.D.
Director of Diagnostic Virology
Kansas Veterinary Diagnostic Laboratory
Diagnostic Medicine/Pathobiology
Kansas State University College of Veterinary Medicine
1800 Denison Ave. / Mosier Hall
Manhattan, KS 66506-5601
Tel : (785) 532-4457
dhesse@vet.k-state.edu

Stephen Higgs, Ph.D., FRES, FASTMH
Associate Vice President for Research
Research Director, Biosecurity Research Institute
Peine Professor of Biosecurity
Professor, Diagnostic Medicine and Pathobiology
Kansas State University
Pat Roberts Hall, Denison Ave. / Mosier Hall
Manhattan, KS 66506-5601
Tel: 785-532-0970
shiggs@bri.ksu.edu

Larry Hollis, D.V.M., M.Ag.
Animal Sciences and Industry
137 Call Hall
Kansas State University
Manhattan, KS 66506-8028
Tel: (785) 532-6533
Fax: (785) 532-7059
lhollis@k-state.edu

Elizabeth W. Howerth, D.V.M., Ph.D., DACVP
Department of Pathology
College of Veterinary Medicine
The University of Georgia
501 D.W. Brooks Dr.
Athens, GA 30602-7388
Tel: (706) 542-5837
Fax: (706) 542-5828
howerth@uga.edu

Donna Johnson, M.S.
Microbiologist, Equine and Ovine Viruses Section, Diagnostic Virology Laboratory
USDA-APHIS-VS, National Veterinary Services Laboratories
1920 Dayton Ave.
Ames, IA 50010
Tel: (515) 337-7551
Fax; (515) 337-7348
Donna.J.Johnson@aphis.usda.gov

Randall L. Levings, D.V.M., Ph.D. (Organizing Committee)
USDA-APHIS-VS, National Center for Animal Health Emergency Management
1920 Dayton Ave.
Ames, IA 50010
Tel: 515 450-8509
randall.l.levings@aphis.usda.gov

Tim Lysyk, Ph.D.
Agriculture and Agri-Food Canada
5403-1 Ave. South
PO Box 3000
Lethbridge, Alberta T1J 4B1
Tel: (403) 317-2259
Fax: (403) 382-3156
Tim.lysyk@agr.gc.ca

Michael T. McIntosh, Ph.D.
Head, Proficiency and Validation Services & Microbial Forensics
Foreign Animal Disease Diagnostic Laboratory
USDA-APHIS- VS-NVSL
Plum Island Animal Disease Center
40550 Route 25
Orient Point NY 11944
Tel: (631) 323-3159
Fax (631) 323-3366
Michael.T.McIntosh@aphis.usda.gov

N. James MacLachlan, D.V.M., Ph.D. (Organizing Committee)
Department of Pathology, Microbiology, and Immunology
Center for Vectorborne Diseases
College of Veterinary Medicine
University of California, Davis
5325 Vet Med 3A
Davis, CA 95616
Tel: (530) 754 8125
njmaclachlan@ucdavis.edu

Scott McVey, D.V.M., Ph.D. (Organizing Committee)
Arthropod-Borne Animal Diseases Research Unit
Center for Grain and Animal Health Research (CGAHR)
Agricultural Research Service
United States Department of Agriculture
1515 College Ave.
Manhattan, Kansas 66502
Phone: (785) 537-5561
scott.mcvey@ars.usda.gov

Peter P. C. Mertens BSc. D.Phil. CBiol, FSB, FHEA
The Pirbright Institute, Pirbright Laboratory
Ash Road, Pirbright, Surrey, GU24 0NF
Tel: +44 (0)1483 232441
peter.mertens@pirbright.ac.uk

Igor Morozov, D.V.M., Ph.D.
Science Projects Manager
Center of Excellence for Emerging and Zoonotic Animal Diseases (CEEZAD)
A Department of Homeland Security
Science and Technology Center of Excellence
Kansas State University College of Veterinary Medicine
Diagnostic Medicine/Pathobiology
1800 Denison Ave. K-224A Mosier Hall
Manhattan, KS 66506-5601

Tel: (785) 532-4255
imorozov@vet.k-state.edu

Brad Mullens, Ph.D.
Department of Entomology
University of California
Riverside, CA 92521
Tel: (951) 827-5800
Fax: (951) 827-3086
bradley.mullens@ucr.edu

Dana Nayduch, Ph.D.
Molecular Biologist
Arthropod-Borne Animal Diseases Research Unit
Center for Grain and Animal Health Research (CGAHR)
Agricultural Research Service, United States Department of Agriculture (USDA)
1515 College Ave.
Manhattan, KS 66502
dana.nayduch@ars.usda.gov

Bob Pfannenstiel, Ph.D.
Research Entomologist
Research Microbiologist
Arthropod-Borne Animal Diseases Research Unit
Center for Grain and Animal Health Research (CGAHR)
Agricultural Research Service, United States Department of Agriculture (USDA)
1515 College Ave.
Manhattan, KS 66502
bob.pfannenstiel@ars.usda.gov

Juergen A. Richt, D.V.M., Ph.D. (Organizing Committee)
Regents Distinguished Professor
Kansas State University College of Veterinary Medicine
Diagnostic Medicine/Pathobiology Dept.
K224B Mosier Hall
Manhattan, KS 66506-5601
Tel: (785) 532-4408
jricht@vet.k-state.edu

Mark G Ruder, D.V.M., Ph.D.
Veterinary Medical Officer
Arthropod-Borne Animal Diseases Research Unit
Center for Grain and Animal Health Research (CGAHR)
Agricultural Research Service
United States Department of Agriculture
1515 College Ave.
Manhattan, KS 66502
Tel: (785) 537-5571
Fax: (785) 537-5560
mark.ruder@ars.usda.gov

David E. Stallknecht, MS, Ph.D.
Professor
589 DW Brooks Dr.
Wildlife Health Building
College of Veterinary Medicine
The University of Georgia
Athens, GA 30602
Tel: (706) 542-1741
Fax: (706) 542-5865
dstall@uga.edu

Shawn Schafer
Executive Director
North American Deer Farmers Association
4501 Hills & Dales Rd. NW
Canton, OH 44708
Tel: (651) 212-1315
Fax: (330) 454.3950
schafer@nadefa.org

Dan Strickman, M.S., Ph.D.
National Program Leader
Veterinary, Medical, and Urban Entomology
Acting Director Overseas Labs
USDA-REE-ARS, Office of National Programs
5601 Sunnyside Ave.
Beltsville, MD 20705
Tel: (301) 504-5771
Fax: (301) 504-5467
daniel.strickman@usda.gov

Jason Sumners, Ph.D.
Resource Scientist/Deer Biologist
Missouri Department of Conservation
Central Regional Office and Research Center
3500 East Gans Road
Columbia, MO 65201
Tel: (573) 815-7901, ext. 3620
jason.sumners@mdc.mo.gov

Piet A. van Rijn, Ph.D.
Project Leader of Viral Exotic Diseases
Central Veterinary Institute of Wageningen UR (CVI)
Department of Virology
P.O. Box 65, 8200 AB Lelystad, The Netherlands
Houtribweg 39, 8221 RA Lelystad, The Netherlands
Tel: +31.320.238.686
Fax: +31.320.238668
piet.vanrijn@wur.nl

Chris Whitehouse, Ph.D. (Organizing Committee)
National Wildlife Health Center
United States Geological Survey
Department of the Interior
Phone: **(**608) 270-2460
cwhitehouse@usgs.gov

Bill Wilson, Ph.D.
Research Microbiologist
Arthropod-Borne Animal Diseases Research Unit
Center for Grain and Animal Health Research (CGAHR)
Agricultural Research Service, United States Department of Agriculture (USDA)
1515 College Ave.
Manhattan, KS 66502
william.wilson@ars.usda.gov

Alan Young, Ph.D.
Professor and Research Leader, Department of Veterinary and Biomedical Science
South Dakota State University
Box 2175, ARW168F
Brookings, SD 57007
Tel: (605) 688-5982
Fax: (605) 688-6133
Alan.Young@sdstate.edu

AD HOC CONTRIBUTORS

Stéphan Zientara, D.V.M., Ph.D.
Directeur de l'UMR 1161 ANSES/INRA/ENVA
Orbivirus Reference Laboratory
23 Avenue du Général de Gaulle
94703 Maisons-Alfort
Tél: 33 01 43 96 72 80
szientara@vet-alfort.fr

REFERENCES

Alexander, R.A., D.A. Haig. 1951. The use of egg-attenuated bluetongue virus in the production of a polyvalent vaccine for sheep: a propagation of the virus in sheep. Onderstepoort J. Vet. Sci. Anim. Ind. 25:3-15.

