Agenda for PALE-Blu meeting to be held at Institut agronomique et vétérinaire Hassan II, September 19th and 20th September 2018

Day1 (19th September 2018)		
Time	**Speaker and (Institution)**	**Title of talk**
9:00-9:30	**Registration and Coffee**	
Session 1 :	**Chairs: P. Mertens & Ouafaa Fassa Fihri**	
9:30 – 09:45	**Peter Mertens** (Coordinator) (UNott – UK)	**Talk 1**: PALE-Blu: welcome and introduction
9:45 -10:00	**Bernd Hoffman** (FLI - Germany)	**Talk 2**: Atypical Bluetongue viruses from Mongolia – an update
10:00 - 10:15	**Christina Ries** (FLI - Germany)	**Talk 3**: "BlueTYPE - from BTV positive field sample to serotype identification in 2 hours"
10:15 - 10:35	**Giovanni Savini** Alessio Lorusso, Giuseppe Mancini, Barbara Alessandrini (IZSAM – Italy)	**Talk 4:** Application of the NanoString Technologies nCounter Platform in the Bluetongue diagnostics
10:35 - 10:55	**Antoine Mignotte, Julie Reveillaud and Karine Huber** (CIRAD - France)	**Talk 5**: Population genetics and bacterial community structures of *Culicoides obsoletus*: a mirror of European geography?
10:55-11:30	**Coffee break**	
Session 2 :	**Chairs: Stephan Zientara & Bernd Hoffmann**	
11:30 -11:50	**Serafin Gutierrez and Virgine Dupuy** (CIRAD, France)	**Talk 6**: Developments in virome analysis from *Culicoides* samples
11:50- 12:10	**Assane Gueye FALL and Modou Moustapha Lo** (ISRA –Senegal)	**Talk 7**: Progress in PALE-Blu activities in Senegal
12:10-12:30	**Mirazimi Ali** (SVA – Sweden)	**Talk 8**: Haploid embryonic stem cells as tool for the identification of host factors relevant for Bluetongue virus (BTV)
12:30-12:50	Zati Vatansever (KAU – Turkey)	**Talk 9**: *Culicoides* species monitoring studies in Turkey
12:50-13:10	(IAV - Morocco)	**Talk 10**: TBC
13:10 - 14:40	**Lunch break**	
Session 3:	**Chair: Piet Van Rijn & David Haig**	
14:40-15:00	**Lyndsay Cooke**, Karin Darpel, Simon Carpenter (TPI - UK)	**Talk 11**: Investigating virus-vector-host interactions that alter BTV infectivity
15:00-15:20	Alexandra Hardy, Meredith Stewart, Aislynn Taggart, Mariana Varela, Andrew Shaw, Sam Wilson, **Massimo Palmarini** (UGLA-UK)	**Talk 12**: Host "resilience" to bluetongue virus infection correlates with viral evasion of the type I interferon response
15:20-15:40	**Theocharis Tsoleridis**, Richard Urbanowicz, Johnathan Ball (UNott - UK)	**Talk 13**: Development of a sheep monoclonal antibody platform against Bluetongue virus.
15:40-16:00	David Haig UNott	**Talk 14**: Vaccination strategy for cross-serotype BTV control

16:00-16:30		**Coffee break**
Chair: David Haig & Piet Van Rijn		
16:30 – 16:50	**Piet A. van Rijn**, and René G.P. van Gennip (WBVR)	**Talk 15**: Preparation of virus stocks of DISA vaccine candidates
16:50 - 17:10	**Javier Ortego** (INIA - Spain)	**Talk 16**: CD8 T CELL RESPONSES TO THE NON-STRUCTURAL PROTEIN NS1 PROVIDES WIDE IMMUNOPROTECTION AGAINST BLUETONGUE VIRUS IN IFNAR(-/-) MICE.
17:10 – 17:30	**Hela Kallel** (Institut Pasteur de Tunis)	**Talk 17**: Expression of chimeric VP2 protein of different serotypes of bluetongue virus in methylotrophic yeast *pichia pastoris*
End of Scientific session for Day 1		
Dinner: 19:30		

