REPORT OF THE COMMITTEE ON BLUETONGUE AND RELATED ORBIVIRUSES

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The Committee met on October 21, 2014 at the Sheraton Hotel in Kansas City, Missouri, from [1 – 5 PM]. There were at least ?? members and at least ?? guests present. James Maclachlan as Committee Chair introduced the meeting, with apologies from Vice-Chair William Wilson. The following presentations were made to the committee:

Presentations

Recent impacts of bluetongue on livestock exports from the United States

Gordon Thornhill
Managing Director of T.K. Exports, Culpeper, Virginia

T.K. Exports, Inc (TKE) is a full service company dedicated to the export of live animals to international destinations. For the past 32 years, TKE has shipped US and Uruguayan animals to some 45 countries world-wide. These animals have been used mainly to improve meat and milk production by clients in their respective countries. For many years, livestock shipments from the USA were very sporadic. In the last 8-10 years, these exports have grown in volume tremendously. Our business increased from 3,500 exported in 2007 to over 20,000 animals in 2013. Today, as economies grow and the demand for better food, coupled with food security, many countries have started developing their own sources of meat and milk. Policies of Food Security have led to the development of livestock sectors within many countries which did not exist before. So now there is a world-demand for quality breeding stock. No one wants to import a dreaded disease. So every national veterinarian in charge of imports of live animals wants to guard against importing some dreaded disease. We now have higher demands for health background for the animals we export.

Today, the USA has become a major player to supply countries with animals. However, exporting of live animals still remains more of a burden to veterinarians rather than an additional source of income. Many of the challenges that exporters face are challenges that are related to science and not the actual sale of the animals. The USA national health program is superior to any that I know on the planet, but it is vastly different from most other countries in the world. So to combat the sometimes adverse import policies and rules, we must better understand the diseases that are important to them and learn what we might do to reduce risks of transmitting them. Many times these diseases are not ones we believe to be important to us in the USA.

As exporters of live animals, we therefore must be very conscious of animal health issues and the introduction of any new diseases both to our country and the countries of our clients. In reality, biosecurity is of the highest importance to what we are doing in the field of export. Since every country who imports animals wants some type of statements and or testing done, we have to be very conscious of not only what tests we do but also how we maintain the animals after we do this testing so that we do not re-infect the animals with the same disease after clearing them. TKE has designed our business model to minimalize these risks.

Our system was designed to bring together animals negative to both Leucosis and Bluetongue. Additionally we have placed these isolations in areas where the incidences of Bluetongue are minimal so

that when we clear the animals with a negative Bluetongue test, then the chances of re-infecting should be minimal.

Bluetongue is a disease which we have known about in the field of exporting for many years. It was the one which the Europeans used to eliminate American animals from entrance into EU countries in the late 1970's. Other countries have also used blue tongue as a reason to block imports of live animals from the USA. Initially, it became a non-tariff barrier to protect markets who politically did not want to allow large numbers of animals to be imported into their country. It was an easy one to use against the USA due to fact that there are areas which are endemic, but we have other areas where the vector does not exist. However, since animals freely move around the USA without being tested for this disease, other countries pointed out that we could not protect animals being exported from exposure since we did not have complete records as to where the animals have been prior to their export. In other words no national ID system or national data on movements of animals left some holes as to determine where animals had been moved during their lifetimes.

Over the years the knowledge grew about the disease and regionalization practices were accepted, so the USA was able to show that there are indeed areas where the vector does not exist. This enabled animals to be moved to these areas that are zero-negative and re-test them again negative without much risk of the animals being re-infected. However, in recent years, the EU has been adversely affected by some lethal strains of BTV which has increased awareness in every country to be on the alert to not import these strains. So BTV has reappeared as an important disease for exporting animals.