Allison, A.B., V. Goekjian, A. Potgieter, W. Wilson, D. Johnson, P.P.C. Mertens, and D. Stallknecht. 2010. Detection of a novel reassortant epizootic hemorrhagic disease virus (EHDV) in the USA containing RNA segments derived from both exotic (EHDV-6) and endemic (EHDV-2) serotypes. J. Gen. Virol. 91: 430-439.

Allison, A.B., E.C. Holmes, A. C. Potgieter, I. M. Wright, C. Sailleau, E. Breard. M. G. Ruder, and D. E. Stallknecht. 2012. Segmental configuration and putative origin of the reassortant orbivirus, epizootic hemorrhagic disease virus serotype 6, strain Indiana. Virology 424: 67-75.

Afshar, A., J. Anderson, B. Eaton, and G. Gustafson. 1991. Serological diagnosis of bluetongue by blocking or competitive ELISA by four laboratories. J. Vet. Diagn. Invest. 3(3): 255-257.

Afshar, A., F.C. Thomas, P.F. Wright, J.L. Shapiro, and J. Anderson. 1989. Comparison of competitive ELISA, indirect ELISA and standard AGID tests for detecting blue-tongue virus antibodies in cattle and sheep. Vet. Rec. 124(6): 136-141.

Afshar, A., H.C. Trotter, G.C. Dulac, and J.J. Reddington. 1993. Evaluation of a commercial competitive ELISA test kit for the detection of group-specific antibodies to bluetongue virus. J. Vet. Diagn. Invest. 5(3): 336-340.

Akita, G.Y., J. Chinsangaram, B.I. Osburn, M. Ianconescu, and R. Kaufman .1992. Detection of bluetongue virus serogroup by polymerase chain reaction. J. Vet. Diagn. Invest. 4(4): 400-405.

Akita, G. Y., J. Glenn, A. E. Castro and B. I. Osburn. 1993. Detection of bluetongue virus in clinical samples by polymerase chain reaction. J. Vet. Diagn. Invest. 5: 154-158.

Aradaib, I. E., J. W. McBride, W. C. Wilson and B. I. Osburn.1995. Development of polymerase chain reaction for specific identification of epizootic hemorrhagic disease virus serotype 1. Arch. Virol. 140(12): 2273-2281.

Anthony, S.J., S. Maan, N. Maan, L. Kgosana, K. Bachanek-Bankowska, C. Batten, K.E. Darpel, G. Sutton, H. Attoui, and P. P. Mertens. 2009. Genetic and phylogenetic analysis of the outer-coat proteins VP2 and VP5 of epizootic haemorrhagic disease virus (EHDV): comparison of genetic and serological data to characterise the EHDV serogroup. Virus Res. 145: 200-210.

Aradaib, I. E., W. C. Wilson, C. E. Schore, M. E. Mohammed, T. D. Yilma, J. S. Cullor and B. I. Osburn. 1998. PCR detection of North American and Central African isolates of epizootic hemorrhagic disease virus (EHDV) based on genome segment 10 of EHDV serotype 1. J Clin Microbiol (9): 2604-2608.

Atchley, W.R. 1967. The *Culicoides* of New Mexico (Diptera: Ceratopogonidae). Kans. Univ. Sci. Bull. 46: 937-1022.

Backx, A., R. Heutink, E. van Rooij, P. van Rijn. 2009 . Transplacental and colostral transmission of wild-type bluetongue virus serotype 8 in cattle after experimental infection. Vet. Microbiol. 138, 235-243.

Balasuriya, U., S. Nadler, W. Wilson, L. Pritchard, A. Smythe, G. Savini, F. Monaco, P. De Santis, N. Zhang, W. Tabachnick, and others. 2008. The NS3 proteins of global strains of bluetongue virus evolve into regional topotypes through negative (purifying) selection. Vet. Microbiol. 126:91–100.

Barber, T.L. 1979. Temporal appearance, geographic distribution, and species of origin of bluetongue virus serotypes in the United States. Am. J. Vet. Res. 40: 1654-1656.

Barnard, B., G. Gerdes, and R. Meiswinkel. 1998. Some epidemiological and economic aspects of a bluetongue-like disease in cattle in South Africa-1995/96 and 1997. Onderstepoort J. of Vet. Res. 65:145–151.

Barrette, R.W., Metwally, S.A., Rowland, J.M., Xu, L., Zaki, S.R., Nichol, S.T., Rollin, P.E., Towner, J.S., Shieh, W.J., Batten, B., Sealy, T.K., Carrillo, C., Moran, K.E., Bracht, A.J., Mayr, G.A., Sirios-Cruz, M., Catbagan, D.P., Lautner, E.A., Ksiazek, T.G., White, W.R., McIntosh, M.T. 2009. Discovery of swine as a host for the Reston ebolavirus. Science 325, 204-206.

Becker, M. E., W. K. Reeves, S. K. Dejean, M. P. Emery, E. N. Ostlund and L. D. Foil. 2010. Detection of bluetongue virus RNA in field-collected *Culicoides* spp. (Diptera: Ceratopogonidae) following the discovery of bluetongue virus serotype 1 in white-tailed deer and cattle in Louisiana. J. Med. Entomol. 47: 269-273.

Beer, M., B. Hoffman, P.S. Mellor, P.P.C. Mertens. 2008.Sequence analysis of bluetongue virus serotype 8 from the Netherlands 2006 and comparison to other European strains. Virology 377(2):308-18.

Belaganahalli, M. N., S. Maan, N.S. Maan, K. Nomikou, I. Pritchard, R. Lunt, P.D. Kirkland, H. Attoui, J. Brownlie, P.P. C. Mertens. 2012. Full Genome Sequencing and Genetic Characterization of Eubenangee Viruses Identify Pata Virus as a Distinct Species within the Genus *Orbivirus* PLoS One. 7(3): e31911. Published online 2012 March 15.

Belhouchet, M., F.M. Jaafar, A. E. Firth, J.M. Grimes, P. P. C. Mertens, H. Attoui. 2011. Detection of a Fourth Orbivirus Non-Structural Protein PLoS One 6(10): e25697.

Berry, B.S., K. Magori, A.C., Perofsky, D. E. Stallknecht, and Park A.W. 2013. Wetland cover dynamics drive hemorrhagic disease patterns in white-tailed deer in the United States. J. Wild. Dis. 49: 501-509.

Bonneau, K.R., DeMaula, C.D., Mullens, B.A., Maclachlan, N.J. 2002. Duration of viraemia infectious to Culicoides sonorensis in bluetongue virus-infected cattle and sheep. Vet. Microbiol. 88, 115–125.

Boone, J.D., Balasuriya, U.B., Karaca, K., Audonnet, J.C., Yao, J., He, L., Nordgren, R., Monaco, F., Savini, G., Gardner, I.A., Maclachlan, N.J. 2007. Recombinant canarypox virus

vaccine co-expressing genes encoding the VP2 and VP5 outer capsid proteins of bluetongue virus induces high level protection in sheep. Vaccine 25, 672–678.

Boyce, C., C. P. Celma, P. Roy. 2008. Development of Reverse Genetics Systems for Bluetongue Virus: Recovery of Infectious Virus from Synthetic RNA Transcripts J. Virol. 82(17): 8339–8348.

Boyer, T. C., M. P. Ward, R. L. Wallace, E.M. Zhou, and R. S. Singer. 2008. Exploratory spatial data analysis of regional seroprevalence of antibodies against epizootic hemorrhagic disease virus in cattle from Illinois and Indiana. Am. J. Vet. Res. 69: 1286-1293.

Boyer, T. C., M. P. Ward, and R. S. Singer. 2010. Climate, landscape, and the risk of Orbivirus exposure in cattle in Illinois and western Indiana. Am. J. Trop. Med. Hyg. 83:789–794.

Bréard, E., C. Sailleau, J. Gourreau, S. Zientara, C. Hamblin, and S. Graham. 2004. Outbreak of epizootic haemorrhagic disease on the island of Réunion. Vet. Rec. 155: 422-423.

Brito, B. P., I. A. Gardner, S. K. Hietala and B. M. Crossley. 2011. Variation in Bluetongue virus real-time reverse transcription polymerase chain reaction assay results in blood samples of sheep, cattle, and alpaca. J. Vet. Diagn. Invest. 23(4): 753-756.