Day 2 - Session 4 :	Chair: Roman Biek & Massimo Palmarini	
09:00-09:20	**Kyriaki Nomikou** (UNott - UK)	**Talk 18: BTV-GLUE: New features and genotyping**
09:20-09:40	**Nicholas Svitek**, Robert Muriuki, Lucilla Steinaa	**Talk 19**: BTV Status in Kenya, Insights from Machakos
09:40-10:00	**David Pascall**, Maude Jacquot, Kyriaki.Nomikou, Peter Mertens, Massimo Palmarini, Roman Biek (UGLA -UK)	**Talk 20**: Uncovering mechanisms of BTV persistence and spread using virus genomic data
10:00-10-20	**William Wint** (ERGO - UK)	**Talk 21**: Project websites and data archives.
10:20-10:40	**Marius Gilbert** (ULB – Belgium)	**Talk 22**: Defining generic ecoregion for bluetongue vectors in Europe.
10:40-11:20	**Coffee**	
Session 4 (continued):	**Chair: Massimo Palmarini & Houssam Attoui**	
11:20 – 11:40	**Sofian Sghaier** (Laboratoire de virologie, Institut de la Recherche Vétérinaire de la Tunisie)	**Talk 23**: Bluetongue sentinel surveillance and monitoring of the genus culicoides in relation of orbiviruses in Tunisia during 2017
11:40-12:00	**S. Zientara** [a], E. Breard[a], C. Viarouge[a], A. Gorlier[a], Y. Blanchard[b], M. Grancollot[c], D. Vitour [a], C. Sailleau[a], (Anses / INRA – France)	**Talk 24**: Emergence of BTV-4 in Mainland France.
12:00-12:20	C. Aguilar-Vega[1*], E. Fernández-Carrión[1], **J.M. Sánchez-Vizcaíno**[1] (UCM - Spain)	**Talk 25:** Application of the ADS model to study the introduction of BTV-3 in Sicily
12:20-12:40	**Houssam Attoui** (Anses / INRA - France)	**Talk 26:** Species barrier to orbiviruses and antiviral strategies
12:40-13:00	**Lesley Bell-Sakyi** (University of Liverpool – UK)	**Talk 27:** Culturing cells from different Culicoides species
13:00 - 14:30	**Lunch break**	
14:30 – 17:30 **Discussion of collaborations for Objectives 1 to 6 (up to 30 minutes each)** **Initiated by 5 minute talk by each work package leaders**		
Objective 1: (WP1 & WP2) (Chairs: Kyriaki Nomikou & Roman Biek) Develop a more detailed and up-to-date molecular-epidemiology map of BTV strains circulating in domesticated and wild ruminants in Europe and neighbouring countries:		
Objective 2: (WP3 & WP4) (Chairs Thomas Balenghien & Marius Gilbert) Map interactions between the environment, the composition of the *Culicoides* species community, the genetic characteristics of Culicoides populations and their microbiomes, to quantify connectivity between midges and virus sub-populations:		
Objective 3: (WP5) (Chairs: Peter Mertens and Stephan Zientara) Develop innovative multiplex diagnostic tools for BTV identification and typing:		
16:00 – 16:30	**Coffee**	
Objective 4: (WP6, WP7, WP8, WP9) (Chairs: Peter Mertens & Karin Darpel) Characterise orbivirus-related genetic control of infection and horizontal transmission in vertebrate hosts, as well as infection replication and vector competence in European Culicoides spp.:		
Objective 5: (WP10, WP11) (Chairs: David Haig & Massimo Palmarini) Develop novel BT vaccines, vaccination strategies and antiviral approaches for BTV:		
Objective 6: (WP12) (Chairs: Peter Mertens David Haig)		

Project management, coordination and communications:
17:30 End of meeting

Please note:

PALE-Blu: participants/Contact/Mailing list: * indicates contributors not attending the meeting

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Abstracts

Talk 1: PALE-Blu: welcome and introduction:

Peter Mertens (coordinator)

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Talk 2: "Atypical Bluetongue viruses from Mongolia – an update"

Christina Ries and Bernd Hoffmann

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ABSTRACT

The FLI (project partner 5) will be involved in the working packages 1, 2, 5 and 8 of the project consortium. That includes the genetic and epidemiological characterisation of BTV strains circulating in Europe as well as in source regions in the world. In this context, we analysed blood samples from goat, sheep and cattle from Mongolia. All samples were screened by BTV group-specific RT-qPCR based on the segment 10. BTV genome positive strains were detected in goat and sheep samples. The positive samples were partial sequenced on the VP2 genome and a virus isolation on BSR cells were performed. The sequence data confirm the identification of at least three novel serotypes of BTV with less than 75% sequence identity to the published serotypes. The sequencing of the complete genomes is ongoing. Furthermore, two of the three novel BTV serotypes could be propagated in cell culture. Based on the generated cell propagated virus strains additional work on the pathogenesis, transmission and host-dependent replication will be possible.

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Talk 3: "BlueTYPE - from BTV positive field sample to serotype identification in 2 hours"

Christina Ries, Martin Beer, Bernd Hoffmann

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Background: Bluetongue virus (BTV; genus Orbivirus, family Reoviridae) is a double-stranded (ds) RNA virus with 10 genome segments. VP2 is the primary target for neutralizing antibodies and defines the serotype. Today, more than 27 serotypes are known and new serotypes are under investigation. Beside the group-specific BTV-genome detection, further serotype characterization is of importance to understand the epidemiology of the disease and for efficient outbreak control.

Methods: A low-density RT-qPCR array representing a panel of group- and serotype specific assays was combined with an internal control system. For BTV serotype detection both published (Maan et al., 2016) and newly developed in-house PCR-systems were combined. The different primer-probe-mixes were placed in 48 wells of a 96 well plate stored at -20 degree until use. The RT-qPCR array can be started in less than 15

minutes. First, the template RNA will be added to the prepared primer-probe-mixes in the PCR plate and heat denatured at 95°C for 3 min. After cooling, the RT-qPCR mastermix will be added in each well and a PCR run of around 90 min can be started. Beside the fast identification of the BTV-serotype in clinical cases, the developed low-density RT-qPCR-array can be easily extended with novel BTV serotype assays or assays for differential diagnosis like EHDV or FMDV.

Results: So far, we analyzed the analytical sensitivity of the array system with 24 typical BTV serotypes using log 10 dilution series. Loss of analytical sensitivity around 1 to 2 Cq-values compared to the group-specific BTV assay was observed, like BTV 1, 4, 13, 19, 23 and 24. For serotypes 2, 15, 16, 17, 20 and 21 similar sensitivity like in the pan BTV-assay could be observed. In addition, the serotype specific assays for serotypes 6, 8, 9, 10, 11, 12, 14, 18 and 22 were even more sensitive than group-specific BTV assay.

Conclusions: A panel of serotype assays could be successfully established, and to our experience, it is feasible to obtain fast and reliable results for serotype identification using the multi-well BTV low-density PCR-array. However, assay parameters for several serotypes need to be changed for further improving the sensitivity of selected serotype-specific PCRs within the BTV-typing array. The adaption of the primer/-probe concentration as well as the split of published and in-house assays in independent wells on the array are possible solutions. For BTV serotype 23 newly designed primers might be necessary.