Bluetongue diagnosis, ways of transmission, early detection and prevention have always been clouded with mystery and perhaps ignorance across international borders. Over the years, more and more knowledge has been gained about this subject but there still exists quite a bit of uncertainty at least in the field of export. Perhaps it is a complex disease which has many different forms and these forms affect many animals in different ways. Some species are not affected, but others may carry the disease and infect other species. Bluetongue transmission continues to evolve due to climate change and animal management procedures. So the study of the science of this disease is very important to exporting livestock and needs to be a main focal point for animal exports.

I do not need to tell you the difficulties which arise from doing any kind of biosecurity. Many times there are trade-offs between practicality and reducing risks to zero. Frankly I am not sure with living animals, there is such a thing as zero risks! However with Bluetongue, the costs of reducing the risks are high, especially when you are dealing with several thousand animals on one shipment. Additionally, the stress of multiple blood sampling and meeting very specific timetables are stressful and costly to the animals and exporters.

So the work of this committee needs to be supported by our government, industry and, in turn, private veterinarians if we are going to meet the challenges ahead. We need science based knowledge on Bluetongue as to the types which are present in the USA, how it spreads, where it exists, what methods of detection are the most useful and how to protect transmission to animals. As a starting point, I believe the most important factor for all of us in the animal industry is to agree or disagree that exporting our breeding stock abroad is something that we want to support. If we support it, then it is not so hard to understand that to keep the markets, we have to meet their demands.

Today, I want to leave you with an idea of what it costs to buy an animal in Turkey or Russia. If the beginning price of an animal is about $2,000, then the end user will pay about double this amount at his farm. At a price of approximately $4,000 per animal, it is a very important investment to the buyer and the need for this healthy animal to produce for their operation. Within our economy the exporter utilizes many domestic operations such as cattle farmers, truckers, feed yards, laboratories, veterinarians, port operators, feed manufacturers and even APHIS who is paid user fees that generate revenue within the agricultural economy. The export industry it is an important part of the livestock sector today and I believe it will grow in the future. Exporting is good for our economy and something we, as a country, should continue. Therefore, it is important that we treat my business, exporting, as an industry that makes an important contribution to the agricultural economy. If we do this, then what this committee does is important and should be supported as such.

USDA Gap Analysis of Orbivirus Diseases: Report Highlights

D. Scott McVey, USDA-ARS, Arthropod-borne Animal Diseases Unit, Manhattan, Kansas

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).

The report of this workshop can be obtained through:

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

Epizootic hemorrhagic disease virus from white-tailed deer show limited genome constellations and preferential reassortment

Srivishnupriya Anbalagan, Elyse Cooper, Pat Klumper, Ben M. Hause, Newport Laboratories, Worthington, Minnesota

Epizootic hemorrhagic disease virus (EHDV) causes hemorrhagic disease in wild and domestic ruminants however infection of bovines is typically subclinical. A collection of 44 EHDV isolated from white tailed deer (WTD) (2008-2012) was fully sequenced and analyzed phylogenetically. High genetic similarity (>94% identity) between serotype 1 (ST1) and ST2 viruses VP1, VP3, VP4, VP6, NS1, NS2, and NS3 segments prevented identification of reassortment events for these segments. Additionally, there was little genetic diversity (>96% identity) within serotypes for VP2, VP5 and VP7. Preferential reassortment within the homologous serotype was observed for VP2, VP5 and VP7 segments for ST1 and ST2 viruses. In contrast, ST6 viruses were all reassortants containing VP2 and VP5 derived from an exotic ST6 with the remaining segments most similar to ST2 viruses. These results suggest that reassortment between ST1 and ST2 viruses requires conservation of VP2, VP5 and VP7 segment constellation while ST6 viruses only require VP2 and VP5 and are restricted to ST2-lineage VP7. As ST6 VP2 and VP5 segments were exclusively identified in viruses with ST2-derived VP7, these results suggest functional complementation between ST2 and ST6 VP7 proteins. In addition to WTD, EHDV was isolated from a pregnant cow in Indiana, U.S.A., exhibiting clinical symptoms of excessive salivation, pyrexia, nasal mucosa epithelial sloughing and abortion. VP2, VP5, and VP7 sequences of the bovine EHDV showed 97.7, 97.4, and 97.9% identity to ST2 reference virus. When compared to WTD EHDV sequences, the bovine EHDV was most homologous (>99.9% identity) to an EHDV isolate from Iowa and showed less than 2.1% divergence to EHDV collected from WTD across the United States in 2013. The high genetic identity between bovine and WTD EHDV isolates suggests indirect, via the *Culicoides* vector, interspecies transmission and given the widespread distribution of similar viruses, the possibility of further incursions into bovines.