Burgin, L. E., J. Gloster, C. Sanders, P. S. Mellor, S. Gubbins, and S. Carpenter. 2013. Investigating incursions of bluetongue virus using a model of long-distance *Culicoides* biting midge dispersal. Transbound. Emerg. Dis. 60:263–272.

Calvo-Pinilla, E., T. Rodríguez-Calvo, J. Anguita, N. Sevilla, J. Ortego.2009. Establishment of a Bluetongue Virus Infection Model in Mice that Are Deficient in the Alpha/Beta Interferon Receptor. PLoS One. 4(4)

Caporale, M., R. Wash, A. Pini, G. Savini, P. Franchi, M. Golder, J. Patterson-Kane, P. Mertens, L. Di Gialleonardo, G. Armillotta, R. Lelli, P. Kellam, M. Palmarini.2011Determinants of Bluetongue Virus Virulence in Murine Models of Disease. J. Virol. 85:21.

Carpenter, S., H. L. Lunt, D. Arav, G. J. Venter, and P. S. Mellor. 2006. Oral susceptibility to bluetongue virus of Culicoides (Diptera: Ceratopogonidae) from the United Kingdom. J. Med. Entomol. 43:73–78.

Carpenter, S., C. McArthur, R. Selby, R. Ward, D. V. Nolan, A. J. M. Luntz, J. F. Dallas, F. Tripet, and P. S. Mellor. 2008. Experimental infection studies of UK *Culicoides* species midges with bluetongue virus serotypes 8 and 9. Vet. Rec. 163:589–592.

Carpenter, S., P. S. Mellor and S. J. Torr. 2008. Control techniques for *Culicoides* biting midges and their application in the UK and northwestern Palearctic. Med. Vet. Entomol. 22: 175-187.

Carpenter, S., A. Wilson, P. S. Mellor. 2009. *Culicoides* and the emergence of bluetongue virus in northern Europe. Trends in Microbiol. 17: 172-178.

Castillo-Olivares, J., E. Calvo-Pinilla, I. Casanova, K. Bachanek-Bankowska, R. Chiam, S. Maan, J. M. Nieto, J. Ortego, P. P. Clement Mertens A Modified Vaccinia Ankara Virus (MVA)

Vaccine Expressing African Horse Sickness Virus (AHSV) VP2 Protects Against AHSV Challenge in an IFNAR −/− **Mouse** Model PLoS One. 2011; 6(1)

Celma, C., M. Boyce, P. van Rijn, M. Eschbaumer, K. Wernike, B. Hoffmann, M. Beer, A. Haegeman, K. De Clercq, and P. Roy. 2013. Rapid generation of replication-deficient monovalent and multivalent vaccines for bluetongue virus: protection against virulent virus challenge in cattle and sheep. J. Virol. 87:9856-9864.

Clavijo, A., F. Sun, T. Lester, D. C. Jasperson and W. C. Wilson. 2010. An improved real-time polymerase chain reaction for the simultaneous detection of all serotypes of Epizootic hemorrhagic disease virus. J Vet Diagn Invest 22(4): 588-593.

Cheney, I.W., Larson, M.D., Mecham, J.O., Wilson, W.C. 1995. Geographical genetic variation in the gene encoding VP3 from the Alberta isolate of epizootic hemorrhagic disease virus. Virus Res. 36, 279-286.

Cheney, I., M. Yamakawa, P. Roy, J. Mecham, and W. Wilson. 1996. Molecular characterization of the segment 2 gene of epizootic hemorrhagic disease virus serotype 2: gene sequence and genetic diversity. Virology 224: 555-560.

Couvillion, C.E., V.F. Nettles, and W. R. Davidson. 1981. Hemorrhagic disease among white-tailed deer in the Southeast from 1971 through 1980. pp. 522 – 537 *In*: Proceedings of the 85th United States Animal Health Association, St. Louis, Mo.

Dangler, C. A., C. A. de Mattos, C. C. de Mattos and B. I. Osburn. 1990. Identifying bluetongue virus ribonucleic acid sequences by the polymerase chain reaction. J. Virol. Meth. 28: 281-292.

DeMaula, C.D., K.R., Bonneau, N.J., Maclachlan. 2000. Changes in the outer capsid proteins of bluetongue virus serotype 10 that abrogate neutralization by monoclonal antibodies. Virus Res. 67, 59–66.

DeMaula, C.D., M.A., Jutila, D.W., Wilson, N.J., Maclachlan. 2001. Infection kinetics, prostacyclin release and cytokine-mediated modulation of the mechanism of cell death during bluetongue virus infection of cultured ovine and bovine pulmonary artery and lung microvascular endothelial cells. J. Gen. Virol. 82, 787–794.

DeMaula, C.D., C.M., Leutenegger, K.R.,Bonneau, N.J., Maclachlan. 2002. The role of endothelial cell-derived inflammatory and vasoactive mediators in the pathogenesis of bluetongue. Virology 296, 33–337.

Doherty, W.M., A.L., Bishop, L.F., Melville, S.J., Johnson, G.A., Bellis, N.T., Hunt. 2004. Protection of cattle from Culicoides spp. in Australia by shelter and chemical treatments. Vet. Ital. 40, 320–323.

Downes, J. A. 1978. The *Culicoides variipennis* complex: a necessary re-alignment of nomenclature (Diptera: Ceratopogonidae). Can. Entomol. 110:63–69.

Drew, C.P., M.C., Heller, C., Mayo, J.L., Watson, N.J., Maclachlan. 2010b. Bluetongue virus infection activates bovine monocyte-derived macrophages and pulmonary artery endothelial cells. Vet. Immunol. Immunopathol. 136, 292–296.

Drolet, B. S., K. W. Mills, E. L. Belden and J. O. Mecham .1990. Enzyme-linked immunosorbent assay for efficient detection of antibody to bluetongue virus in pronghorn (Antilocapra americana). J. Wildl. Dis. 26(1): 34-40.

Drolet, B.S., L.M. Reister, J.O. Mecham, W.C. Wilson, P., Nol, K.C. VerCauteren, P.A. van Rijn, Richard A. Bowen. 2013. Experimental infection of white-tailed deer with bluetongue virus serotype 8. (Accepted in Vet Microbiol)

Dudley, R. 2013. Report of the Committee on Bluetongue and Related Orbiviruses, *In* 116th Annual Meeting of the United States Animal Health Association, October 18-24, Greensboro, NC. *In Press.*

Dungu, B, T., Gerdes, T., Smit. 2004. The use of vaccination in the control of bluetongue in southern Africa. Vet. Ital. 40(4):616-622.

EFSA Panel on Animal Health and Welfare (AHAW). 2009. Scientific Opinion on Epizootic Hemorrhagic Disease. EFSA Journal 7(12):1418. [67 pp.]. doi:10.2903/j.efsa.2009.1418. Available online: www.efsa.europa.eu/efsajournal

Elbers, A., A. Backx, A. van der Spek, M. Ekker, P. Leijs, K. Steijn, H. van Langen and P. van Rijn .2008. Epidemiology of bluetongue virus serotype 8 outbreaks in the Netherlands in 2006. Tijdschr Diergeneeskd 133(6): 222-229.

Enserink, M., 2006. Emerging infectious diseases. During a hot summer, bluetongue virus invades northern Europe. Science 313, 1218–1219.

Eschbaumer, M, K, C.A. Wernike Batten, G. Savini, L. Edwards, A. L. Di Gennaro Teodori, C.A. Oura, M. Beer, B. Hoffmann. 2012. Epizootic hemorrhagic disease virus serotype 7 in European cattle and sheep: diagnostic considerations and effect of previous BTV exposure. Vet Microbiol. 2012 Oct 12;159(3-4):298-306. doi: 10.1016/j.vetmic.2012.04.020. Epub 2012 Apr 20.

Eschbaumer, M., K. Wernike, C. A. Batten, G. Savini, L. Edwards, A. Di Gennaro, L. Teodori, C. A. Oura, M. Beer and B. Hoffmann .2012. Epizootic hemorrhagic disease virus serotype 7 in European cattle and sheep: diagnostic considerations and effect of previous BTV exposure. Vet Microbiol 159(3-4): 298-306.

Favero, C.M., A.C.D. Matos, F.S. Campos, M.V. Cândido, E.A. Costa, M.B. Heinemann, E.F. Barbosa-Stancioli, and Z. Lobato. 2013. Epizootic hemorrhagic disease in Brocket deer, Brazil. Emer. Inf. Dis. 19: 346.