Key words: BTV, serotypes, qRT-PCR, sensitivity, array

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Talk 4: Application of the NanoString Technologies nCounter Platform in the Bluetongue diagnostics

Giovanni Savini
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Bluetongue virus (BTV) is a segmented double-stranded RNA virus belonging to the genus *Orbivirus* of the family *Reoviridae* and it is transmitted mainly by *Culicoides* spp midges. Up to 2008, 24 serotypes of BTV were officially recognized. However, in the last years novel and generally mild pathogenic BTV serotypes, so far identified as small ruminant adapted BTV, have been described in the field. These include BTV-25 (TOV strain) from Switzerland, BTV-26 from Kuwait, BTV-27 (variants 01, 02 and 03) from Corsica (France), BTV-XJ1407 from China, a BTV strain isolated from a sheep pox vaccine (SP vaccine derived BTV), BTV-X ITL2015 from Sardinia (Italy), BTV-Y TUN2017 from Tunisia and BTV-Z ITA2017 from Italy. Genome constellation of BTV is composed by 10 segments and among them, Seg-2 encodes for the VP2, the outermost protein of the virion which determines serotype specificity eliciting the production of specific protective antibodies. Multiple BTV serotypes can co-circulate in the same region, as seen in Italy with BTV-1 and BTV-4 during 2014. Identification of BTV serotype uses multiple typing assays, that tend to be executed based on the known epidemiological situation within a given country. Samples containing multiple serotypes (particularly those including newly introduced strains) may therefore be missed. The complete identification of even a single BTV strain can therefore be expensive and time consuming. The aim of this work is to develop a diagnostic test, based on the nCounter® Analysis System platform (Nanostring technologies), that would all at once and simultaneously identify all BTV serotypes, in biological samples, including internal organs and blood specimens and in tissue culture adapted BTV strains, saving time and operator steps. The nCounter® Analysis System uses the principle of molecular hybridization of sequence-specific color code (that works as a molecular barcode) and capture probes. This technology is actually, mainly used for gene expression experiments. Probes were designed according to all Seg-2 sequences available on line. The accuracy of this new diagnostic system has therefore been tested with reference and atypical BTV strains and a panel of of field biological samples including some infected with historical BTV serotypes (BTV-1, BTV-2, BTV-4, BTV-9,

BTV-16), some with novel BTV serotypes and some with two or more BTV serotypes. All samples included in the diagnostic trial were correctly identified no matter whether they were infected with one or more serotypes. Aiming at using this test in the routine diagnostic, further investigations are currently ongoing to assess the specificity with a larger panel of BTV infected samples collected worldwide, to improve its sensitivity of the test and to define the sensitivity threshold. .

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Talk 5: Population genetics and bacterial community structures of *Culicoides obsoletus*: a mirror of European geography?

Antoine Mignotte[1], Karine Huber[2], Adrien André[3], Johan Michaux[1,3], Etienne Loire[1], Frederic Mahé[4], Laetitia Gardès[1], Thomas Balenghien[3], Maxime Duhayon[1], Ignace Rakotoarivony[1], Laura Tabourin[1], Josue Martinez de la Puente[4], Javier Lucientes[5], Adolfo Ibañez-Justicia[6], Helge Kampen[7], Doreen Werner[8], Marion England[9], Simon Carpenter[9], Julie Reveillaud[2], Claire Garros[10].

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Understanding how geographical and environmental features, landscape patterns and in particular those created by human land uses structure host and their associated microbial communities is key to understand the spread of vector-borne diseases. *Culicoides* dispersal capacities are described as 'dispersive stratified' which results from the combination of processes occurring at a short distance combined with jumps over long distances. Although winds and local air movements have an undeniable role in the dispersal of *Culicoides*, anthropogenic factors also play a predominant role, particularly at the local level (host distribution, landscape patterns). *Culicoides obsoletus* is reported in sympatry with one morphologically indistinguishable species, *C. scoticus*, and other morphologically related species, *C. chiopterus*, *C. dewulfi* and *C. montanus*. Moreover, recently, several authors have reported the existence of cryptic diversity within the species commonly called *C. obsoletus*. Our first overall objective was to characterize the dispersal abilities of two main vector species in Europe, *C. obsoletus sensu stricto* and *C. chiopterus*, showing different host-vector behaviors.

Sequencing a portion of the Cox1 mitochondrial gene of 3,200 *C. obsoletus sensus lato/C. scoticus* individuals from 17 European countries revealed two clades within *C. scoticus* and confirmed the presence of at least three undescribed phylogenetic clades *(C. obsoletus* clade O2*, C. obsoletus* clade "Dark" and one not yet named) close to *C. obsoletus s.s*. These results are reinforced by rDNA16S mitochondrial gene sequences and a gene coding for ribosomal rDNA18S, over the entire haplotypic diversity resulting from Cox1 barcoding. Then, we investigated how dispersion shapes the spatial arrangement of *C. obsoletus s.s* genetic diversity in Europe using 13 microsatellite markers by observing patterns of allelic frequency distributions. This work is the first step to a more comprehensive study on the landscape genetics of two main vector species in the Palearctic region, *C. obsoletus s.s* and *C. chiopterus*.

In addition, in order to assess connectivity between midges and their associated bacterial communities, we investigated the bacteriome of 88 *Culicoides obsoletus s. s.* from six European countries using 16S rRNA gene amplicon high-throughput sequencing. Even though the Northern countries exhibited distinct bacterial assemblages than the Southern ones, data suggested that factors other than geography drive the structure of bacterial communities. These preliminary results set the stage for additional analyses of the microbiome of *Culicoides* at finer scales and the factors influencing its community structure.