References:

1. Srivishnupriya Anbalagan, Elyse Cooper, Pat Klumper, and Ben M. Hause. Whole genome analysis of epizootic hemorrhagic disease virus identified limited genome constellations and preferential reassortment. *Journal of General Virology,*2014, 95:434-441
2. Srivishnupriya Anbalagan and Ben M. Hause. Characterization of epizootic hemorrhagic disease virus from a bovine with clinical disease with high spatial-temporal similarity to white-tailed deer isolates. *Archives of Virology,* 2014, 159:2737–2740.

Epizootic Hemorrhagic Disease Vaccination and Titer Response in Cervidae

Douglas Wagner, Tammy Kolander, Ron Batman, Newport Laboratories, Worthington, Minnesota

The objectives of the study were to determine optimal antigen titers, minimum numbers of diseases, whether antigen interference occurs, and the best adjuvant. 35 does were split into 7 groups each of 5 animals, with each group given one vaccine as 3 doses 21 days apart. A final bleed was done 20 days after the 3rd dose. The specific groups were:

1. 4-way 9 log multivalent with TS6
2. 9 log monovalent with TS6
3. 4-way 9 log multivalent with Trigen
4. 9 log monovalent with Trigen
5. 8 log monovalent with Trigen
6. 7 log monovalent with Trigen
7. Non-vaccinated controls (NVC)

Immune responses were quantitated using ELISA (EHDV & BTV) and serum neutralization (SN) (EHDV) assays.

EHDV CONCLUSIONS

- 8 to 9 logs antigen concentrations promote a more rapid and robust immune response than 7 log concentrations.
- At least 2 doses are necessary.
- There does not appear to be antigen interference when multiple antigens are combined into one vaccine.
- TS6 and Trigen are comparable.

BTV CONCLUSIONS

- The vaccinated animals developed positive titers after 1 dose.
- There doesn't appear to be much difference in BTV titers after 2 or 3 doses at 9 logs.
- TS6 and Trigen are comparable.

Mechanism of Overwintering of Bluetongue Virus in Temperate Zones

Christie E. Mayo, Cameron Osborne, N. James Maclachlan
School of Veterinary Medicine, University of California, Davis, California

Recent field studies on commercial dairy farms have further identified how bluetongue virus is sustained in temperate regions between seasonal periods of transmission, which has been a topic of much conjecture and speculation for over 100 years. Earlier claims from the mid-20th century that the virus persisted in livestock were eventually disproven, and attention has increasingly focused on the insect vector. In a recent publication Mayo at al. reported that host-seeking parous female *Culicoides* midges were caught during daylight hours in midwinter in CO_2 baited traps. Using RT-PCR, she then confirmed these traps midges had strong CT values for BTV RNA. The finding that bluetongue virus can "overwinter" in long-lived female midges has important ramifications for predicting the occurrence of bluetongue in livestock and to its eventual control. Subsequent studies have failed to show vertical transmission of the virus in either laboratory reared *Culicoides* midges or field-collected larvae.

Mayo CE, Mullens BA, Reisen WK, Osborne CJ, Gibbs EP, Gardner IA, MacLachlan NJ. 2014. Seasonal and interseasonal dynamics of bluetongue virus infection of dairy cattle and Culicoides sonorensis midges in Northern California – implications for virus overwintering in temperate zones. PLoS One 9: e106975

Bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) isolations/PCR positives Calendar year 2013

Eileen Ostlund, USDA-APHIS-VS National Veterinary Services Laboratory, Ames, Iowa

Bluetongue virus or RNA was detected in 102 samples submitted or collected during calendar year 2013. The positive bluetongue virus isolation (VI) and polymerase chain reaction (PCR) test results from submissions to the National Veterinary Services Laboratories (NVSL) in 2013 are listed in Table 1.