Ferrari, G., C. De Liberato, G.Scavia, R. Lorenzetti, M. Zini, F.Farina, A.Magliano, G.Cardeti, F.Scholl, M.Guidoni, M.T.Scicluna, D.Amaddeo, P.Scaramozzino, G.L. Autorino. 2005. Active circulation of luetongue vaccine virus serotype-2 among unvaccinated cattle in central Italy. Prev. Vet. Med. 68, 10–13.

Flacke, G.L., M.J. Yabsley, B.A. Hanson, and D.E. Stallknecht. 2004. Hemorrhagic disease in Kansas: enzootic stability meets epizootic disease. J. Wildl. Dis. 40: 288-293.

Flanagan, M., S.J. Johnson. 1995. The effects of vaccination of Merino ewes with an attenuated Australian bluetongue virus serotype 23 at different stages of gestation. Aust. Vet. J. 72, 455–457.

Foster, N. M., R. H. Jones, and B. R. McCrory. 1963. Preiminary investigations on insect transmission of bluetongue virus in sheep. Amer. J. Vet. Res. 24:

Gard, G.P., L.F. Melville. 1992. Results of a decade's monitoring for orbiviruses in sentinel cattle pastured in an area of regular arbovirus activity in northern Australia. In: T.E. Walton and B.I. Osburn (Editors), *Bluetongue, African Horse Sickness and Related Orbiviruses*, CRC Press, Boca Raton, 85-89.

Gard, G.P., Weir, R.P., Walsh, S.J. Arboviruses recovered from sentinel cattle using several isolation methods. Vet Microbiol., **18**, 119–125.

Gaydos, J. K., W. R. Davidson, F. Elvinger, D. G. Mead, E. W. Howerth, and D. E. Stallknecht. 2002b. Innate resistance to epizootic hemorrhagic disease in white-tailed deer. J. Wildl. Dis. 38: 713–719.

Gaydos, J. K., D. E. Stallknecht, D. Kavanaugh, R. J. Olson, and E. R. Fuchs. 2002c. Dynamics of maternal antibodies to hemorrhagic disease viruses (Reoviridae: Orbivirus) in white-tailed deer. J. Wildl. Dis. 38:253–257.

Gerry, A. C., B. A. Mullens. 2000. Seasonal abundance and survivorship of *Culicoides sonorensis* (Diptera: Ceratopogonidae) at a Southern California dairy, with reference to potential bluetongue virus transmission and persistence. J. Med. Entomol. 37:675–688.

Gerry, A. C., B. A. Mullens, N. J. Maclachlan, and J. O. Mecham. 2001. Seasonal transmission of bluetongue virus by *Culicoides sonorensis* (Diptera: Ceratopogonidae) at a Southern California dairy and evaluation of vectorial capacity as a predictor of bluetongue virus transmission. J. Med. Entomol. 38:197–209.

Gibbs, E.P.J., E. C. Greiner. 1989. Bluetongue and epizootic hemorrhagic disease. pp. 39 – 70 *In:* T. Monath (ed.) Arboviruses: Epidemiology and Ecology, Vol. 2, CRC Press, Boca Raton Florida.

Gibbs, E.P.J., W.J. Tabachnick, T.J. Holt, D.E. Stallknecht. 2008. US concerns over bluetongue. Science 320: 872-872.

Gibbs, E. P. J., Greiner, E. C. The epidemiology of bluetongue. Comp. Immun. Microbiol. Inf. Dis. 17: 207-220.

Gibbs, E., E. Greiner, W. Taylor, T. Barber, J. House, and J. Pearson. 1983. Isolation of bluetongue virus serotype 2 from cattle in Florida: serotype of bluetongue virus hitherto unrecognized in the Western Hemisphere. Am. J. Vet. Res. 44: 2226.

Gibbs, E. P. J., W. J Tabachnick, Holt, T. J. and Stallknecht, D. E. 2008. US concerns over bluetongue. Science 320: 872.

Green, A. L., D. A. Dargatz, E. T. Schmidtmann, M. V. Herrero, A. H. Seitzinger, E. N. Ostlund, B. A. Wagner, K. M. Moser, N. E. Wineland, and T. E. Walton. 2005. Risk factors associated with herd-level exposure of cattle in Nebraska, North Dakota, and South Dakota to bluetongue virus. Am. J. Vet. Res. 66:853–860.

Goulad, A.R. 1987. The complete nucleotide sequence of bluetongue virus serotype 1 RNA3 and a comparison with other geographic serotypes from , and the , and with other orbivirus isolates. Virus Res., **7**, 169–183.

Hoffmann, B., M. Beer, S. M. Reid, P. Mertens, C. A. Oura, P. A. van Rijn, M. J. Slomka, J. Banks, I. H. Brown, D. J. Alexander and D. P. King. 2009. A review of RT-PCR technologies used in veterinary virology and disease control: sensitive and specific diagnosis of five livestock diseases notifiable to the World Organisation for Animal Health. Vet Microbiol 139(1-2): 1-23.

Hofmann, M., C, Griot, V. Chaignat, L.Perler, B.Thur.2008. Bluetongue disease reaches Switzerland. *Schweiz. Arch. Tierheilk.*, 150, 49-56.

Hawkes,R.A., P.D. Kirkland, D.A. Sanders, F. Zhang, Z. Li, R.J. Davis, N.Zhang. 2000. Laboratory and field studies of an antigen capture ELISA for bluetongue virus. *J. Virol. Methods*, 85, 137–149.

Hofmann M.A., S. Renzullo, M. Mader, V. Chaignat, G. Worwa, B. Thuer. 2008. Genetic Characterization of Toggenburg Orbivirus, a New Bluetongue Virus, from Goats, Switzerland Emerg Infect Dis. December; 14(12): 1855–1861. doi: 10.3201/eid1412.080818

Hofmann, M. A., S. Renzullo, M. Mader, V. Chaignat, G. Worwa, and B. Thuer. 2008. Genetic characterization of toggenburg orbivirus, a new bluetongue virus, from goats, Switzerland. Emer. Inf. Dis. 14:1855 - 1861.

Hunt, G. J., B. A. Mullens, W. J. Tabachnick. 1999. Colonization and maintenance of species of *Culicoides*. *In:* Maintenance of Animal/Human and Plant Pathogen Vectors. (F. Mahmood and K. Maramarosch, eds.). Oxford and IBH Publ. Co. Pvt. Ltd. New Delhi. pp. 33-55.

Holbrook, F. R., W. J. Tabachnick, E. T. Schmidtmann, C. N. McKinnon, R. J. Bobian and W. L. Grogan. 2000. Sympatry in the *Culicoides variipennis* complex (Diptera: Ceratopogonidae): a taxonomic reassessment. J. Med. Entomol. 37: 65-76.

Howerth, E.W., D.E. Stallknecht, and P.D. Kirkland. 2001. Bluetongue, Epizootic Hemorrhagic Disease, and Other Orbivirus-Related Diseases. pp. 77-97. *In* E. Williams and I. Barker (eds.), Infectious Diseases of Wild Mammals, Third Edition. Iowa State Press, Blackwell, Ames, IA.

Huismans H., N.T. van der Walt, M. Cloete, B.J. Erasmus.1987. Isolation of a capsid protein of bluetongue virus that induces a protective immune response in sheep. Virology 157: 172–179.

Kusay J.T., E. Calvo-Pinilla, F. Mateos, S. Gubbins, A. Bin-Tarif, K. Bachanek-Bankowska, O. Alpar, J. Ortego, H.H. Takamatsu, P. P. Clement Mertens, J. Castillo-Olivares, Protection of IFNAR (−/−) Mice against Bluetongue Virus Serotype 8, by Heterologous (DNA/rMVA) and

Homologous (rMVA/rMVA) Vaccination, Expressing Outer-Capsid Protein VP2, PLoS One. 2013; 8(4): e60574. Published online 2013 April 12. doi: 10.1371/journal.pone.0060574.

Jack, P.J., R.N. Amos-Ritchie, A. Reverter, G.Palacios, P.L. Quan, O. Jabado, T. Briese, W.I. Lipkin, D.B. Boyle. 2009. Microarray-based detection of viruses causing vesicular or vesicular-like lesions in livestock animals. Veterinary microbiology 133, 145-153.

Jeggo, M., P. F. Wright, J. Anderson, B. Eaton, A. Afshar, J. E. Pearson, P. Kirkland and Y. Ozawa. 1992. Review of the IAEA meeting in Vienna on standardization of the competitive ELISA test and reagents for diagnosis of bluetongue. Bluetongue, African Hose Sickness, and Related Orbiviruses. T. E. Walton and B. I. Osburn. Boca Raton, FL, CRC Press.