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Talk 6: Developments in virome analysis from *Culicoides* samples

Serafin Gutierrez and Virgine Dupuy (CIRAD, France)

The identification of viruses associated to animals and plants has been boosted by the implementation of virus metagenomics using high-throughput sequencing technologies. The interest of this methodology for both virus ecology and diagnostics has triggered technical improvements in most steps required in virus metagenomics, from nucleic-acid extraction to bioinformatics analysis of sequencing results. Nevertheless, there is yet a key step in virus metagenomics that has received little attention, that of library preparation. Here, we will present a new library preparation for virus metagenomics. Contrary to commercial kits, our protocol allows high-throughput processing of samples at a low cost, together with an excellent output of virus sequences. We will show challenges in virus metagenomics associated to Culicoides samples that have required protocol optimisation and how protocol improvements allow for virus discovery and sensitive arbovirus detection from wild-caught midges.

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Talk 7: Progress in PALE-Blu activities in Senegal

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Under the Pale-Blu project, studies are conducted to identify and characterise BTV strains circulating in Senegal through animals and local competent vector populations that are potentially capable of transmitting the virus to ruminants. For that EDTA-blood samples and sera samples will be collected from sheep and goats throughout all fourteen Senegalese regions, along with local populations of *Culicoides.* These vectors will be collected using both OVI light / suction traps and CDC light CO2 baited traps set during overnight from one hour before sunset to one hour after sunrise. Captured individuals species will be identified morphologically using morphological keys. The commercial kit ID Screen® Bluetongue Competition will be used for sera screening and positive samples tested by RT-PCR to determine genome and serotype circulation.

Currently a total of 245 sera collected from three regions from sheep and goats have been tested for anti-VP7 antibodies detection. Results shows a global seropositivity of 57.55% (n=141) with 62% in sheep and 48.8% in goat. The seropositivity varied significantly according to regions. Of the 141 positive animals, BT virus was detected in 73 (51.77%). Circulating strains will be characterize through serotyping. *Culicoides* species potential BTV vectors (*C. kingi, C. imicola* and *C. oxystoma*) accounted among the dominant species. The abundance of these species varied significantly according to the locality. *Culicoides* pools will be proceed for BTV detection.

Collaboration will be established with partners to screening for all the serotypes well documented for positive animal and *Culicoides* samples.

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Talk 8: Haploid embryonic stem cells as tool for the identification of host factors relevant for Bluetongue virus (BTV)

Lijo John[1], Ulrich Elling[2], Caroline Venersson[1], Josef Penninger[2] and Ali Mirazimi[1]

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We are using genetically barcoded haploid embryonic stem cells to uncover the genes that are relevant for BTV infection. Currently we are screening for the surviving, BTV resistant embryonic stem cells and the selected hits will be chosen forward for the functional validation. Additionally, we are also studying the vector-pathogen molecular interactions of BTV in tick and midget cells. Identification of these interactions will define the vector competence, and this will assist to develop new strategies to prevent these diseases.

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Talk 9: *Culicoides* species monitoring studies in Turkey

Zati Vatansever, Ahmet Deniz, Berna Demirci and Hilal Bedir

Culicoides species monitoring studies has been carrying out in 62 cities with 118 light traps (onderstepoort type), in Turkey. Samples were collected two times in a month. Animal type, altitude, coordinates and geographic information were recorded before trapping. In 2017 192.170 individuals were collected and 33 species were identified. Samples were divided in to 550 pools which contains 100-150 individuals each of them. Pools were tested for Blue tongue (BT), Epizootic Hemorrhagic Diseases (EHD), Bovine Ephemeral Fever (BEF), AKABANE viruses by Real Time PCR. Six of the *C. shultzei* pools were determined positive for AKABANE virus and nine pools (7 *C.imicola*, 1 *C. punctatus*, 1 *C.newsteadi*) founded positive for BT virus.

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Talk 10: TBC

Talk 11: Investigating virus-vector-host interactions that alter BTV infectivity

Lyndsay Cooke, Simon Carpenter, Lara Harrup and Karin Darpel

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Culicoides biting midges serve as the biological vector for Bluetongue virus (BTV) transmission to the vertebrate host. During midge feeding BTV is deposited into the ruminant skin alongside biologically active *Culicoides* saliva. BTV therefore forms part of a complex set of interactions whereby virus infectivity may be influenced by viral, vector and vertebrate host factors. To facilitate the successful acquisition of a blood meal, *Culicoides* midges must overcome the host homeostatic processes that are initiated as a result of midge feeding by depositing anti-inflammatory and immune-modulatory saliva into the bite site. In addition to saliva induced disruption of host physiological and immunological processes, which likely indirectly contribute to BTV infectivity, it is known that *Culicoides* saliva can directly alter BTV infectivity through protease activity that it directed towards VP2. The cleavage of this BTV outercoat protein results in the generation of infectious subviral particles (ISVPs) that have a modified structure and also an increased infectivity for *Culicoides* spp. and *Culicoides* derived cells. The proteolytic ability of *Culicoides* saliva to cleave BTV VP2 has been previously characterised for serotype 1 and serotype 16, while its abilities and efficiency to cleave VP2 of other BTV serotypes has not been determined. Additionally it is currently unknown if saliva cleaved ISVPs also demonstrate modified infectivity across the wide range of natural ruminant target cells. Within this project we are investigating the effect of serotype 1 VP2 cleavage on viral infectivity for selected ruminant target cells and furthermore assess the cleavability of VP2 from additional serotypes and in doing so investigate cross serotype variability in VP2 size and physical properties.