Table 1. BT virus isolation (VI) / PCR positives, Calendar year 2013

State	*No.*	*Species*	*PCR*	*VI*	
CA	5	Cattle	Positive	Negative	Unable to type; 1 also EHDV-2
CA	2	Cattle	BTV-11	Negative	1 also EHDV-2
CA	3	Pronghorn	BTV-13	BTV-13	1 also EHDV-2
CA	3	Cattle	BTV-17	BTV-17	
CA	6	Cattle	BTV-17	Negative	2 also EHDV-2
FL	10	Nubian Goat (single herd)	Positive	Negative	Unable to type
FL	1	Nubian Goat	BTV-1	BTV-1	
FL	1	Nubian Goat	BTV-2	BTV-2	
FL	2	Nubian Goat (1), Deer (1)	BTV-3	BTV-3	Deer isolate (SCWDS)
FL	1	Deer	BTV-11	BTV-11	
IN	1	Saanen Goat	BTV-11	Negative	
IA	3	Bison (1), Cattle (2)	Positive	Not done	Unable to type
IA	2	Cattle	BTV-13	Negative	
MO	1	Cattle	BTV-17	BTV-17	
NE	8	Bison (7), Cattle (1)	Positive	Negative or not done	Unable to type; 1 bison also EHDV
NE	4	Bison (single herd)	BTV-10	Not done	
NE	1	Bison	BTV-11	Negative	
NE	1	Cattle	BTV-13	Negative	
NE	4	Bison (single herd)	BTV-13	BTV-13 (2)	1 also EHDV-2
NE	7	Bison (single herd)	BTV-17	Negative or not done	
NE	1	Bison	BTV-17		SCWDS isolate; also EHDV-2 & BHV-4

State	No.	Species	PCR	VI	
NE	1	Bison	BTV-13 & 17	Not done	
OH	1	Deer	BTV-17	BTV-17	
OK	1	Cattle	Positive	Negative	Unable to type
PA	13	Cattle	Positive	Negative	Unable to type
PA	4	Cattle	BTV-13	BTV-13	
SD	3	Pronghorn	BTV-11	BTV-11	
SD	3	Deer	BTV-17	BTV-17	
SD	7	Deer	BTV-17	Negative or not done	2 also EHDV-2
TX	1	Sheep	BTV-13	Negative	
WA	2	Llama (1), Sheep (1)	BTV-11	BTV-11	
WA	1	Alpaca	BTV-11	Negative	

During calendar year 2013, 113 samples tested positive for EHDV by virus isolation and/or PCR. The positive EHDV isolation and PCR test results from submissions to NVSL in 2013 are listed in Table 2.

Table 2. EHDV isolation (VI)/ PCR positives, Calendar year 2013

State	No.	Species	PCR	VI	
CA	5	Cattle (single herd)	EHDV-2	EHDV-2 (1) Not done (4)	1 also BTV-pos 1 also BTV-11 2 also BTV-17
CA	3	Pronghorn	EHDV-2	EHDV-2	1 also BTV-13
FL	1	Deer	EHDV-6		SCWDS isolate
IL	1	Deer	EHDV-2	EHDV-2	
IL	1	Deer	EHDV-2	Not done	
IA	1	Cattle	Positive	Not done	No type
IA	14	Cattle (10), Deer (4)	EHDV-2	EHDV-2	
IA	34	Bison (5), Cattle (20), Deer (9)	EHDV-2	Negative or not done	
MN	1	Cattle	EHDV-2	Negative	
MS	1	Deer	EHDV-2		Isolate submitted for typing
MO	1	Cattle	EHDV-1	Not done	Insufficient sample
MO	2	Deer (1), Sheep (1)	EHDV-2	EHDV-2	
MT	1	Deer	EHDV-2	EHDV-2	
MT	2	Bison	EHDV-2	Negative	
NE	3	Bison	Positive	Not done	No type; 1 also