Johnson, D.J., W.C. Wilson and P. S. Paul. 2000. Validation of a reverse transcriptase multiplex PCR test for the serotype determination of U.S. isolates of bluetongue virus. Vet. Microbiol. 76(2): 105-115.

Johnson D.J., E.N. Ostlund, D.E.Stallknecht, V.H. Goekjian, M. Jenkins-Moore, S.C.Harris .2006. First report of bluetongue virus serotype 1 isolated from a white-tailed deer in the United States. J Vet Diagn Invest. Jul;18(4):398-401.

Jorgensen, N. M. 1969. The systematics, occurrence, and host preference of *Culicoides* (Diptera: Ceratopoginidae) in southeastern Washington. Melanderia 3:1–47.

Katz, J. B., A. D. Alstad, G. A. Gustafson and K. M. Moser. 1993. Sensitive identification of bluetongue virus serogroup by a colorimetric dual oligonucleotide sorbent assay of amplified viral nucleic acid. J. Clin. Microbiol. 31(11): 3028-3030.

Katz, J. B., G. A. Gustafson, A. D. Alstad, K. A. Adler and K. M. Moser. 1993. Colorimetric diagnosis of prolonged bluetongue viremia in sheep, using an enzyme-linked oligonucleotide sorbent assay of amplified viral nucleic acids. Am. J. Vet. Res. 54(12): 2021-2026.

Kedmi, M., M. E.Van Straten, N. Ezra, Galon, E. Klement. 2010. Assessment of the productivity effects associated with epizootic hemorrhagic disease in dairy herds. J. Dairy Sci. 93: 2486–2495.

Lunt, R.A., J.R., White, S.D. Blacksell.1988. Evaluation of a monoclonal antibody blocking ELISA for the detection of group-specific antibodies to bluetongue virus in experimental and field sera. *J. Gen. Virol.*, 69, 2729–2740.

Lysyk, T. J., and T. Danyk. 2007. Effect of temperature on life-history parameters of adult *Culicoides sonorensis* (Diptera: Ceratopogonidae) in relation to geographic origin and vectorial capacity for bluetongue virus. J. Med. Entomol. 44:741–751.

Maan, N. S., S. Maan, M. N. Belaganahalli, E. N. Ostlund, D. J. Johnson, K. Nomikou and P. P. Mertens .2012. Identification and differentiation of the twenty six bluetongue virus serotypes by RT-PCR amplification of the serotype-specific genome segment 2. PLoS One **7**(2): e32601.

Maan, S., N. S. Maan, K. Nomikou, E. Veronesi, K. Bachanek-Bankowska, M. N. Belaganahalli, H. Attoui, and P. P. Mertens. 2011. Complete genome characterisation of a novel 26th bluetongue virus serotype from Kuwait. PLoS One 6:e26147.

Sushila M., S.M. Narender, K. Nomikou, C. Batten, F. Antony, M. N. Belaganahalli, A.M. Samy, A. A. Reda, S. Ahmed Al-Rashid, M.E. Batel, A.L. Chris Oura, P.C. Peter Mertens. 2011. Novel Bluetongue Virus Serotype from Kuwait Emerg Infect Dis. 17(5): 886–889. doi: 10.3201/eid1705.101742

Sushila M., N.S. Maan, N. Ross-Smith, C.A. Batten, A.E. Shaw, S.J. Anthony, A.R. Samuel, K.E. Darpel, E.Veronesi, C.A.L. Oura, K.P. Singh, K. Nomikou, A.C. Potgieter, H. Attoui, E.van Rooij, P. van Rijn, K. De Clercq, F.Vandenbussche, S. Zientara, E.Bréard, C. Sailleau, M.Beer, B. Hoffman, P.S. Mellor & P.P.C. Mertens. 2008. *S*equence analysis of bluetongue virus serotype 8 from the Netherlands 2006 and comparison to other European strains. Virology 1;377(2):308-18.

Sushila, M., S.M. Narender, P.A. van Rijn, R.G. P. van Gennip, A. Sanders, I.M. Wright, C.Batten, B. Hoffmann, M. Eschbaumer, C.A. L. Oura, A.C. Potgieter, K. Nomikou, P.P.C. Mertens.2010. Full Genome Characterisation of Bluetongue Virus Serotype 6 from the Netherlands 2008 and Comparison to Other Field and Vaccine Strains. PLoS One 5(4): e10323.

Maan, S., N. Maan, A. Samuel, S. Rao, H. Attoui, and P. Mertens. 2007. Analysis and phylogenetic comparisons of full-length VP2 genes of the 24 bluetongue virus serotypes. J. Gen. Virol. 88: 621–630.

MacLachlan, N.J., Nunamaker, R.A., Katz, J.B., Sawyer, M.M., Akita, G.Y., Osburn, B.I., Tabachnick, W.J., 1994. Detection of bluetongue virus in the blood of inoculated calves: comparison of virus isolation, PCR assay, and in vitro feeding of Culicoides variipennis. Arch. Virol. 136, 1-8.

Maclachlan, N.J., 2004. Bluetongue; pathogenesis and duration of viraemia. Vet. Ital. 40, 462–467.

Maclachlan, N.J., Schore, C.E., Osburn, B.I. 1984. Antiviral responses of bluetongue virus infected bovine fetuses and their dams. Am. J. Vet. Res. 45, 1469–1473.

Maclachlan, N.J. C.M. Mayo. Potential strategies for control of bluetongue, a globally emerging, Culicoides-transmitted viral disease of ruminant livestock and wildlife. Antiviral Research 99 (2013) 79–90.

MacLachlan, N. J., S. Zientara, D. E. Stallknecht, J. D. Boone, V. H. Goekjian, C. Sailleau, and U. B. Balasuriya. 2007. Phylogenetic comparison of the S10 genes of recent isolates of bluetongue virus from the United States and French Martinique Island. Virus Res. 129:236–240.

Mars, M. H., C. van Maanen, P. Vellema, J. A. Kramps and P. A. van Rijn. 2010. Evaluation of an indirect ELISA for detection of antibodies in bulk milk against bluetongue virus infections in the Netherlands. Vet Microbiol 146(3-4): 209-214.

McColl, K.A., A.A., Gould.1991. Detection and characterisation of bluetongue virus using the polymerase chain reaction. *Virus Res.*, **21**, 19–34.

Matsuo, E., P. Roy. 2013. Minimum requirements for bluetongue virus primary replication in vivo. J. Virol. 87, 882–889.

Mayo, C.E., B.M. Crossley, S.K. Hietala, I.A. Gardner, R.E. Breitmeyer, N.J. Maclachlan. 2010. Colostral transmission of bluetongue virus nucleic acid among newborn dairy calves in California. Transbound. Emerg. Dis. 57, 277–281.

Mayo, C. E., I. A. Gardner, B. A. Mullens, C. M. Barker, A. C. Gerry, A. J. Guthrie, and N. J. MacLachlan. 2012. Anthropogenic and meteorological factors influence vector abundance and prevalence of bluetongue virus infection of dairy cattle in California. Vet. Microbiol. 155:158 – 164.

Mead, D., D.E. Stallknecht, J. Phillips-Brantley, S. Vigil, and J. Corn. 2013. SCWDS update: hemorrhagic disease and *Culicoides* sp. surveillance. *In* United States Animal Health Association Annual Meeting, 2012; October 18-24, Greensboro, NC. *In Press.*

Mecham, J. O. 1993. Detection of bluetongue virus from blood of infected sheep by use of an antigen-capture enzyme-linked immunosorbent assay after amplification of the virus in cell culture. Amer. J. Vet. Res. 54(3): 370-372.

Mecham, J. O.1997. Epizootic hemorrhagic disease of deer virus: Does it pose a risk to U.S. livestock? U.S. Animal Health Assoc.

Mecham, J. O., V. C. Dean, M. M. Jochim.1986. Correlation of serotype specificity and protein structure of the five U.S. serotypes of bluetongue virus. J. Gen. Virol. 67: 2617-2624.

Mecham, J. O., M. M. Jochim.1992. Comparison of the neutralizing immune response of sheep and cattle to bluetongue viruses. Bluetongue, African Horse Sickness, and Related Orbiviruses. T. E. Walton and B. I. Osburn, eds. Boca Raton, FL, CRC Press**:** 768-775.

Mecham, J. O. M. M. Jochim. 2000. Development of an enzyme-linked immunosorbent assay for the detection of antibody to epizootic hemorrhagic disease of deer virus. J. Vet. Diag. Investig. 12(2): 142-145.