Viral factors, such as VP2 cleavability, may also impact on the ability of selected strains to be acquired by, replicate within and be transmitted by *Culicoides* midges. A panel of BTV strains which displayed highly divergent ability to replicate within the *C. sonorensis* model have indicated a potential role for VP2 in viral determinants of observed vector competence (VC) rates. Additional comparison of *C. sonorensis* infection across strains from the same serotype allow for further investigations of VP2 role in vector infection. Trials are currently underway as part of this project to enable the standardisation of VC determination and to highlight the ability to compare VC estimates that are determined across various methods of midge processing.

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Talk 12: Host "resilience" to bluetongue virus infection correlates with viral evasion of the type I interferon response

Alexandra Hardy, Meredith Stewart, Aislynn Taggart, Mariana Varela, Andrew Shaw, Sam Wilson, **Massimo Palmarini** (UGLA-UK)

Abstract not yet available

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Talk 13: "Development of a sheep monoclonal antibody platform against Bluetongue virus"

Tsoleridis T, Urbanowicz RA, McClure CP, Ball JK

Bluetongue (BT) is a non-zoonotic arboviral disease of wild ruminants and livestock in many parts of the world with a very high economic impact on the livestock industry. It is, thus, important to develop efficient diagnostic and therapeutic tools against the virus. In this study, three different sheep (Ovis aries) were immunised with different BT virus (BTV) VP2 proteins to generate antibodies. The immunisation process included a primary inoculation followed by 3 boosts on days 18, 32 and 46 post immunisation respectively. Blood was collected from the immunised sheep and peripheral blood mononuclear cells (PBMCs) were isolated. Single B-cells were sorted with flow cytometry and PCR was performed to amplify their immunoglobulin heavy (IgH) and light (Igλ) chain genes. Heavy and light chains of interest would be then cloned into an expression vector for antibody production. Simultaneously, we developed a deep amplicon sequencing-based method to generate the antibody repertoire of the sheep throughout the immunisation process. Eventually, we compared the single-cell PCR results to the antibody repertoire, and we proceeded with cloning and expressing the most frequent ones. Monoclonal antibodies able to neutralise BTV could be used as therapeutics against the virus and antibodies able to efficiently bind to the virus could be used as diagnostic tools. This study describes the development of a novel cutting-edge platform for generating monoclonal antibodies against BTV.

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Talk 14:
David Haig

Develop novel BT vaccines and vaccination strategies for bluetongue virus (BTV): cross-serotype protection.

David Haig; Elizabeth Reid; Petra Fay; Janet Daly, Sarah Gilbert* and Peter Mertens

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The current commercial BTV vaccines consist of either live attenuated viruses, or inactivated tissue culture 'grown' viruses. They may be effective at preventing disease but are serotype-specific and do not allow discrimination of infected from vaccinated animals (DIVA). We are attempting to design novel subunit vaccines that are cross-serotype-specific, by generation of cross-reactive virus-neutralising antibodies (nAb)

to the VP-2 capsid proteins (responsible for serotype specificity), in combination with cell-mediated immunity to non-structural protein 1 (NS1) that is relatively conserved amongst BTV serotypes and is cross-serotype protective.

~20 plant-expressed recombinant VP2s of different BTV serotypes were used to immunise rabbits, and the resulting antisera tested by VP2 ELISAs and in serum-neutralising tests (SNT). In addition, antisera from sheep infected with each reference strains of BTV (BTV-1 to BTV-26) were tested by VP2 ELISA and SNT.

A small pilot sequential immunisation experiment was performed in sheep using a selection of the VP2s and antisera analysed as above. The results show that there are several cross-serotype VP2-specific antibody responses detected by ELISA, and importantly, some examples of cross-serotype neutralising antibody responses. Current experiments are exploring the nAb responses in more detail. Finally, genes coding for a range of VP2s are being cloned into the chimp adenoviral (ChAdv) and modified vaccinia virus (Ankara) vectors in preparation for mouse and sheep immunisation experiments. These vectors have already proved successful in vaccination studies for a range of human and animal diseases.

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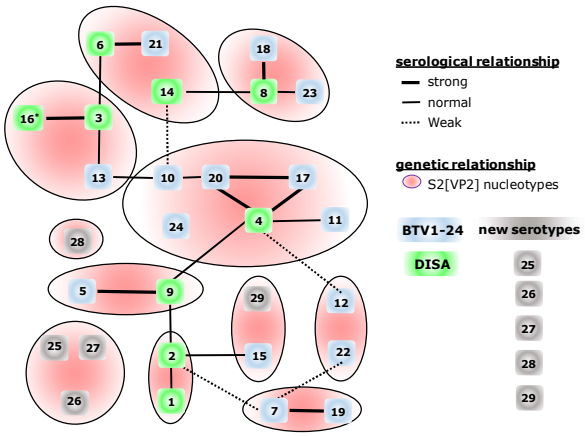
Talk 15: Preparation of virus stocks of DISA vaccine candidates

Piet A. van Rijn, and René G.P. van Gennip (WBVR)