State	No.	Species	PCR	VI	
					BTV positive
NE	3	Bison	EHDV-2	EHDV-2	1 also BTV-13
NE	9	Bison	EHDV-2	Negative or not done	
NE	1	Bison	EHDV-2		SCWDS isolate; also BTV-17, BHV-4
ND	1	Cattle	EHDV-2	Negative	
OK	1	Deer	EHDV-2	EHDV-2	
SD	2	Deer	EHDV-1	EHDV-1	
SD	12	Cattle (6), Deer (5), Elk (1)	EHDV-2	EHDV-2	2 Deer also BTV-17
SD	14	Cattle (9), Deer (4), Elk (1)	EHDV-2	Negative or not done	
SD	1	Deer	EHDV-6	EHDV-6	
WI	1	Cattle	Positive	Negative	No type

Part-year 2014 data for NVSL orbivirus identifications is shown in Table 3. As of October 10, BTV has been identified in 7 samples from 5 states and EHDV has been identified in 4 samples from 3 states.

Table 3. Bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) isolations/PCR positives during Calendar year 2014 (January 1 through October 10)

State	No.	Species	PCR	VI	
CO	1	Goat	BTV-11	BTV-11	
ID	1	Alpaca	BTV Positive	Not done	High Ct; insufficient virus for typing
MO	1	Cattle	BTV Positive	Not done	Insufficient virus for typing
NE	1	White-tailed Deer	BTV-17	Negative	
NJ	3	White-tailed Deer	BTV-17	BTV-17 (2), *1 pending*	2 were SCWDS submissions
FL	1	Deer	EHDV-2	EHDV-2	
FL	1	White-tailed Deer	EHDV-6	EHDV-6	
NC	1	White-tailed Deer	EHDV-6		Isolate submitted
TX	1	Eld's Deer	EHDV-2	EHDV-2	

SCWDS Update: Hemorrhagic Disease and *Culicoides* sp. Surveillance

Dr. Danny Mead, Southeastern Cooperative Wildlife Disease Study (SCWDS), University of Georgia

Dr. Daniel Mead presented the SCWDS hemorrhagic disease report as well as an update on the SCWDS *Culicoides* survey. SCWDS has received samples (mostly WTD) from 13 states for HD testing. EHDV-2 has been detected in samples submitted from GA, LA, and OR. EHDV-6 has been detected in samples submitted from FL and NC. BTV-17 was detected in NJ. SCWDS has been conducting surveys to determine what *Culicoides* spp. are present in the SE since 2007. Since 2007 they have collected at 307 sites and have collected over 227,196 biting midges. Dr. Mead provided a list of species that were found outside of their previously recorded ranges.

The Arthropod-borne Animal diseases Research Unit: research Program Update and Current Status

D. Scott McVey

USDA-ARS, Arthropod-borne Animal Diseases Research Unit, Manhattan, Kansas

The Arthropod Borne Animal Diseases Research Unit's (ABADRU) research mission is to solve major endemic, emerging, and exotic arthropod-borne disease problems in livestock. The Unit completed the move to Manhattan, KS in 2010 and now the ABADRU is well established at the Center for Grain and Animal Health Research (CGAHR). Five new scientists that were hired to replace the scientific staff that did not relocate to KS are well on the way to establish new research ABADRU programs under the ARS National Research Programs: NP103 and Animal Health and NP104, Veterinary, Medical, and Urban Entomology. The areas of research range from vector biology to understand virus-host interactions to better control these important diseases.

Committee Business:

1. Seconded nominations were received and passed by unanimous vote of the committee membership that Dr E. Paul Gibbs serve as incoming Chair of the Committee and Dr. D. Scott McVey as Vice Chair.
2. A resolution regarding a proposed national strategy for orbiviruses to support the international export of ruminant livestock from the United States was moved, seconded and passed unanimously.

With no further business the meeting was adjourned.