Mecham, J. O., D. Stallknecht, W. C. Wilson. 2003. The S7 gene and VP7 protein are highly conserved among temporally and geographically distinct American isolates of epizootic hemorrhagic disease virus. Virus Res. 94: 129–133.

Mecham, J. O. W. C. Wilson. 1994. Strategies for improved bluetongue diagnostic strategies for improved bluetongue diagnostics. Proc. U.S. Anim. Health Assoc. 98: 43-50.

Mecham, J. O., W. C. Wilson. 2004. Antigen capture competitive enzyme-linked immunosorbent assays using baculovirus-expressed antigens for diagnosis of bluetongue virus and epizootic hemorrhagic disease virus. J. Clin. Microbiol. 42(2): 518-523.

Mellor, P. S., J. Boorman, M. Baylis. 2000. *Culicoides* biting midges: their role as arbovirus vectors. Ann. Rev. Entomol. 45: 307-340.

Metcalf, H., A. Luedke, M. Jochim. 1992. Epizootic hemorrhagic disease virus infection in cattle. pp. 222-237. *In* T. E. Walton and B. I. Osburn (eds.), Bluetongue, African Horse Sickness, and Related Orbiviruses: Proceedings of the Second International Symposium, CRC Press Inc., Boca Raton, FL.

Metcalf, H. E., J. E. Pearson, A. L. Klingsporn. 1981. Bluetongue in cattle: A serologic survey of slaughter cattle in the United States. Am. J. Vet. Res. 42:1057–1061.

Mertens, P.P.C., S. Maan, A.R. Samuel, H. Attoui. 2005. Orbivirus: Reoviridae. pp. 466 – 483 *In* C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger, and L.A. Ball, (eds.). Virus Taxonomy: VIIIth Report of the ICTV. Elsevier/Academic Press, London.

Mertens. 2007. Development and initial evaluation of a rtRT-PCR assay to detect bluetongue virus genome segment 1. J Virol Methods 145(2): 115-126.

Miller, M. M., J. Brown, T. Cornish, G. Johnson, J. O. Mecham, W. K. Reeves, and W. Wilson. 2010. Investigation of a bluetongue disease epizootic caused by bluetongue virus serotype 17 in sheep in Wyoming. Journal of the American Veterinary Medical Association 237:955–959.

Moulin, V., C.V. Noordegraaf, B. Makoschey, M. van der Sluijs, E. Veronesi, K. Darpel, P. P. C. Mertens. 2011. Hans de Smit Clinical disease in sheep caused by bluetongue virus serotype 8, and prevention by an inactivated vaccine. Vaccine. 30(12):2228-35. DOI:10.1016/j.vaccine.2011.11.100

Mullen, G.R., R.H. Jones, Y. Braverman, and K.E. Nusbaum. 1985. Laboratory infections of *Culicoides debilipalpis* and *C. stellifer* (Diptera: Ceratopogonidae) with bluetongue virus. Progress in Clinical and Biological Research 178: 239-243.

Mullens, B. A., C. E. Dada. 1992. Spatial and seasonal distribution of potential vectors of hemorrhagic disease viruses to peninsular bighorn sheep in the Santa Rosa Mountains of southern California. J. Wildl. Dis. 28:192–205.

Mullens, B. A., A. C. Gerry, T. J. Lysyk, and E. T. Schmidtmann. 2004. Environmental effects on vector competence and virogenesis of bluetongue virus in *Culicoides*: interpreting laboratory data in a field context. Vet. Ital. 40:160–166.

Murphy, M. D., B. A. Hanson, E. W. Howerth, D. E. Stallknecht. 2006. Molecular characterization of epizootic hemorrhagic disease virus serotype 1 associated with a 1999 epizootic in white-tailed deer in the eastern United States. J. Wildl. Dis. 42: 616–624.

Murphy, M. D., E. W. Howerth, N. J. MacLachlan, D. E. Stallknecht. 2005. Genetic variation among epizootic hemorrhagic disease viruses in the southeastern United States: 1978–2001. Inf. Gen. Evol. 5:157–165.

Mullens, B. A. and J. L. Rodriguez. 1988. Colonization and response of *Culicoides variipennis* (Diptera: Ceratopogonidae) to pollution levels in experimental dairy wastewater ponds. J. Med. Entomol. 25: 441-451.

Neng, J., M. H. Harpster, W. C. Wilson and P. A. Johnson. 2013. Surface-enhanced Raman scattering (SERS) detection of multiple viral antigens using magnetic capture of SERS-active nanoparticles. Biosens Bioelectron 41: 316-321.

Nettles V.F., W.R. Davidson, D.E. Stallknecht. 1992. Surveillance for hemorrhagic disease in white-tailed deer and other wild ruminants, 1980-1989. *In*: Proceeding of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies. 46:138-146.

Nettles, V., D. Stallknecht. 1992. History and progress in the study of hemorrhagic disease of deer. Transactions of the North American Wildlife and Natural Resources Conference. 57: 499–516

Niedbalski, W. 2011. Evaluation of commercial ELISA kits for the detection of antibodies against bluetongue virus. Pol J Vet Sci 14(4): 615-619.

Noad, R., P. Roy. 2009. Bluetongue vaccines. Vaccine. 27:D86-D89.

Nunes, et al. 2012. pers comm ds RNA meeting Puerto Rico.

Papadopoulas, O., P.S. Mellor, P.P. Mertens. 2009. Bluetongue control strategies. In: Mellor, P., Baylis, M., Mertens, P. (Eds.), Bluetongue. Elsevier, Amsterdam, pp. 429–452.

Odiawa, G., J. Blue, D. Tyler, and E. Shotts. 1985. Bluetongue and epizootic hemorrhagic disease in ruminants in Georgia: survey by serotest and virologic isolation. Am. J. Vet. Res. 1985. 46: 2193.

Orru, G., P. D. Santis, F. Solinas, G. Savini, V. Piras and V. Caporale. 2004. Differentiation of Italian field and South African vaccine strains of bluetongue virus serotype 2 using real-time PCR. J. Virol. Meth. 122(1): 37-43.

Ostlund, E.N. 2008. Report of the Committee on Bluetongue and Related Orbiviruses, pp. 148-151. *In* 112th Annual Meeting of the United States Animal Health Association, October 23-29, 2008, Greensboro, NC.

Ostlund, E.N. 2010. Report of the Committee on Bluetongue and Related Orbiviruses, pp. 157-158. *In* 114th Annual Meeting of the United States Animal Health Association, November 11-17, 2010, Minneapolis, MN

Ostlund, E., K. Moser, D. Johnson, J. Pearson, B. Schmitt. 2004. Distribution of bluetongue in the United States of America, 1991–2002. Vet. Ital. 40:83–88.

Omori, T., Y. Inaba, T. Morimoto, Y. Tanaka, R. Ishitani. 1969. Ibaraki virus, an agent of epizootic disease of cattle resembling bluetongue. I. Epidemiologic, clinical and pathologic observations and experimental transmission to calves. Japanese J. Microbiol. 13:139-157.

Palacios, G., P.L. Quan, O.J. Jabado, S. Conlan, D.L. Hirschberg, Y. Liu, J. Zhai, N. Renwick, J.Hui, H.Hegyi, A. Grolla, J.E. Strong, J.S. Towner, T.W. Geisbert, P.B. Jahrling, C. Buchen-Osmond, H. Ellerbrok, M.P. Sanchez-Seco, Y. Lussier, P. Formenty, M.S. Nichol, H. Feldmann, T. Briese, W.I. Lipkin. 2007. Panmicrobial oligonucleotide array for diagnosis of infectious diseases. Emerg. Infect. Dis. 13, 73-81.

Pare, J., D. W. Geale, M. Koller-Jones, K. Hooper-McGrevy, E. J. Golsteyn-Thomas, C. A. Power. 2012. Serological status of Canadian cattle for brucellosis, anaplasmosis, and bluetongue in 2007-2008. Can. Vet. J. 53: 949–956.

Pearson, J. E., G. A. Gustafson, A. L. Shafer A. D. Alstad.1992. Diagnosis of bluetongue virus and epizootic haemorrahgic disease. Bluetongue, African Horse Sickness, and Related Orbiviruses. T. E. Walton and B. I. Osburn. Boca Raton, FL, CRC Press.

Pearson, J., G. Gustafson, A. Shafer, A. Alstad, T. Walton, and B. Osburn. 1992. Distribution of bluetongue in the United States, pp. 128-139. *In* T. E. Walton and B. I. Osburn (eds.), Bluetongue, African horse sickness, and related orbiviruses: Proceedings of the Second International Symposium. CRC Press Inc., Boca Raton, FL.