The previously developed BT DISA/DIVA vaccine platform is based on live-attenuated vaccine strain BTV6/Net08 without NS3/NS3a protein. Several deletions in genome segment 10 Seg-10[NS3/NS3a] have been shown genetically unstable. All these derivatives are phenotypically similar and are related to lack of functional NS3/NS3a protein and thus disturbed in virus release from in particular *Culicoides* (KC) cells. However, genetic instability of vaccine virus is a negative feature with regard to vaccine registration. We have improved the genetic stability of the BT DISA/DIVA vaccine platform by use of a small in-frame deletion in Seg-10[NS3/NS3a] encompassing the essential (DISA principle) and immunogenic (DIVA principle) Late Domain. In addition, the in-frame deletion in Seg-10 is genetically stable for subsequent passages of DISA vaccines.
Previous studies suggested that vaccine viruses with exchanged Seg-2[VP2] for some serotypes are less stable, although this has not been conclusively proven. Further, exchange of both outer shell proteins would enhance the serotype specific immune response. Therefore, BT DISA/DIVA vaccines are generated by exchange of genome segments Seg-2[VP2] and Seg-6[VP5] encoding both outer shell proteins for several serotypes, see table. Most selected serotypes are present in or at the border of Europe and are also representing most subgroups / nucleotypes of BTV, see figure. Note, 2IT and 2USA are based on sequences of BTV-2 from Italy and USA, respectively. Since Seg-2 of 2IT in combination with Seg-6 of reference BTV-2 (2SA) grows to low virus titers, we generated BT DISA vaccine 2 based on 2USA. 3SA is based on virulent BTV-3 circulating in South Africa. 1/8 and 1/16 represent chimeric Seg-2 of these serotypes as previously described (Feenstra et al., 2015). BT DISA/DIVA vaccines are produced on BSR monolayers.
Virus stocks of BT DISA/DIVA vaccines are stored at 4°C and -80°C for a longer time period and will be titrated again in order to confirm virus stability. So far, no other serotypes are considered at the moment. Unfortunately, rescue of BT DISA/DIVA vaccine for serotype 7 was not successful so far, see figure.
A selection of BT DISA/DIVA vaccines will be studied in cattle and sheep as mono-serotype prototypes and as cocktail vaccines.

BT DISA	outer shell		check by PCR		
vaccine	Seg-2	Seg-6	Seg-2	Seg-10	remark
1	1	1	1	+	ready
2	2IT	2SA	2	+	low titer
2	2USA	2USA	2		in progress
3	3SA	3	3	+	ready
4	4	4	4	+	ready
6	6	6	6	+	ready
8	8	8	8	+	ready
9	9	1	9		in progress
14	14	6	14	+	ready
1/8	1(8)	1	1(8)	+	ready
1/16	1(16)	1	1(16)		in progress



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Talk 16: ¨ CD8 T CELL RESPONSES TO THE NON-STRUCTURAL PROTEIN NS1 PROVIDES WIDE IMMUNOPROTECTION AGAINST BLUETONGUE VIRUS IN IFNAR(-/-) MICE¨.

Javier Ortego
INIA - Spain

The development of vaccines against Bluetongue, a prevalent livestock disease, has been focused on surface antigens that induce strong neutralizing antibody responses. Because their antigenic variability, these vaccines are usually serotype restricted. We now show that a single highly conserved non-structural protein, NS1, expressed in a modified vaccinia Ankara virus (MVA) vector can provide multiserotype protection in IFNAR(-/-) 129 mice against Bluetongue virus that is largely dependent on CD8 T cell responses. We found that the protective antigenic capacity of NS1 resides within the N-terminus of the protein and is provided in the absence of neutralizing antibodies. The protective CD8 T cell response requires the presence of a specific peptide within the N-terminus of NS1, since its deletion ablates the efficacy of the vaccine formulation. These data reveal the importance of the non-structural protein NS1 in CD8 T cell-mediated protection against multiple BTV serotypes when vectorized as a recombinant MVA vaccine.

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Talk 17: Expression of a chimeric VP2 protein of different serotypes of bluetongue virus in the methylotrophic yeast *Pichia pastoris*

Wafa Tombari[1], Ines Akrouti[1], Mariem Ben Zakour[1], Sofien Sghaier[2] and Héla Kallel[1]

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We studied the expression of domains of VP2 protein (aa 249 to 398) of BTV serotypes 1, 4 and 8 in the methylotrophic yeast *Pichia pastoris*. The expression of the chimeric protein was under the control of AOX1 promoter and targeted to the culture medium using α-factor as a secretion signal. These serotypes were selected since they are circulating in Tunisia and the Mediterranean basin. Peptides were linked by three Glycine polylinkers in order to insure the correct folding of VP2 domains. More specifically, and to improve the expression level of the chemeric VP2 and increase the translational efficiency in Pichia system, a codon-optimized gene was designed based on the protein sequences of VP2 according to the codon bias of *P. pastoris*. The optimized sequence was chemically synthesized.
The 1,384 bp sequence encoding the VP2 polyprotein gene was digested with EcoRI and XbaI restriction enzymes, and then inserted into the expression plasmid pPICZαA, yielding the recombinant plasmid used to transform KM71H strain. Numerous recombinant clones were obtained; randomly selected Zeocin-resistant recombinant clones were tested for the integration into the Pichia genome by PCR and sequencing. Upon induction with methanol, the expression of approximately 55 kDa extracellular protein was visualized by SDS-PAGE, corresponding to the expected size of the target protein. However, the immunoreactivity of the chimeric VP2 still needs to be checked. Western blotting using anti-BTV sera was not conclusive.
Copy number and the transcriptional level of the target gene in the selected recombinant Pichia clones assessment using real-time PCR are in progress.

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Talk 18: BTV-GLUE: New features and genotyping

Josh Singer[1], **Kyriaki Nomikou**[1,2], Rob Gifford[1], Joseph Hughes[1], Peter Mertens[2,3], Massimo Palmarini[1]

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3. The Pirbright Institute, Pirbright, Woking, UK

Recent advances in sequencing technologies has resulted in a wealth of virus genomic data.

Virus genome sequence data is fundamental in understanding virus biology, pathogenesis, evolution, outbreaks and epidemics. To make full use of the available virus sequence data it must be organised within databases.

We have developed BTV-GLUE, a new bioinformatics sequence data resource for bluetongue virus.

Sequences from the NCBI nucleotide database are curated along with complementary and improved metadata. These data are integrated together inside GLUE (http://tools.glue.cvr.ac.uk), a data-centric software package for capturing virus sequence data and organising it along evolutionary lines. The dataset also contains reference sequences with genome feature annotations, multiple sequence alignments and phylogenetic trees, for each BTV segment. Clades at different levels have been defined for BTV genome segment 2 and an automated genotyping tool has been developed and incorporated in BTV-GLUE. Various datasets can be downloaded, FASTA files of nucleotide (nt) and /or amino acid (aa) sequence data and metadata for the sequences. Multiple sequence alignments of nucleotide or amino acid sequences of defined full genome segments or restricted to defined genome segment regions can also be downloaded.

The beta version of the BTV-GLUE dataset is available via a public web server (http://btv.glue.cvr.ac.uk).

We continue with definition of clades for the rest of BTV genome segments and automated genotyping tool for all segments. Additionally, an automated tool for analysis of deep sequencing data will be developed.

BTV-GLUE will help the BTV community to study varying aspects of BTV biology and evolution and will facilitate the adoption of a nomenclature that more easily distinguished the properties of BTV strains circulating worldwide.

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Talk 19: BTV Status in Kenya, Insights from Machakos

Nicholas Svitek, Robert Muriuki, Lucilla Steinaa (ILRI- Keyna)

Bluetongue virus (BTV) has previously been reported to be highly prevalent in Kenya. Recent studies have shown that animals sampled in Western Kenya show around 90% exposure to the virus as determined by competitive ELISA (cELISA), and about 50% of them demonstrate on ongoing infection as measured by quantitative PCR (qPCR). We sought to investigate what is the level of BTV exposure in farm animals in other regions of the country. Therefore, during the first year of the project, the goal was to first obtain reagents and setup assays for diagnosing BTV at ILRI from samples collected in the field and attempt virus isolation on KC cells. For that purpose, a scientist from our group went to the Pirbright Institute to follow a training on BTV molecular diagnostics and implement these diagnostic assays at ILRI. Following this, we went to Machakos county, located in the Eastern province of Kenya, during the long rainy season, to sample cattle (n=182), sheep (n=170), goats (n=121) and camels (n=44). We are currently performing cELISA and qPCR assays with these samples to evaluate the level of exposure to the virus in these animals and identify the most prevalent circulating BTV strains in this region. The results of this investigation will be presented at the PALE-Blu annual meeting in Rabat, Morocco.

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Talk 20: "Uncovering mechanisms of BTV persistence and spread using virus genomic data"

David Pascall, Maude Jacquot, Kyriaki.Nomikou, Peter Mertens, Massimo Palmarini, Roman Biek

(UGLA -UK)

The European BTV-8 outbreak, the largest single serotype outbreak affecting Europe to date, has yet to be studied from an evolutionary perspective using full genomes. Using complete viral genomes sampled serially since 2006, we are using phylogenetic analyses to investigate its origins, demography and geography. Our ultimate aims are to understand the persistence of the virus across winters and over the period after 2009 during which no clinical cases were described. In this talk, we illustrate how phylodynamics approaches can allow questions such as this to be answered, and present preliminary results from an analysis of the demography of the virus from the beginning of the outbreak in 2006 to its re-emergence in 2015 based on over 70 full genomes. These results will be placed in the context of how they are progressing our initial aims: to test whether the virus experienced a seasonal reduction in

population size during the winter months when the midge vector is inactive, and to estimate where in space the virus persisted during the period from 2009-2015.

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Talk 21: Project websites and data archives.

William Wint

(ERGO - UK)

ERGO has contributed to a wide range of project outputs for the first period – these include the project website – www.paleblu.eu and the spatial data website www.palebludata.com of which overviews of content and future plans are presented. An important focus of the Company's activities has been to populate the spatial data site with data requested by partners, and to update existing datasets where needed. Several significant additions have been to the exiting catalogue of some 2000 covariate and other datasets. These include three global firsts: a categorised road density parameter for Europe and north Africa; an updated 2000-2016 archive of biologically relevant climatic indicators of produced by Fourier analysis of MODIS satellite imagery; and coverages of monthly wind speed and wind direction for 2005 – 2012. In addition, preliminary spatial distribution models of the abundance of several Culicoides vectors have been generated for Europe and north Africa which are currently being assessed by the field data providers.

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Talk 22"Defining generic ecoregion for bluetongue vectors in Europe"

Marius Gilbert[1], Jean Artois[1], Carla Ippoliti[2], Luca Candeloro[2], Annamaria Conte[2]

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ABSTRACT

The main objective of our work package is to disentangle the relative effects of environmental and anthropogenic factors in BTV spread and quantify connectivity between epi-zones over short periods of time. The first step toward this is to define BTV epizones in Europe. We used multivariate and spatial clustering technique to map the distribution of areas that are similar in terms of a number of eco-climatic variables, following an analytical framework that had been developed for vector-borne diseases in Italy. We show that the incorporation of host distribution data allows integrating more specific aspects of the BTV epizones, and significantly influences the spatial pattern of epizones, with areas that share similar conditions in terms of host species compositions and eco-climatic conditions. The future integration of vector distribution data would allow capturing the full environment / host / vector variability in the definition of epizones.