Perez de Diego, A.C., Athmaram, T.N., Stewart, M., Rodriguez-Sanchez, B., Sanchez-Vizcaino, J-M., Noad, R., Roy, P. 2011. Characterization of protection afforded by a bivalent virus-like particle vaccine against bluetongue virus serotypes 1 and 4 in sheep. PLos One.6(10),e26666.

Pritchard, L., A. Gould, W. Wilson, L. Thompson, P. Mertens, and A. Wade-Evans. 1995. Complete nucleotide sequence of RNA segment 3 of bluetongue virus serotype 2 (Ona-A). Phylogenetic analyses reveal the probable origin and relationship with other orbiviruses. Virus Res. 35:247–261.

Purse, B.V., Brown, H.E., Harrup, L., Mertens, P.P., Roger, D.J. 2008. Invasion of bluetongue and other Orbivirus infections into Europe: the role of biological and climatic processes. Rev. Sci. Tech. 27, 427–442.

Purse, B.V., P.S. Mellor, D.J. Roger, A.R. Samuel, P.P. Mertens, M. Baylis. 2005. Climate change and the recent emergence of bluetongue in Europe. Nat. Rev. Microbiol. 3, 171–181.

Quan, P.L., G. Palacios, O.J. Jabado, S. Conlan, D.L. Hirschberg, F.Pozo, P.J. Jack, D. Cisterna, N. Renwick, J. Hui, A. Drysdale, R. Amos-Ritchie, E. Baumeister, V. Savy, K.M. Lager, J.A. Richt, D.B. Boyle, A. Garcia-Sastre, I. Casas, P. Perez-Brena, T. Briese, W.I. Lipkin. 2007. Detection of respiratory viruses and subtype identification of influenza A viruses by GreeneChipResp oligonucleotide microarray. Journal of clinical microbiology 45, 2359-2364.

Quist, C. F., E. W. Howerth, D. E. Stallknecht, J. Brown, T. Pisell, and V. F. Nettles. 1997. Host defense responses associated with experimental hemorrhagic disease in white-tailed deer. J. Wildl. Dis. 33: 584–599.

Ratinier M., M. Caporale, M. Golder, G. Franzoni, K. Allan, S.F. Nunes, A. Armezzani, A. Bayoumy, F. Rixon, A. Shaw, M. 2011. Palmarini Identification and Characterization of a Novel Non-Structural Protein of Bluetongue Virus PLoS Pathog. 7(12): e1002477.

Rodman, D., D. Johnson. 2012. Report of the Committee on Bluetongue and Related Orbiviruses, pp. in press. *In* United States Animal Health Association Annual Meeting, October 18-24, 2012, Greensboro, NC.

Roughton, R.D. 1975. An outbreak of a hemorrhagic disease in white-tailed deer in Kentucky. J. Wildl. Dis. 11: 177-186.

Roy, P. Genetically engineered structure-based vaccine for bluetongue disease. Vet. Ital. 40(4): 594-600.

Sailleau, C., G. Zanella, E. Breard, C. Viarouge, A. Desprat, D. Vitour, M. Adam, L. Lasne, A. Martrenchar, L. Bakkali-Kassimi. 2012. Co-circulation of bluetongue and epizootic haemorrhagic disease viruses in cattle in Reunion Island. Veterinary microbiology 155:191–197.

Samal, S.K., C.W. Livingston,S.McConnell, R.F. Ramig. 1987b. Analysis of mixed infections of sheep with bluetongue virus serotypes 10 and 17; evidence for genetic reassortment in the vertebrate host. J. Virol. 61, 1086–1091.

Savini, G., A. Afonso, P. Mellor, I. Aradaib, H. Yadin, M. Sanaa, W. Wilson, F. Monaco, and M. Domingo. 2011. Epizootic heamorragic disease. Res. Vet. Sci. 91: 1–17.

Savini, G., N.J. MacLachlan, J.M. Sanchez-Vizcaino, S. Zientara. 2008. Vaccines against bluetongue in Europe. Comp. Immun. Microbiol. Inf. Dis. 31:101-120.

Sellers, R. F., A. R. Maarouf. 1991. Possible introduction of epizootic hemorrhagic disease of deer virus (serotype2) and bluetongue virus (serotype 11) into British Columbia in 1987 and 1988 by infected *Culicoides* carried on the wind. Can. J. Vet. Res. 55:367–370.

Sellers, R. F., A. R. Maarouf. 1989. Trajectory analysis and bluetongue virus serotype 2 in Florida 1982. Can. J. Vet. Res. 53: 100-102.

Shad, G., W. C. Wilson, J. O. Mecham and J. F. Evermann. 1997. Bluetongue virus detection: a safer reverse-transcriptase polymerase chain reaction for prediction of viremia in sheep. J. Vet. Diagn. Invest. 9(2): 118-124.

Shaw et al. 2012. Reassortment between two serologically unrelated bluetongue virus 2 strains is flexible and can involve any genome segment. J. Virol (J. Virol. doi:10.1128/JVI.02266-12)

Shapiro, J. L., A. Wiegers, G. C. Dulac, A. Bouffard, A. Afshar, D. J. Myers, C. Dubuc, M. W. Martin, M. Koller. 1991. A surevy of cattle for antibodies against bluetongue and epizootic hemorrhagicdisease of deer viruses in British Columbia and southwestern Alberta in 1987. Can. J. Vet. Res. 55:203–204.

Shaw, A. E., P. Monaghan, H. O. Alpar, S. Anthony, K. E. Darpel, C. A. Batten, A. Guercio, G. Alimena, M. Vitale, K. Bankowska, S. Carpenter, H. Jones, C. A. Oura, D. P. King, H. Elliott, P. S. Mellor and P. P. Mertens. 2007. Development and initial evaluation of a real-time RT-PCR assay to detect bluetongue virus genome segment 1. J. Virol. Methods. 145(2):115-26.

Shope, R.E., L.G. MacNamara, R. Mangold. 1960. A virus-induced epizootic hemorrhagic disease of the Virginia white-tailed deer (*Odocoileus virginianus*). J. Exp. Med. 111: 155-170.

Shulaw, W., Y. Zhang. 2008. EHD in Ohio cattle in 2007: clinical signs and serologic findings. *In*, 112th Annual Meeting of the United States Animal Health Association, October 23-29, 2008, Greensboro, NC.

Shultz, G., P.D. DeLay. 1955. Losses in newborn lambs associated with bluetongue vaccination of pregnant ewes. J. Am. Vet. Med. Assoc. 127, 224–226.

Singer, R.S., N.J. Maclachlan, T.E. Carpenter. 2001. Maximal predicted duration of viremia in bluetongue virus infected cattle. J. Vet. Diagn. Invest. 13, 43–49.

Sleeman, J. M., J. E. Howell, W. M. Knox, P. J. Stenger. 2009. Incidence of hemorrhagic disease in white-tailed deer is associated with winter and summer climatic conditions. EcoHealth 6:11–15.

Smith, K. E., D. E. Stallknecht. 1996. *Culicoides* (Diptera: Ceratopogonidae) collected during epizootics of hemorrhagic disease among captive white-tailed deer. J. Med. Entomol. 33: 507-510.

Smith, K.E., D.E. Stallknecht, C.T. Sewell, E.A. Rollor, G.R. Mullen, and R.R. Anderson. 1996b. Monitoring of *Culicoides* spp. at a site enzootic for hemorrhagic disease in white-tailed deer in Georgia, USA. J. Wildl. Dis. 32:627-642.

Smith, K.E., D. E. Stallknecht, and V.F. Nettles. 1996. Experimental infection of *Culicoides lahillei* (Diptera: Ceratopogonidae) with Epizootic Hemorrhagic Disease Virus serotype 2 (Orbivirus: Reoviridae). J. Med. Entomol. 33: 117-122.

Souza Monteiro, D.M., L.R. Carrasco, L.J. Moffitt, A.J. Cook, 2012. Robust surveillance of animal diseases: an application to the detection of bluetongue disease. Prev. Vet. Med. 105, 17–24.

Sumner, T., L. Burgin, J. Gloster, S. Gubbins. 2013. Comparison of pre-emptive and reactivestrategies to control an incursion of bluetongue virus serotype 1 to Great Britain by vaccination. Epidemiol. Infect. 141, 102–114.

Stallknecht, D.E., E. Howerth. 2004. Epidemiology of bluetongue and epizootic haemorrhagic disease in wildlife: surveillance methods. Vet. Ital. 40: 203-207.

Stallknecht, D. E., E. W. Howerth, J. K. Gaydos. 2002. Hemorrhagic disease in white-tailed deer: our current understanding of risk. Transactions of the North American Wildlife and Natural Resources Conference 67: 75 – 86. .

Stallknecht, D.E., M.P. Luttrell, K.E. Smith, and V.F. Nettles. 1996. Hemorrhagic disease in white-tailed deer in Texas: A case for enzootic stability. Journal of Wildlife Diseases 32:695-700.

Tabachnick, W. J. 1996. *Culicoides variipennis* and bluetongue virus epidemiology in the United States. Ann. Rev. Entomol. 41: 23-43.

Tanya, V., E. Greiner, and E. Gibbs. 1992. Evaluation of *Culicoides insignis* (Diptera: Ceratopogonidae) as a vector of bluetongue virus. Vet. Microbiol. 32: 1-14.

Temizel, E. M., K. Yesilbag, C. Batten, S. Senturk, N. S. Maan, P. P. C. Mertens, and H. Batmaz. 2009. Epizootic hemorrhagic disease in cattle, Western Turkey. Emer. Inf. Dis. 15: 317 – 319.

Theiler, A. 1908. The inoculation of sheep and the results in practice. Vet. J. 64:600-607.

Thorne, E. T., E. S. Williams, T. R. Spraker, W. Helms, and T. Segerstrom. 1988. Bluetongue in free-ranging pronghorn antelope (*Antilocapra americana*) in Wyoming: 1976 and 1984. J. Wildl. Dis. 24:113–119.

Toussaint JF., C. Sailleau, E. Breard, S. Zientara, K. de Clercq. 2007. Bluetongue virus detection by two real-time RT-qPCRs targeting two different genomic segments. J. Virol. Methods, 140(1-2), 115-123.

Vanbinst, T., F. Vandenbussche, E. Dernelle and K. De Clercq. 2010. A duplex rtRT-PCR for the detection of bluetongue virus in bovine semen. J Virol Methods 169(1): 162-168.

Van Donkersgoed, J., A. Gertonson, M. Bridges, D. Raths, D. Dargatz, B. Wagner, A. Boughton, D. Knoop, and T. E. Walton. 2004. Prevalence of antibodies to Bluetongue virus and *Anaplasma marginale* in Montana yearling cattle entering Alberta feedlots: Fall 2001. Can. Vet. J. 45:486–492.

Van Donkersgoed, J., T. F. T. Linfield, M. Bridges, D. Raths, D. Dargatz, B. Wagner, J. Block, D. Knoop, T. E. Walton. 2006. Prevalence of antibodies to serotypes of Bluetongue virus and *Anaplasma marginale* in Montana feeder cattle: 2002–2003. Can. Vet. J. 47:692–694.

van Gennip, H.G.P., S.G.P. van de Water, C.A. Potgieter, I.M. Wright, D. Veldman, P.A. van Rijn. 2012a. Rescue of recent virulent and avirulent field strains of Bluetongue virus by reverse genetics. Plos One 7(2): e30540. doi:10.1371/journal.pone.0030540.

van Gennip, H.G.P., S.G.P. van de Water, M. Maris-Veldhuis, P.A. van Rijn. 2012b. Bluetongue viruses (BTV) based on modified-live vaccine serotype 6 with exchanged outer shell proteins confer full protection in sheep against virulent BTV8. Plos One 7(9): e44619. doi:10.1371/journal.pone.0044619e.

Van Rijn et al. 2011. pers comm 5th EPIZONE meeting Arnhem, Netherlands

Vasagayam, J. A., M. P. Wellby, P. P. Mertens, J. N. Burroughs and J. Anderson. 1996. "Detection and differentiation of epizootic haemorrhagic disease of deer and bluetongue viruses by serogroup-specific sandwich ELISA. J Virol Methods 56(1): 49-57.

Wechsler, S.J., L.E. McHolland, W. J. Tabachnick.1989. Cell lines from Culicoides variipennis (Diptera: Ceratopogonidae) support replication of bluetongue virus Volume 54:3, 385–393

White, J.R., S.DO Blacksell, R.A. G.P. Lunt Gard. 1991. A monoclonal antibody blocking ELISA detects antibodies specific for epizootic haemorrhagic disease virus. *Vet Microbiol*. **29,** 237-250.

Wilson, W. C., J. L. Archer, C. C. L. Chase. 1992. The use of RNA detection techniques to identify bluetongue and epizootic hemorrhagic disease viruses in Culicoides Variipennis. Bluetongue, African Horse Sickness, and Related Orbiviruses., CRC Press**:** 687-693.

Wilson, W. C., B. J. Hindson, E. S. O'Hearn, S. Hall, C. Tellgren-Roth, C. Torres, P. Naraghi-Arani, J. O. Mecham and R. J. Lenhoff .2009. A multiplex real-time reverse transcription

polymerase chain reaction assay for detection and differentiation of Bluetongue virus and Epizootic hemorrhagic disease virus serogroups. J. Vet. Diagn. Invest. 21(6): 760-770.

Wilson, W., H.-C. Ma, E. Venter, A. Van Djik, B. Seal, J. Mecham. 2000. Phylogenetic relationships of bluetongue viruses based on gene S7. Virus Res. 67:141–151.

Wilson, W. C., C. C. L. Chase.1993. Nested and multiplex polymerase chain reactions for the identification of bluetongue virus infection in the biting midge, Culicoides variipennis. J. Virol. Methods 45(1): 39-47.

Wilson, W. C., E. S. O'Hearn, C. Tellgren-Roth, D. E. Stallknecht, D. G. Mead and J. O. Mecham. 2009. Detection of all eight serotypes of Epizootic hemorrhagic disease virus by real-time reverse transcription polymerase chain reaction. J. Vet. Diagn. Invest. 21(2): 220-225.

Wilson, W. C., D. E. Stallknecht and J. O. Mecham. 2004. Field-deployable real-time polymerase chain reaction detection of bluetongue and epizootic haemorrhagic disease viral ribonucleic acid. Vet Ital 40(4): 587-593.

Wirth, W.W., R.H. Jones RH. 1957. The North American subspecies of *Culicoides variipennis* Diptera: Heleidae). United States Department of Agriculture Technical Bulletin No. 1170.

Wirth, W., C. Morris. 1985. The taxonomic complex, *Culicoides variipennis*. Progress in clinical and biological research 178: 165 - 175.

World Health Organization. 1967. Arboviruses and human disease. Report of a WHO scientific group. WHO Technical Report Series 369: 1-84.

Xu, B., M. Madden, D. E. Stallknecht, T. W. Hodler, and K. C. Parker. 2012. Spatial-temporal model of haemorrhagic disease in white-tailed deer in south-east USA, 1983 to 2000. Vet. Rec. 170: 288–288.

Yadin, H., J. Brenner, V. Bumbrov, Z. Oved, Y. Stram, E. Klement, S. Perl, S. Anthony, S. Maan, C. Batten, and others. 2008. Epizootic haemorrhagic disease virus type 7 infection in cattle in Israel. Vet. Rec. 162: 53–56.

Yang, J., Q. Hua, H. Chen, J. Lv, Z. Qin, M. Jin, H. Tao, S. Zeng, Z. Ruan, B. Chen, X. Zhou .2010. Development and evaluation of an immunochromatographic strip for the detection of serum antibodies against bluetongue virus. J Virol Methods 163(1): 68-73.

Yin, H. Q., M. X. Jia, S. Yang, P. P. Jing, R. Wang and J. G. Zhang. 2012. Development of a highly sensitive gold nanoparticle probe-based assay for bluetongue virus detection. J Virol Methods 183(1): 45-48.

Zhang, H., M. H. Harpster, W. C. Wilson, P. A. Johnson. 2012. Surface-enhanced Raman scattering detection of DNAs derived from virus genomes using Au-coated paramagnetic nanoparticles. Langmuir 28(8): 4030-4037.

Zhou, E.M., J. Riva and A. Clavijo. 2001. Development of an immunoglobulin M (IgM) capture enzymelinked immunosorbent assay for detection of equine and swine IgM antibodies to vesicular stomatitis virus. Clin. Diagn. Lab. Immunol. 8(3) : 475-481.

Zientara, S., N.J. MacLachlan, P. Calistri, J.M. Sanchez-Vizcaino, G. Savini. 2010. Bluetongue vaccination in Europe. Expert Rev. Vaccines 9, 989–991.

Zientara, S., J.M. Sanchez-Vizcaino. 2013 in press. Control of bluetongue in Europe. Vet. Microbiol.