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Talk 23: Blutongue sentinel surveillance and monitoring of the genus culicoides in relation of orbiviruses in 2017 in Tunisia

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Abstract :

Since 1999, Bluetongue (BT) outbreaks have occurred in Tunisia and 4 serotypes, BTV2, BTV1, BTV4 and BTV3, were involved in 2000, 2006, 2009, and 2016, respectively. BT surveillance program, based on seroconversion of sentinel cattle and virus detection in blood samples by means of real-time RT-PCR were carried out in 16 herds from the end of 2017. Entomological surveillance has been implemented with the aim to study population dynamics of *C. imicola* and other potential vectors. Two-night catches of midges per site were performed monthly from september to december 2017 on 17 sites through- out the country. The preliminary results of these two monitoring will be presented.

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Talk 24: **"Emergence of BTV-4 in Mainland France"**

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Background:

In mainland France, Bluetongue virus (BTV) emerged in 2006. After several vaccination campaigns of cattle, sheep and goats, no virus had been detected after 2010. France obtained an OIE/EU recognized BTV-free status in December 2012. In 2015, BTV-8 re-emerged in central France. In December 2016, BTV-4 was detected in South of Corsica. At the beginning of November 2017, a calf was tested RT-PCR BTV-4 positive in the context of pre-movement analysis. This presentation describes the detection of this case, the

laboratory investigations that have been carried out to characterize the causative agent and the measures put in place to evaluate the BTV-4 spread and to control the disease.

Materials and methods

A 15-day-old calf, which was born in Haute-Savoie moved to an assembly center in Loire, prior to being exported to Spain. Blood sample was taken and rtRT-PCR analyses and isolation assays on KC, embryonated eggs and BSR cells were carried out. Next-generation sequencing was performed on RNA extracts from infected KC cells.
To evaluate the spread of BTV-4, blood cattle samples were collected in Haute-Savoie and neighboring departments in November and December 2017. Analyses were performed in Departmental Laboratories and BTV-4 positive samples sent to the ANSES National Reference Laboratory for confirmation.

Results:

The calf was detected BTV positive with pan-BTV rtRT-PCR kit, negative with a BTV-8 rtRT-PCR and positive with a specific BTV-4 rtRT-PCR. After isolation of the BTV-4 strain, sequence analysis of the 10 double-stranded RNA segments showed a close relationship with the BTV-4 isolated in Hungary (2014) and in Corsica (2016). The nucleotide and amino-acid sequences identities were for all segments superior to 99.5 % with the homologous sequences published.
To date, 84 BTV-4 outbreaks were detected in Mainland France. Most of them (n =74) were in Haute-Savoie, the 10 others occurred in the departments of Ain, Haute-Saône, Saône-et-Loire, Jura, Maine-et-Loire and Yonne. Only one BTV-4 isolate has been obtained.

Conclusion:

rtRT-PCR analyses from cattle samples collected during survey or investigations showed that BTV-4 was present throughout the Haute-Savoie department in November 2017, suggesting that BTV-4 was introduced several months before the first case detection. A compulsory vaccination campaign was decided in November 2017. However, the limited availability of vaccines led to a change in the BTV control strategy. On the first of January 2018, the whole of mainland France switched to BTV-4/BTV-8 restriction zone.

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Talk 25: Application of the ADS model to study the introduction of BTV-3 in Sicily

C. Aguilar-Vega[1*], E. Fernández-Carrión[1], **J.M. Sánchez-Vizcaíno**[1]
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In October 2017, bluetongue virus serotype 3 (BTV-3) was detected in the western part of Sicily for the first time in Italy and in Europe. In 2016, the strain was circulating in Tunisia. Since the distance between the closest coast of both countries is less than 150 km, we aimed to assess the possible introduction of BTV-3 to Italy by the aerial transportation of infected midges from the Cap Bon peninsula in Tunisia to the western part of Sicily.

For that purpose, we used a recently published model, an advection-deposition-survival (ADS) model, to identify the probable days of infected *Culicoides* introduction, from August 1 to September 28, 2017. Additionally, the Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) model, from the National

Oceanic and Atmospheric Administration (NOAA) Air Resources Laboratory (ARL), was utilized to contrast the results from the former model with a proven reliable tool.

Our findings point to September as the most likely month of introduction with several days of midge deposition in Sicilian territory. The model gives also the exact day and hour of midge deposition. Furthermore, the accumulative deposited particles of this month showed high risk of introduction to the western area of the province of Trapani, where the outbreak was reported.

The ADS model is a versatile model which can be used in any location and could be used as an early warning detection system for the windborne introduction of vector-borne diseases.

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Talk 26: Species barrier to orbiviruses and antiviral strategies.

Houssam Attoui (Anses / INRA - France)

Abstract not yet available

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Talk 27: Culturing cells from different *Culicoides* species

Lesley Bell-Sakyi, The Tick Cell Biobank, University of Liverpool

There is currently only one widely available *Culicoides* cell line, KC, derived from the New World species *Culicoides sonorensis*. Using adaptations of published methods, I have been attempting to set up primary cell cultures from eggs of two UK midge species, *Culicoides nubeculosus* sourced from the laboratory colony at The Pirbright Institute, and field-caught *Culicoides impunctatus* provided by a colleague at the University of Liverpool. Protocols developed through this work will then be applied to BTV vector species sourced from PALE-Blu partners, starting with Senegalese *Culicoides imicola* and *Culicoides oxystoma* to be provided by ISRA. In this talk I will present the latest results of the midge cell culture attempts in Liverpool.

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Speakers unable to attend: